ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 5, Issue – 2, March - 2022, Page No. : 103 - 117

Biofilm and periodontal disease - Then and now

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Citation of this Article: Dr. Raunak Baksh, Dr. Rashmi Hegde, Dr. Satyajit Salve, Dr. Taania Saana Aarif., Dr. Prerna Ghodke., Dr. Sangeeta Muglikar, Dr. Dhanashree Chaudhary, “Biofilm and periodontal disease - Then and now”, IJDSIR- March - 2022, Vol. – 5, Issue - 2, P. No. 103 – 117.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Introduction

Oral cavity has an environment conducive for bacterial community to inhabit as primary niches. These bacterial communities grow, organize and adhere to the tooth surface forming biofilm. This biofilm matures and then form dental plaque[1]. Dental plaque is a clinically structured, resilient, yellow-grayish substance that adheres tenaciously to the intraoral hard surfaces, including removable and fixed restorations[2]. The colonization of various bacteria in the oral cavity of a healthy individual depends on balanced bacteria host and interbacterial interactions i.e symbiosis. The continuous presence of plaque in gingival tissues and interactions of pathobionts with host cell results in inflammation, leading to periodontitis. In this condition there is a shift

in the microbiome and its functions associated with disease which is called dysbiosis[1].

Structure

Biofilms are composed of microbial cells encased within a matrix of extracellular polymeric substances, such as polysaccharides, proteins, and nucleic acids[2]. They have definite architectural structure and there is no uniformity in the distribution of bacteria, however there are aggregates of microcolonies that have variation with size and shape[3]. The microorganisms that are present on the outer surface of biofilm are not strongly attached to the matrix. Bacteria living in the deeper structures have better protection by biofilm rather than planktonic bacteria. Organized structure composed of microcolonies of bacterial cells non-randomly distributed in a shaped matrix or glycocalyx. Biofilm has slimy extracellular matrix produced by bacteria residing in it. This matrix and the microbial community has protective function from the chemotherapeutic agents. The polysaccharide matrix acts as a barrier and makes entry of chemotherapeutic agents difficult which helps bacteria and their colonies in survival. The flushing of bacteria by gingival crevicular fluid is prevented by extracellular matrix as it keeps them bonded together. Bacteria have a communication network to communicate with each other called Quorum Sensing. Through this sensing they keep monitoring each other's presence as well as modulate their gene expression in response to the number of bacteria[2]. Other than interbacterial communication, bacterial –host cell cross talk is also evident. Bacteria must export enzymes to break up nutrients and absorb and assimilate the essential carbohydrates and amino acids in order to grow and reproduce. By joining together and adhering to a surface, biofilm bacteria gain a survival advantage and improve their access to nutritional supplies. Using products exported by neighbours which have a symbiotic benefit[4].

Stages of biofilm formation

1) Adherence phase

Deposition of supragingival and subgingival biofilm is the initiation of formation of dental plaque. Deposition of glycoproteins from the oral environment on tooth surface takes place. Various proteins and peptides are secreted from salivary glands which contributes in the formation of biofilm. The thin layer that is adsorbed to tooth surface is called acquired pellicle. This acquired pellicle makes oral cavity more receptive to colonization by bacteria. The chemical and physical characteristic of acquired pellicle varies by different glycoproteins coated on different surfaces like enamel, dentin, cementum and restoration. The composition of pellicle has its effect on the types of bacteria that attach to different surfaces and microbial composition of the biofilm[5, 6, 7].

2) Lag phase

In this phase there is a shift from planktonic to sessile life which results in change in the bacterial phenotype. During this phase a temporary lag in growth of bacteria is seen because of the shift in their genetic expression. Genes in the polysaccharide production are increased while the genes involved in flagella production will turn off. The lag phase sets the course for further development of the biofilm[8].

3) Rapid growth phase

A large amount of water insoluble extracellular polysaccharide is secreted from glycocalyx during this phase. Alginates that are acetylated polymers of uronic acid which is secreted by gram negative bacteria. Microcolonies grow within the matrix and co-aggregation; co-adhesion leads to increase in thickness of biofilm[9, 10, 11].

4) Steady state phase

The growth of bacteria is steady during this phase. Internal transfer of nutrient takes place. Bacteria present deep in matrix shows sign of death and disruption of cell wall while the bacteria present close to the surface remain intact. Inter bacterial matrix shows crystals that show initiation of calculus mineralization. Static condition within a biofilm may cause bacteria to shrink into forms unresolvable with a light microscope[12]. Many broken bacterial cells and ghost cell walls devoid of cytoplasm can be found deep within the interior of old dental plaques. Crystals are frequently detected in the interbacterial matrix near the pellicle, which may be the early phases of mineralization that lead to calculus formation[13].

