Acta Scientific Dental Sciences (ISSN: 2581-4893)

Volume 6 Issue 5 May 2022

Review Article

Interspecies Communication in Oral Biofilm

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DOI: 10.31080/ASDS.2022.06.1361

Received: March 01, 2022

Published: April 19, 2022

Abstract

Periodontitis is a multifactorial disease that results in alveolar bone loss and attachment loss. Plaque biofilm and calculus were the basic culprits that creates the inflammatory state and causes host and microbial interactions within the periodontium. There are several species present within the oral cavity which makes necessary arrangements for survival within the host. Various modes of communication were played by these gram positive and negative species so that they can act together against the single host i.e., human. They release many substances which inhibit the host substances and alter the immune system of host. Moreover, they also help the selective survival of adjacent microbial species. Present review gives a brief description of some modes of communication within in oral biofilm, methods to study the bacterial interactions and controlled community-based pathogenesis of oral microbiota.

Keywords: Biofilms; Dental Plaque; Microbiota; Periodontal Diseases; Periodontitis; Quorum Sensing

Introduction

Any effective organization relies heavily on communication. The oral microflora have developed the means by which they communicate and thereby form successful organizations which in our oral cavity is referred to as the biofilm. In general it can be defined as a congregation of microbiota in biotic and abiotic surfaces covered with extracellular polymeric matrix substance and establishes new characteristics with respect to expression of gene, growth rate, metabolic activities and protein synthesis [1,2]. Oral microbial communities, which contain about 700 different bacterial species, are among the most complex microbial floras in the human body [3].

Plaque production requires interactions between human oral microorganisms. From a vast array of bacterial species colonize into densely populated communities from the early stages of colonization to the production of mature supragingival and subgingival plaque.

Interactions among different bacterial cell types are proposed to drive the maturation of plaque. Physical contact, metabolic exchange, small-signal-molecule-mediated communication, and genetic material exchange are all examples of these interactions [4]. Social interactions are most intense when individuals live side by side in a group, which for many microbes will mean a biofilm. Still there is a need to continue our search regarding the various mechanisms how species communicate among themselves. Hence present review discusses briefly regarding some modes of communication within in oral biofilm, methods to study the bacterial interactions and controlled community-based pathogenesis of oral microbiota Coaggregation **[5].**

The term co-aggregation in biofilms is a process of adhesion of genetically distinct microbial species by some specific molecules to form multi species biofilms [6]. The biofilm communities are complex and dynamic structures that accumulate through the sequential and ordered colonization of multiple oral bacteria. A highly selective mechanism of co-aggregation between species is involved in the development of multispecies communities. Many of these interspecies and intra-species coaggregations are reversible by the addition of simple sugars such as lactose.

In the 1970s, Gibbons and Nygaard discovered coaggregation among plaque bacteria when they conducted pairwise testing of 23 strains. Only five of the 253 pairs showed strong coaggregation, and these five were pairs composed of a *Streptococcus* and an *Actinomyces* or a *Coccobacillus*. Gibbons and Nygaard called it Interbacterial aggregation.

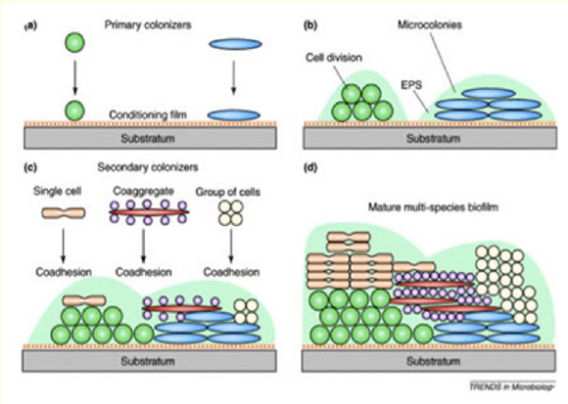

Figure a

Trends in microbiology

Streptococci and Gram positive rods such as *Actinomyces naeslundii* were the first bacteria to colonize on the tooth surface. *Streptococcus* mitis, *Streptococcus* sanguinis and *Streptococcus* oralis represent 60-90% of the cultivable streptococci [4] within the first four hours of plaque formation. Early colonisers of teeth associate with each other and with Fusobacteria, whereas late colonisers linked with disease associate with Fusobacteria but rarely with each other or with initial colonisers. In the absence of *F.nucleatum* many other secondary colonizers cannot become part of the dental plaque community. The surface of mature plaque, on the other hand, contains many more morphological varieties of bacteria after 24 hours, which coaggregate to form complicated structures like "corn cobs" and "bristle brush forms".

Certain cell components are known to mediate these interactions, namely adhesins and receptors which allow for bacterial surface attachment to host tissue, solid surfaces or other microorganisms. Bacteria can express multiple adhesins.

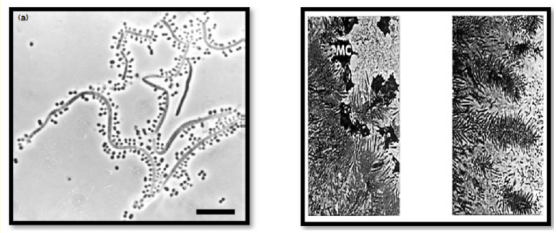

Figure b

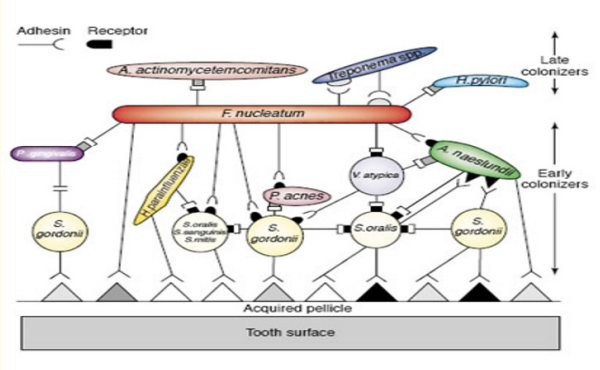

Figure c

Quorum sensing [7]

Some bacteria use sophisticated cell-cell communication mechanisms within biofilms to coordinate gene expression. Gene expression is initiated when the signalling molecules reach a certain threshold level.

The quorum-sensing mechanism of the bioluminescent sea bacterium *Vibrio fischeri* was the first to be characterised, and it is regarded the model for quorum sensing in most gram-negative bacteria (Nealson and Hastings 1979) [8] The light organ of the Hawaiian squid *Euprymna scolopes* is colonised by *V. fischeri*. To disguise its shadow and prevent predation, the squid employs the bacteria's light as counter lighting.

Nealson and Hastings 1979 [8] first demonstrated that a cell-free supernatant from a culture of *V. fischeri* contained a substance that stimulated the production of light when added to cultures at

low cell density. This signal was subsequently chemically characterized and shown to be N-acyl homoserine lactone, and the sensory mechanism that produced and responds to the signal was found to consist of only two proteins, which were designated LuxI and LuxR.

Till date, 40 different LuxI/LuxR-like quorum sensing systems have been identified in gram-negative bacteria and they regulate a number of important physiologic and virulence- related properties. The LuxS protein is required for the biosynthesis of the type 2 autoinducer, AI-2, which is involved in quorum sensing in a wide range of bacterial species. This system may be involved in cross-communication among both Gram-positive and Gram negative bacteria, as homologues of LuxS are widespread within the microbial world.