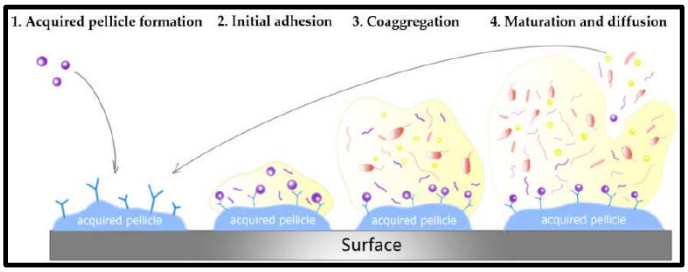

Figure 1: Stages of biofilm formation. (Illustration from Influence of Dental Prosthesis and Restorative Materials Interface on Oral Biofilms 2018)

Properties of Biofilm -Life in "Slime City"

Antibiotic resistance

Antibiotic resistance is almost 1000-1500 times higher in biofilms than in bacteria in a planktonic condition. Increased resistance mechanisms differ from species to species, antibiotic to antibiotic, and biofilm growth in various habitats. The resistance of bacteria to antibiotics is affected by nutritional status, growth rate, temperature, pH, prior exposure to sub-effective concentrations of antimicrobial agents. The susceptibility of bacteria to antimicrobial drugs has been assessed by measuring the Minimum inhibitory concentration (MIC) or Minimum bactericidal concentration (MBC) on cells cultured in liquid culture. The MIC of an organism growing on a surface can be 2 to 1000 times higher than that of the same cells developing planktonically.

Given an organism's lower surface sensitivity, determining an agent's "Biofilm inhibitory concentration" (BIC) or "Biofilm eliminating concentration" (BEC) or "Biofilm killing concentration" (BKC) is more appropriate[14,15].

Mechanism of antimicrobial resistance

Bacteria in biofilms develop at a slower rate, making them less vulnerable to many antibiotics, but not all. Strongly charged or chemically highly reactive agents can fail to reach deeper zones of biofilm because it acts as an ion exchange resin, removing such molecules from solution. Antimicrobial agents may be repelled by the matrix's structure; charged inhibitors can bind to oppositely charged polymers that make up the biofilm matrix. Extracellular enzymes that neutralise antibiotics, such as lactamase, formaldehyde lyase, and formaldehyde dehydrogenase, may become trapped and concentrated in the extracellular matrix, rendering some antibiotics inactive. The chemical may adsorb to the surface of the biofilm and inhibit organisms there, while leaving cells deeper in the biofilm unharmed. Bacteria growing on a surface have a new phenotypic change, which can lead to lower inhibitor sensitivity. The drug target may be modified or not expressed in a biofilm as a result of growth on a surface, or the organism may adopt different tactics[15,16].

"Super resistant" bacteria were identified in biofilm. These bacteria have multi drug resistant pumps and can extrude antimicrobial agents from the cell. Antibiotics are placed outside the outer membrane by these pumps, providing protection against antibiotics that target cell wall production. Bacteria develop slowly in nutrient-

depleted environments, making them far less susceptible than faster-dividing cells. The environment at the depths of a biofilm may be unfavorable with some drugs optimal action[2, 14,15.]

Quorum sensing

Different chemical communication methods, commonly referred to as quorum sensing, are often used to regulate biofilm formation. These density-dependent cell-to-cell signaling systems, which require the production of specialised signaling molecules known as autoinducers, enable bacteria to establish coordinated social behaviour. Quorum sensing systems extensively regulate biofilm development and maturation, generating changes in bacterial surface and metabolism that allow for the formation of a biofilm matrix. Since QS-related genes have been discovered to regulate the development of biofilms in a variety of oral infections, QS is being increasingly acknowledged as a key element in pathogenic oral biofilm development[17].

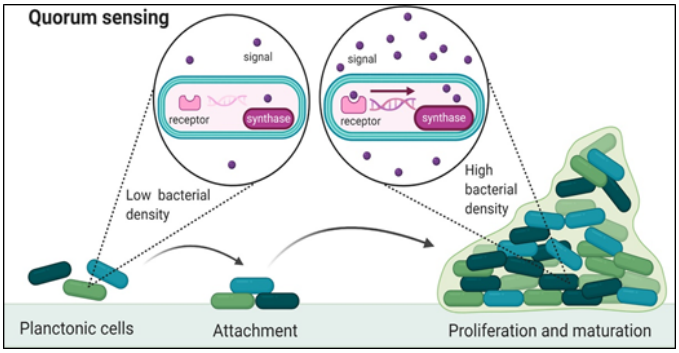

Figure 2: Quorum Sensing (Illustration from Quorum sensing systems as a new target to prevent biofilm-related oral diseases 2020).

Table 1: Key Players in Quorum Sensing.

Key Players in Quorum Sensing	
1	Autoinducers
	• Acyl homoserine lactones
	• Autoinducer 2
	• Cyclic dipeptides
	• Bradyoxetin
	• Other types of autoinducers
2	Autoinducer Synthases
	• AHL synthases
	• AI-2 synthase
	•Synthases for other types of autoinducers
3	Quorum Sensing Regulators
	• Lux R-type regulators
	• Lux P/Q-type regulators

Quorum quenching

By alarming the quorum sensing system used by the many species of bacteria that make up the plaque biofilm, the biofilm production can be disrupted. Quorum quenching is the process of inhibiting quorum sensing. Quorum sensing can be inhibited in a variety of methods such as enzymatic degradation of signaling molecules; blocking signal generation; and blocking signal reception. Quorum sensing inhibitors can be divided into two groups. The first group includes compounds that structurally resemble quorum sensing signals, such as halogenated furanones and synthetic Auto Inducer Peptides (AIPs), which are structurally similar to Acyl-Homoserine Lactones (AHL) and Auto Inducer Peptide (AIP) signals, respectively. These inhibitors prevent the relevant signal from binding to the receptor or lower the receptor concentration. The other groups of small chemicals include enzyme inhibitors. Triclosan, a potent inhibitor of the enoyl-acyl carrier protein (ACP) reductase that is involved in the synthesis of acyl-ACP, one of the essential intermediates in AHL biosynthesis, reduces AHL production, and closantel is a potent inhibitor of histidine kinase sensor of the two-component system[18].

Gene Transfer

Cells also “communicate” and interact with each other via horizontal gene transfer. Biofilms provide optimal

conditions for horizontal gene transfer due to the close closeness of cells (HGT). HGT requires obtaining DNA from either co-existing species or from exogenous sources. Transduction by bacterial viruses (bacteriophages), conjugation by bacterial pili, and transformation by DNA uptake involving biologically competent bacteria are all methods for transferring DNA.In Gram-negative bacteria, DNA can also be transmitted via membrane vesicles. HGT allows oral bacteria to sample from a massive metagenome, increasing their ability to adapt to changes in the oral environment. Antibiotic resistance genes (ARGs) are abundant in the oral cavity, and HGT is assumed to be the principal route for acquiring them. Extracellular DNA (eDNA) is a component of the biofilm matrix that is important for adhesion, possibly nutrition storage, and as a source of phosphate and other ions. Extracellular DNA (eDNA) has been seen with S. mutans, S. gordonii, Veillonella species and P. gingivalis. The fact that both resident and pathogenic bacteria isolated from the naso-pharyngeal region had genes imparting penicillin resistance that have a shared mosaic structure suggests HGT in dental biofilms[19].

Hypothesis

- **Specific plaque hypothesis (Loesche 1976)**

it states that pathogenicity of dental plaque is dependent on the presence of or an increase in specific microorganisms this theory conclude that certain microorganism present in the plaque are responsible for periodontal disease[20] van Palenstein Helderman,1984 stated that disease producing specific microorganisms are part of indigenous microflora and cannot be eliminated from oral microflora[21]. Miller in 1890 in his book 'The Microorganisms of the Human Mouth' stated that all bacterial species induce inflammation and cause destruction of periodontal tissue[22]. P. gingivalis, T. Forsythia, and A. actinomycetemcomitans, strongly associated with disease status, progression and unsuccessful therapy. Moderate evidence of etiology was seen with P. intermedia, P. nigrescens, C. rectus, P. micros, F. nucleatum, E. nodatum, and various spirochaetes[23]. Plaque associated with crevicular epithelial cells shows dominance of species such as S. oralis, S. intermedius, Parvimonas micra, P. gingivalis, P. intermedia, Tannerella forsythia, and F. nucleatum. Viruses, fungi, archaea, and protozoa can be encountered in the oral cavity of humans. Herpesviruses, human papillomaviruses, picornaviruses, and retroviruses can all contribute to the development of oral ulcers, tumors, mononucleosis, Sjögren syndrome, osteomyelitis, osteonecrosis, oral leukoplakia, and oral lichen planus[2].