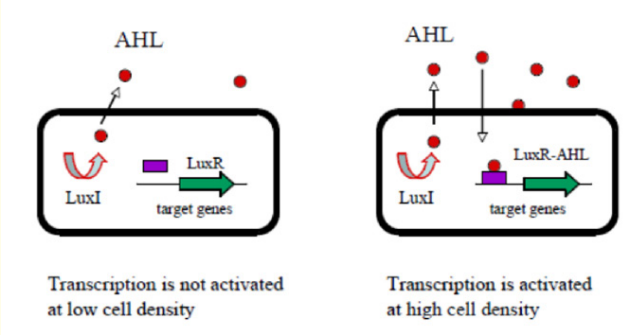

Figure 1: The LuxI/LuxR-type quorum sensing in Gram-negative bacteria. The LuxI-like protein is an autoinducer synthase that catalyzes the formation of a specific acyl-hormoserine lactone(AHL. The AHL) freely diffuses through the cell membrane at high cell density. The LuxR is a transcriptional regulator that binds to the diffusing AHL and it turn activities the transcription of its target genes.

Tomasz, 1965 [9] stated that a hormone- like extracellular product helped regulate competence in *Streptococcus* pneumonia. The signal was later identified as a peptide, Competence stimulating peptide (CSP).

It was discovered that when CSP levels hit a certain level, a subpopulation of bacteria died. They also released DNA into the environment, which was taken up by other bacteria with the necessary skills. Because bacteria cannot reproduce sexually, DNA exchange through this competence may result in alterations in the bacterial genome that are required for evolution. CSP has been proven to govern biofilm formation in addition to modulating competence: CSP-deficient mutants displayed lower biofilm-forming properties, but synthetic CSP was shown to boost the biomass of several streptococcal species. Competence was induced by the addition of exogenous CSP. The products of at least six genes, *comAB, comX*, and *com CDE* are involved in CSP signaling [10].

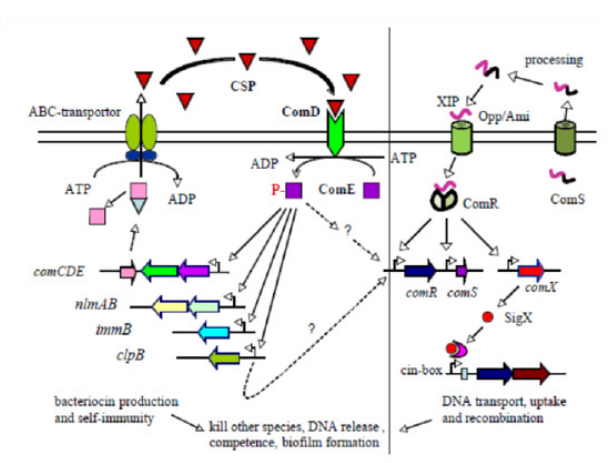

Figure 2: A schematic diagram indicating two types of siganaling peptide-mediated quorum-sensing in Gram-positive bacterium, S. mutans. The comCDE quorum-sensing primarily regulates production of bacteriocins self-immunity proteins, while the newly identified ComRS quorum-sensing system proximally controls competence development via in the control if sigX that encodes an alternative sigma factor, SigX (ComX). CSP is ComC signal peptide; XIP is mature sigX-induced peptide. Opp/Aml is an ABC transporter (Peptide importer).

Bacterial interactions have now become a therapeutic target, causing biofilm development to be disrupted and a cascade of events to occur as a result. Quorum quenching is the therapeutic enzymatic degradation of the signaling molecules will prevent the formation of biofilms and possibly weaken established biofilms.

Antibiotic resistance [7,11]

Reduced sensitivity of cells to antimicrobial drugs is a significant clinical consequence of both the structural arrangement of biofilms and the resulting altered pattern of gene expression.

Cells conventionally become resistant because of mutations affecting the drug target, to the presence of efflux pumps or to the production of modifying enzymes, etc., but even innately sensitive bacteria become less susceptible when growing on a surface. The structure of a biofilm may limit antimicrobial agent penetration; charged inhibitors can bind to oppositely charged polymers in the biofilm matrix (diffusion-reaction theory). Haque M., *et al.* [12] 2019 in their review concluded that mouth is a territory of various microbes and irrational use of antibiotics by dental surgeons in their clinical practice may also become a reason for antibiotic resistance and evolution of drug resistant destructive pathogens. According to recent meta-analysis and review done by Teughels W., *et al*. [13] 2020 and Abdulkareem A., *et al*. [14] 2021, stated that adjunctive use of antibiotics alongside periodontal therapy a significant improvement has been reported in clinical outcomes in periodontitis stage III/IV and grade C with absence of risk factors.