Table 2: Overview of Primary and Secondary Colonizers in Dental Plaque.

Primary colonizers	Secondary colonizers
• Streptococcus gordonii	• Campylobacter gracilis
• Streptococcus intermedius	• Campylobacter rectus
• Streptococcus mitis	• Campylobacter showae
• Streptococcus oralis	• Eubacterium nodatum
• Streptococcus sanguinis	• Aggregatibacter actinomycetemcomitans serotype b
• Actinomyces gerencseriae	• Fusobacterium nucleatum spp. nucleatum
• Actinomyces israelii	• Fusobacterium nucleatum spp. vincentii

• Actinomyces naeslundii	• Fusobacterium nucleatum spp. polymorphum
• Actinomyces oris	• Fusobacterium periodonticum
• Aggregatibacter actinomycetemcomitans serotype a	• Parvimonas micra
	• Prevotella intermedia
• Capnocytophaga gingivalis	• Prevotella loescheii
• Capnocytophaga ochracea	• Prevotella nigrescens
• Capnocytophaga sputigena	• Streptococcus constellatus
• Eikenella corrodens	• Tannerella forsythia
• Actinomyces odontolyticus	• Porphyromonas gingivalis
• Veillonella parvula	• Treponema denticolaVeillonella

Non-specific plaque hypothesis (Theilade 1986)

The nonspecific plaque hypothesis stated that the microorganisms present in plaque that are accumulated on tooth surfaces and in the gingival crevice leads to the development of periodontal disease. The pathogenicity and the noxious products of these organisms initiate inflammation and host defense system, leading to the destruction of periodontal tissues. This hypothesis considered the amount of plaque accumulated as a disease producing factor. Thus, increase in the amount of plaque (quantity), as opposed to presence of certain specific pathogens present in plaque is responsible for inducing disease and its progression[24].

Ecological plaque hypothesis (Marsh 1991)

He proposed that a change in a key environmental factor (or factors) will trigger a shift in the balance of the resident plaque microflora, and this might predispose a site to disease. This hypothesis combines the two hypotheses specific plaque hypothesis and non-specific plaque hypothesis[17]. The total amount of dental plaque and the specific microbial composition of plaque contributes to the transition of periodontium from health to disease. Marsh expanded this theory and related the changes in microbial composition to changes in ecological factors such as the presence of nutrients and essential cofactors, pH and redox potential[25,26].

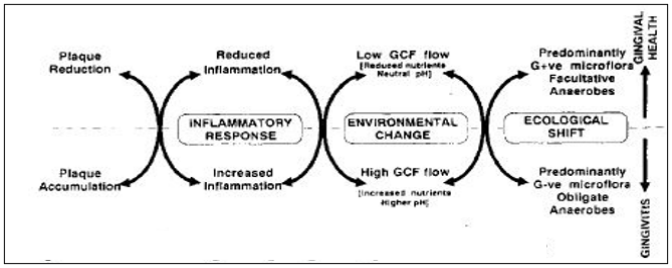

Figure 3: Ecological Plaque Hypothesis (Illustration from Microbial Ecology of Dental Plaque and Its Significance in Health and Disease Marsh 1994)

Due to Inflammation and tissue degradation, there is change in host environment leading to the shift in plaque microbiome. This shift shows a decrease in beneficial species whereas increase in the pathogenic species. This dysbiotic change is responsible for the chronic disease like periodontitis[27]. Benefit of this hypothesis is its therapeutic intervention by eliminating the cause such as microbial, host environmental which will help in restoring microbial homeostasis[2].

Keystone Pathogen hypothesis (G Hajishengalis 2012)

The concept of keystone pathogen is derived from basic ecological studies. George Hajishengalis proposed the Keystone Pathogen hypothesis. It indicates that certain low abundance pathogens disrupt tissue homeostasis into

dysbiotic diseases[28]. Evidence indicates that keystone pathogens can trigger inflammation when they are present in low numbers leading to periodontal disease. Studies in mice exposed to P. gingivalis developed periodontitis even when the pathogen was less than 0.1%. Whereas, in germ free mice disease did not occur in absence of other bacteria[2]. Hastruck et.al. 2007 found evidence of P. gingivalis as keystone pathogen in rabbit[29]. P. gingivalis was declared a "keystone" species in this study because it was identified in such low abundance but had such a significant impact on the number and composition of the oral microbiota, resulting in periodontal disease. P. gingivalis can activate the immune system by acting as an agonist of TLR4, or it can dampen the immunological response by acting as an antagonist of TLR4. Interleukin-8 synthesis can be inhibited by P. gingivalis. This condition is known as "local chemokine paralysis," and it causes polymorphonuclear leukocyte transmigration to be delayed, making the host less capable of dealing with the pathogenic onslaught. P. gingivalis has the ability to disrupt the complement system, which is part of the innate immune system. On the one hand, it inhibits activation by degrading C3 and trapping C4b-binding protein, but on the other hand, it uses the proinflammatory C5a through a crosstalk mechanism between its receptor and TLR2[2].

Hajishengallis and Lamont 2021 with “Black Queen Hypothesis” showed the similarities of the functional interdependences of the microbial constituents of the subgingival microbiome. Where functions which require energy are discarded by some organism considering it dispensable, these organisms are labeled as ‘cheaters’, as long as they are not lost completely and are retained by other “helper” organisms. This lays the theoretical groundwork for the establishment of keystone pathogens (and possibly other specialised functions) that benefit the community. Endogenous bacteria with pathogenic potential, such as keystone pathogens, use the metabolic and/or colonisation properties of their microbial neighbours (accessory pathogens) to increase the nososymbiocity of the community, whereas exogenous pathogens responsible for classic infectious diseases use strategies to overcome colonisation resistance[30].

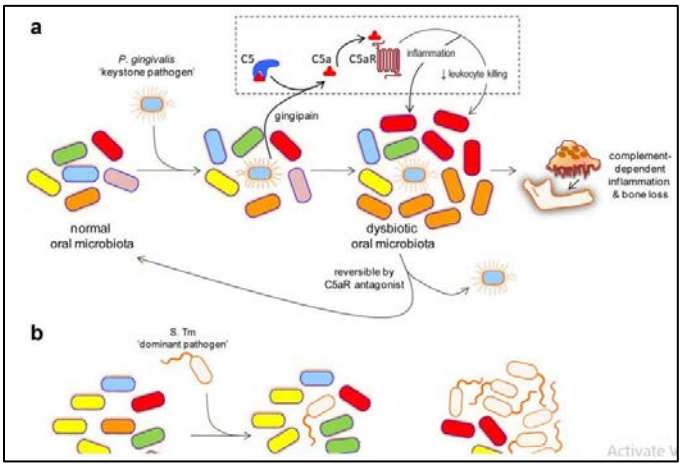

Figure 4: Keystone Pathogen Hypothesis. (a) Keystone Pathogen-Induced Dysbiotic Disease (b) Dominant Pathogen-Induced Inflammation and Effects on The Microbiota. (Illustration from Keystone Pathogen Hypothesis Hajishengallis 2012)

Polymicrobial Synergy and Dysbiosis Model (PSD)

Periodontitis is triggered by a broadly-based dysbiotic, synergistic microbiota, as opposed to the classic concept of a conventional infectious disease caused by a few or even several select bacteria periopathogens, such as ‘red complex’. Hajishengallis proposed polymicrobial synergy and dysbiosis model (PSD). A varied microbiota colonises the gingival crevice, and suitable species form heterotypic communities. The host and these communities are in symbiosis. Overgrowth and overt pathogenicity are controlled by the host, despite the fact that they are proinflammatory and can release harmful chemicals like as proteases. The microbial composition

of communities can change over time, from person to person, and from location to location. Following interactive communication with accessory pathogens such as mitis group streptococci, colonisation by keystone pathogens such as Porphyromonas gingivalis raises the pathogenicity of the entire population. The host immune system is compromised, and the dysbiotic population grows in number, disturbing tissue homeostasis and causing periodontal tissue damage[31].

Abuselem et.al. 2021 discussed the relevance of microbial compositional shifts as they can lead to both gingivitis and periodontitis. When dental plaque is viewed as a tissue, both gingivitis and periodontitis can be viewed as manifestations of the dental plaque becoming dysfunctional, such that healthy homeostasis is no longer maintained. Hence, the dental plaque biofilm in its entirety fits the definition of a diseased tissue. The microbial compositional changes and community characteristics for each clinical disease state. Here, bacterial community interactions also mimic the cell-to-cell interactions well described in host tissues: each cell of host origin in gingival tissue or each bacterium in the biofilm tissue senses its local environment and behaves transcriptionally in an appropriate way. Likewise, changes in the microbial composition can be likened to cancer or virally induced phenotypic changes in cells that alter tissue function[32].