Metabolic communication [15]

In the oral cavity, endogenous proteins and glycoproteins (mucins) are the main sources of carbon and nitrogen for the resident oral microflora. Beneficial interactions may occur through the excretion of a metabolite by one organism that can be used as a nutrient by a different organism or through the breakdown of a substrate by the extracellular enzymatic activity of one organism that creates biologically available substrates for different organisms.

Bacteriocins

Bacteria are also able to generate products which may exert either specific or nonspecific effects on other bacteria. They frequently have a narrow killing spectrum and impede the growth of related species, unlike standard antibiotics. Oral bacteria's bacteriocins, notably mutacins generated by *S.mutans,* are thought to influence the creation and development of dental plaque [16] Bacteriocins may influence interspecies relationships by functioning as signalling molecule mimics. *Streptococcus pyogenes* and *Streptococcus salivarius,* for example, create lantibiotic bacteriocins that are structurally similar and can interact with each other's two-component signalling systems [17].

Genetic exchange

Signaling is not the only way of transferring information in biofilms. The high density of bacterial cells growing in biofilms facilitates the exchange of genetic information between cells of the same species and across species or even genera Potential mechanisms mediating genetic exchange in biofilms could include conjugation, transformation, and transduction. Plasmid DNA transfer from *S.gordonii* to S.mutans was detected in mixed cultures of *S. mutans* and *S.gordonii* expressing a shuttle plasmid in a CSP- and mutacin IV-dependent manner [18]

Methods to study bacterial interactions in oral biofilm [19]

It's critical to develop community-based assays that allow researchers to identify the microbial composition, multispecies architecture, and associated physiology, because interactions between different components create many new physiological functions that can't be observed with individual components. Recently developed software add capabilities for visually exploring three-dimensional data contained in confocal image stacks and thus greatly facilitate the understanding of complex spatial structures of biofilms. Confocal laser scanning microscopy, DNA microarray are being used to study these interactions.

Confocal laser scanning microscopy is an extensive tool to study biofilms in flow cell reactors under continuous flow. Several advantages like live biofilms can be properly imaged, spatial cell and matrix distribution and multiple time point images can be obtained. In combination with image analysis software like COSMAT this method can be helpful for quantification of biofilm matrix. This method was applied by Tseng., *et al.* [20] 2013 and Sonderholm., *et al*. [21] 2017 for monitoring the spatial temporal effects of different nutritional environments or antimicrobial treatments. Colvin., *et al.* 2012 in his study investigated the matrix composition of Pseudomonas aeurginosa and concluded the role of EPS psl and pel for the role of biofilm formation.

A study done by Wang H., *et al*. [22] 2017 untilized this micro array technique and total RNA of bacteria was treated with or without 6.25 microgram/milliliter NAl-P-113 and converted to cDNA, labelled and further hybridization was done by p gingivalis W83. Gene expression profiling and validation was also performed that NAL-P-113 at a low dose significantly down regulated genes regarding to mobile and extrachromosomal element functions, transport and binding of p gingivalis w83. Further concluded that NAL-P-113 exerted the anti-biofilm, anti-microbial properties regarding the inhibition of biofilms by mediating the energy metabolism and binding proteins to bacteria

Controlling community-based oral microbial pathogenesis [23]

With a better understanding of the oral microbial community, particularly the importance of the balance between oral pathogens and commensal residents, new approaches for selective inhibition of oral pathogens and modulation of the microbial composition of dental plaque to control community-based oral microbial pathogenesis are becoming more appealing. The various methods are as follows

Inhibiting adherence with antagonists

This is one of the most common approach as it is known that inhibition of entry of pathogenic bacteria into biofilms reduce the destruction. As adherence occurs through mechanical interlocking in inter dental areas, chemical binding to pellicle with sucrose mediated gluan attachments and non-specific binding [24,25] This method is better explained in dental caries caused by *Streptococcus* mutans. Studies also stated that a cell surface protein of *S. mutans* termed SpaP or Ag I/II has been identified as an adhesin which interacts with the tooth pellicle [26] Further dodecapeptide of SpaP inhibits S mutans attachment to teeth both in-vivo and in-vitro thus indicating their uses in toothpastes, mouth washes for inihibition of caries and control community based pathogenesis [27].