Current concepts

Pathobionts

The microorganism living as normal residents in oral cavity, skin, and internal cavities are called commensals. In these commensal organisms there are some organisms which cause and promote disease these are called as Pathobionts. Pathobionts include opportunistic pathogen that reside in healthy and cause disease in susceptible host. Chow et. al 2011 stated that the growth of these pathobionts is triggered by immunodeficiency, pathogen infection, treatment with antibiotic and host-damaging drugs[1, 33].

Antibodies Against Citrullinated Peptide Antigens (ACPAs)

It was thought that pathogenic bacteria, bacterial cells, and/or toxin/metabolic byproducts may enter the systemic circulation from a clinically asymptomatic localised lesion and spread to discrete anatomical sites, causing disease[34]. Oral dysbiosis as a precursor to pathogenic autoimmunity, which leads to the development of rheumatoid arthritis. The prevalence of P. gingivalis in periodontitis lesions, in particular, has been linked to both chronic inflammatory disorders[35]. However, neither oral bacteria nor their toxin and byproducts have been found in the rheumatoid joint's focus, but the presence of P. gingivalis DNA in synovial fluid has been found by Reichart et.al. in 2013. Immunological consequences associated with oral infection caused by pathobionts are formation of antibodies against citrullinated peptide antigens (ACPA) that precede the development of rheumatoid arthritis and a molecular mimicry of bacterial proteins against host proteins, both of which raises the production of autoantibodies. The synthesis of ACPAs is linked to the development of RA, and their presence can be used as a reliable diagnostic marker for the disease. The pathology takes place due to dysregulated citrullination, followed by release of neo-epitopes that break immunity and trigger autoantibody formation[1]. Kopesky et. al. 2014 stated ACPAs binds to osteoclast precursor cells and cause production of IL-8 which acts on growth factor and carries differentiation into osteoclast[36]. But this could be only bone resorption mechanism, and it is not sufficient to produce chronic synovial inflammation[37]. Sohn et al. 2015 in animals model found that single dose

administration of ACPA did not induce arthritis. But if mild synovial inflammation is already present severe joint disease similar to rheumatid arthritis develops in presence of ACPA. It suggests that ACPAs alone are not sufficient to produce chronic inflammation but they do play an active role in disease process causing rheumatid arthritis[38]. Specific oral pathobionts that aid in the synthesis of ACPA and disease progression influence atherosclerosis, infective endocarditis, diabetes, unfavorable pregnancy outcomes, and respiratory illnesses[1].

Complex Microbial Consortium

Mark Welch in 2016 detected plaque as a complex microbial consortium. It consists of mass of Corynebacterium filaments with Streptococcus at the periphery. This structure is called as "Hedgehog" because of its spiny radially oriented filaments. He identified 9 taxa which are present in hedgehog structures.Corynebacterium,Streptococcus,Porphyromonas,Heamophilus/Aggregatibacter,Neisseriaceae,Fusobacterium, Leptotrichia, Capnocytophaga and Actinomyces. Corynebacterium filaments radiate outward from near the center and the coccoid Streptococcus cells are arranged around the distal tips of the Corynebacterium filaments. Also located at the periphery of the structure, in the same region as the Streptococcus, are cells of Aggregatibacter and Porphyromonas. Capnocytophaga occupies a wide band just inside the periphery. To summarize Corynebacterium is a filament rich annulus with periphery of corncob structures. Corncobs are structures consisting of coccoid cells kernels surround a central filament.

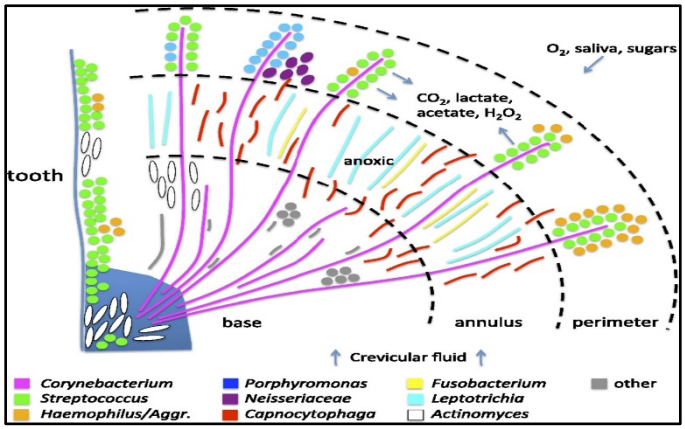

Figure 5: Interpretation of Hedgehog structure (Illustration from. Biogeography of a human oral microbiome at the micron scale. Proceedings of the National Academy of Sciences. Mark Welch 2016)

Usually, corncob consist of single layer of Streptococcus kernels surrounded by partial or a single layer of Aggregatibacter or Hemophilus porphyromonas kernels can be seen along with Streptococcus in same filament and even can form entire corncob by their own[39].

Symbiosis To Dysbiosis

A shift in the periodontal microbiome shows Symbiosis to Dysbiosis. Oral microbiome contains various microorganism with a balanced composition. Symbionts are organism that are good for health. Commensals are organism that neither are constant residents in oral cavity nor are good or bad for host. Pathobionts are permanent residents of oral microbiome and they have potential to induce disease. Dysbiosis occurs when there is shift in the composition of Microbiota with reduction in symbionts and increase in the number of pathobionts. This shift in microbiome affects host immune response and leads to dysbiosis leading to establishment of gingivitis and progression into periodontitis[2, 31, 40].

Bridge Species- Fusobacterium

Bacteria belonging to the genus of Fusobacterium, like Fusobacterium nucleatum can co-aggregate with both early and late colonizers, hence they are called as Bridge

species and they promote development of biofilm. Kaplan et.al 2010 stated for bridging F. nucleatum require molecules like Rad D, arginine-inhibitable adhesin and fusobacterial apoptosis protein Fap2[1, 41].

P. gingivalis And Oral Epithelium

Many studies have been performed on how P. gingivalis affects gingival epithelium and utilizes it for its survival[1]. Dogan et. al 2000 found that epithelial cells get invaded by P. gingivalis and quantity of invasion varies in different cells[42]. Dorn et. al. 2000 found that different P. gingivalis species has different adhesion rates. 0.5% in W50 strain. Adhesion capacity varies on different cell surfaces[43]. Pinnock et. al. 2014 examined survival and toxin of P. gingivalis in 3D organotypic mucosal model, which resemble oral environment. He found that there was increase in in intracellular survival and release in toxin during incubation. This suggested that interaction of epithelial cell and bacteria depend on various factors[44]. Muzio et.al in 2000 and Sugawara et. al 2006 found that Toll like receptors are pattern recognition receptor of epithelial cells and immune cells to recognize microbial toxin[45]. O'Connell et. al. in 2007 and Aberdam et al. in 2008 found that P. gingivalis has effects on miRNA in host cells. Degradation and change in expression of miRNA leads to dysregulation of proliferation and host cell immune responses[46, 47]. Moffat and Lamont in 2011 found that infection in gingival epithelial cells with P. gingivalis organism has shown altered miRNA involved in regulation of apoptosis and cytokine secretion. To survive in oral cavity P. gingivalis utilizes epithelial cells[48].

Gingipains are a series of arginine or lysine specific cysteine proteinases that have been identified as important virulence factors in Porphyromonas gingivalis. Gingipains are involved in epithelial cell adhesion and colonisation, erythrocyte haemagglutination and haemolysis, inflammatory response disruption and manipulation, and destruction of host proteins and tissues. Lysine-specific gingipain (Kgp), arginine-specific gingipain A (Rgp A), and arginine-specific gingipain B (Rgp B) are the three forms of gingipains found in P. gingivalis. Gingipains are mostly found on the outer membranes and outer membrane vesicles of all P. gingivalis strains except for HG66, which generates and secretes soluble forms of gingipains into the extracellular environment[49].

Oral Bacteria and Stem Cells

Mesenchymal stem cells (MSCs) are commonly referred as a group of cells that hold the capacity to self-renew for the life time and to differentiate to multilineage cells. Stem cells are mobilized towards the site of injury, and they come in contact with bacteria and bacterial components. Bacterial infection of stem cells could have long-term effects on the host's ability to operate. By triggering the host's defences, periodontal infections cause tissue damage which leads to the production of bacterial leucotoxins, collagenases, fibrinolysins, and other proteases, which break down host tissues and cause gingival inflammation. Microbial components, such as lipopolysaccharide (LPS), have the ability to activate macrophages and lymphocytes, causing them to synthesize and secrete a wide range of molecules, including cytokines, prostaglandins, hydrolytic enzymes, and tumor necrosis factor alpha, which stimulate periodontal tissue breakdown effectors. LPS derived from perio-pathogenic bacteria induces effects on the proliferation of periodontal ligament fibroblasts. P. gingivalis LPS promoted cell proliferation in periodontal ligament stem cells (PDLSCs). In both PDLSCs and dental follicle progenitor cells DFPCs, P. gingivalis LPS has been demonstrated to decrease osteoblastic differentiation. After stimulation with A.

actinomycetemcomitans LPS, Nomiyama et al. suggested that Gram-negative bacterial infection might down-regulate the odontoblastic characteristics of rat pulp progenitor cells[50].