Replacement therapy

This method was initially introduced by J Hillman and colleagues in prevention of dental caries where non-cariogenic bacteria were introduced which inhibit the cariogenic bacteria and reduce the incidence of dental caries [28] This was even supported by Seema M and Marwah N [29] 2010 where strain replacement therapy is a novel approach for inhibition of dental caries. Thus non-cariogenic bacteria would replace the cariogenic one. In recent trends nano particles, small molecules, Quaternary Ammonium Salts (QAS), and natural products like Arginine, Tea, Propolis and Cranberry have been utilized for inhibition of Oral biofilms [30].

Probiotic approaches [31]

This is one of the recent approaches for prevention of dental biofilms and their pathogenesis. Several trials have been performed to depict this probiotic approaches some of them are: An in-vitro study done by Wu CC., *et al*. [32] 2015 utilized Lactobacillus salivarius strains for inhibition of S mutans and achieved decreased levels of the same and reduced expression of virulence genes of S mutans. A controlled trial done by Inverenici MM., *et al*. [33] 2018 utilized Bifidobacterium animalis and subspecies lactis with lozenges as adjuvant in treating periodontitis and achieved a lower level of pro-inflammatory cytokines and delayed recolonization of periodontal pocket. Later Benic GZ., *et al.* [34] 2019 used *Streptococcus* salivarius M 18 for treating halitosis and prevention of formation of dental biofilms. They have concluded that probiotics have reduced the halitosis in patients with orthodontic braces.

Interference with signaling mechanisms

Presence of some organisms that inhibit the occurrence of signaling mechanism of pathogenesis is a basic mode. This is better explained for prevention of dental caries where S. gordonii was demonstrated to interfere with quorum sensing in *S. mutans* by inactivating the CSP of the latter organisms [35] Ghosh A., *et al.* [36] 2020 in their review concluded that new smaller precise therapeutic anti-biofilm agents should be generated that would interfere in the maturation of biofilms at various levels and prevent the drug resistance too.

Targeted antimicrobial therapy via a novel STAMP technology

A STAMP is a fusion peptide with two moieties: akilling moiety made of a nonspecific antimicrobial peptide anda targeting moiety made of a specific species binding peptide. The targeting moiety provides specific binding to a selected pathogen and facilitates the targeted delivery of the attached antimicrobial peptide [37] The STAMPS were capable of eliminating *S. mutans* from biofilms of multispecies without having much effect on non-cariogenic bacteria of oral cavity thus indicating the positive effects of this technology in probiotic approaches too. This proof helped in demonstration of constructing specific STAMPS for other pathogens of oral biofilms. Guo L and Edlung A [38] in 2017 developed a targeted antimicrobial peptide C16G2 to eradicate harmful S. mutans where in lab studies successfully inhibited the pathogen without any threats of drug resistance. STAMP C16G2 is developed under an Investigational New Drug authorization with the U.S. FDA and is currently in Phase 2 clinical trials. This technology will revolutionalize in near future and many materials can be introduced for oral microbiota related diseases.

Conclusion

Developing oral prophylactic strategies through interference with communication systems of biofilm micro-organisms repre-

sents an interesting future challenge. Unlike strategies that target microbial viability, such approaches may interfere with microbial adaptive pathways without killing the micro-organisms. A better understanding of these processes is necessary for the development of novel strategies for oral disease prevention and control based on interference in these interspecies communication systems.

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