Biederman et.al 2014 his study revealed that human dental follicle stem cells (hDFSCs) elicit a reduced pro-inflammatory response following bacterial infection, as compared to differentiated cells. Hieke et al. 2016 found that infection with viable bacteria induced distinct reactions by stem cells that were different from reactions to a single administration of LPS. Thus, infected stem cells showed a reduced capacity for migration[1].

P. gingivalis And Macrophages

Macrophage functions are also modulated by P. gingivalis. Macrophage migration-inhibitory factor (MIF) is involved in killing of bacteria by recruitment and activation of macrophages[1]. Li et al 2013 showed that in deep pockets P. gingivalis reduced the expression of MIF mRNA[51]. Castro et. al 2017 in his in vitro study showed that treatment of macrophages with the lysine-specific gingipain Kgp impaired its migration to apoptotic neutrophils and reduced he anti-inflammatory effect of apoptotic cells, resulting in a rapid inflammatory response. P. gingivalis promotes chronic inflammation by a gingipain mediated defect in apoptotic cell clearance and resolution of tissue restoration. P. gingivalis modifies antimicrobial host response and causes an imbalance in immune responses, leading to prolongation of inflammatory status and continuous damage against periodontal tissues[52].

Extracellular Compartment of Dental Plaque

Jakubovics et al. 2021 describes the extracellular compartment of the dental plaque biofilm and emphasizes the highly structured nature and coordinated functions of this matrix. The term glycocalyx originated as a description of the extracellular layer surrounding both prokaryotic and eukaryotic cells. In both instances, the glycocalyx provides a local environment that favors adhesion, community cohesion, and communication through highly specific interactions between and within cells of bacterial and host origin. In combination, these properties help to determine the number, type, and functions of the resident cells within each bacterially or host-derived tissue[53].

Biofilm And Homeostasis

Hathaway-Schrader and Novice 2021 found that the oral biofilm maintain tissue homeostasis with the adjacent gingival tissue, it also contributes to alveolar bone turnover. Microbial biofilm tissue is shown to contribute to alveolar bone turnover. The contribution of the oral microbiome to the RANKL/osteoprotegerin axis is viewed, and its contribution to alveolar bone remodeling, was observed by comparing bone heights. Hence, bone turnover can be seen as a probable function of the biofilm tissue. But it is dependent on the composition of the biofilm and hence bone turnover, and indeed net bone loss may be responsive to the change in composition which accompany microbial dysbiosis of the biofilm tissue[54].

Biofilm And Peri-Implantitis

Kotsakis and Olmedo 2021 described the differences between the pathologies exhibited in periodontal disease and peri implantitis. They found that the metal corrosion or release of ions from implants affects the homeostatic balance between microbial biofilm tissue and the adjacent host tissues. This happens due to the immunomodulatory effect of the implant corrosion or an impact on the structure of the biofilm in contact with the corroding implant surface. Exogenous products disturb the function of biofilm and host tissue disrupting the homeostasis. Other exogenous factors that influence the balance between the microbial tissue and the host tissue

includes the genetic of the host, deficiencies in the immune and innate systems, and the use of broad-spectrum antibiotics and antimicrobial agents[55].

Resilient Oral Microbiome

Wade 2021 in his review concluded that oral microbiome is resilient. The bacteria present in oral cavity are selected by the host immune system. When it is disturbed by immune deficiency or by reduced delivery of immune factors through a reduced saliva, colonization with non-oral bacteria takes place. In periodontal disease periodontal pocket is colonized with anaerobic bacteria which subvert the host response which leads to chronic non-healing lesion. This host and external factors can be seen as loss of health associated reselience[56].

Conclusion

Concepts on etiology of periodontal diseases have evolved through years and dental plaque has been considered the primary cause of various inflammatory periodontal diseases. Its action to cause disease has been studied widely through various hypotheses. The occurrence of periodontal diseases is influenced by numerous other factors, such as the virulence and resistance mechanisms of involved microbial species but also host-related factors. The complex organization of dental plaque biofilms, the interactions between commensal and pathobionts and also the relation with the host is important for mechanisms of periodontal diseases and their effect on systemic health. An in-depth knowledge about biofilm and its role in initiation and progression of periodontitis will aid in the treatment, prognosis and prevention of the disease.

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