

Interspecies Communication In Oral Biofilm

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Abstract: Oral cavity contains large numbers of microorganisms. Dental plaque is composed of bacteria in a matrix of salivary glycoproteins and extracellular polysaccharides which displays extensive interactions while forming biofilm structures, carrying out physiological functions, and inducing microbial pathogenesis. Our emerging view of the microbial world is one in which individual bacteria exert their influence within communities through producing, sensing and responding to an array of chemical signals.

Keywords: biofilm, dental plaque, microflora.

I. Introduction

Most microbial ecosystems, like the human mouth, contain large numbers of genetically distinct microorganisms. Oral microbial communities are some of the most complex microbial floras in the human body, consisting of more than 700 different bacterial species¹. The oral microbial flora is responsible for two major human diseases: dental caries and periodontitis. For a very long time, oral microbiologists endeavoured to use reductionism to identify the key pathogens responsible for oral microbial pathogenesis. But rather dental plaque is an established sophisticated microbial community with novel functions that are essential for biofilm architecture and microbial physiology².

Oral Microflora

The mouth can be viewed as an island, which is characterized by near-constant presence of saliva, by short-term but extreme temperature fluctuation, by an externally exposed hard surface (teeth) and only limited bacterial energy sources¹. The human foetus inside the uterus is sterile. Within hours after birth, mouth becomes colonized by a number of facultative and aerobic bacteria. Beginning the second day, anaerobic bacteria can be detected in infant's mouth. After weaning, the entire human microflora is formed by a complex collection of approximately 10¹⁴ microorganisms consisting of about 400 different types of bacteria.³

Plaque Hypothesis:

Dental plaque is defined clinically as a structured, resilient, yellow grayish substance that adheres tenaciously to the intraoral hard surfaces, including removable and fixed restorations. It is mainly composed of bacteria in a matrix of salivary glycoproteins and extracellular polysaccharides³.

Plaque hypothesis is of following types^{3,4}:

1. Non specific Plaque Hypothesis: (W.D. Miller, 1890)⁵

Miller in his book "The Microorganisms of the Human Mouth" described how the accumulation of many bacterial species collectively induces inflammation and destruction of periodontal tissue. He believed that the virulence factors of the community as a whole produced the observed pathology.

2. Specific Plaque Hypothesis: (Walter J. Loesche 1979)⁵

Loesche in his article "Clinical and Microbiological Aspects of Chemotherapeutic Agents Used According to the Specific Plaque Hypothesis" described how periodontal disease can be attributed to individually identifiable bacterial species unlike the non-specific plaque hypothesis which suggested that all plaque was pathogenic.

3. Ecological plaque hypothesis: (P.D. Marsh ,1991)^{5,6,7,8}

Marsh in his work "Sugar, fluoride, pH and microbial homeostasis in dental plaque" proposed a modified hypothesis of the "specific plaque hypothesis" and "non-specific plaque hypothesis", bridging the gaps in these theories with evidence supporting the significance that environmental factors rather than just bacteria, have in relation to caries and periodontal disease.

Biofilm:

A biofilm can be defined as matrix enclosed bacterial populations adherent to each other and/or to surfaces or interfaces^{3,4}.

Tooth-associated oral biofilms can be roughly divided into : supragingival biofilms (on exposed enamel surfaces) and subgingival biofilms (within the periodontal pocket or sulcus)^{1,2,8,9}.

Transitions Of Oral Biofilms:

Transitions in supragingival biofilms:

The crucial pH in dental plaques of each individual is modulated by salivary concentrations of Ca²⁺, Po₄ and OH⁻, salivary production rate, dietary acid intake, host immune response and plaque community composition. Some Streptococci metabolize arginine present in salivary oligopeptides which results in the production of ammonium ions, thereby increasing pH within the biofilm.

Transitions in subgingival biofilms:

Subgingival anaerobic bacteria exposed to gingival crevicular fluid occupy a niche that is characterized by catabolism of amino acids from exogenous protein through secreted proteases, and the overall species diversity is higher than that of supragingival biofilms. Some of these anaerobic bacteria are considered to be periodontopathogens (for example, Porphyromonas gingivalis) and cause periodontitis. Periodontopathogens are thought to misdirect host defence and increase tissue-destructive inflammation. Poor oral hygiene changes the environment to support increased periodontopathogen biomass, leading to gingival detachment and bone and tooth loss¹.

Interactions Between Residents In Dental Plaque:

The oral cavity contains complex, multispecies microbial communities. This suggests that the residents in this community should display extensive interactions while forming biofilm structures, carrying out physiological functions, and inducing microbial pathogenesis. Our emerging view of the microbial world is one in which individual bacteria exert their influence within communities through producing, sensing and responding to an array of chemical signals^{10,11}.

Nutrients as the Basis for Bacterial Interspecies Interactions within Biofilms:

Organisms that have adapted to oral cavity have evolved metabolic pathways to utilize the available nutrients in specific ecological niche. Porphyromonas gingivalis often coexists with other bacteria, such as Prevotella intermedia, Fusobacterium nucleatum and Treponema denticola. Grenier and Maryland(1986)¹¹ observed that when grown in co culture, P. gingivalis can promote its own growth by metabolizing the succinate produced by T.denticola. In addition, isobutyric acid excreted by P. Gingivalis can stimulate the growth of T. denticola. Yoneda et al (2001)¹² demonstrated that cell extracts from both F. nucleatum and T. forsythia can also stimulate the growth of P. gingivalis.

General Metabolic Products Which Influence Biofilm Resident Interactions:

The secondary metabolites of one organism have effects on other organisms within the same biofilms. Dental plaque which contains high proportions of S. mutans generally yields low levels of S. sanguinis .The former organisms can metabolize sugars to lactic acid and S. sanguinis produce hydrogen peroxide, which has an antagonistic effect on S. mutans¹³.

The metabolic products of one organism may promote the growth of other organisms. The lactic acid produced by S. mutans can be readily metabolized by Veillonella family. Streptococcus oligofermentans converts lactic acid into hydrogen peroxide which is highly toxic to S. mutans¹⁴.

Bacteriocins role in Interspecies Interaction within Oral Biofilms:

Bacteriocins are proteinaceous toxins that inhibit the growth of microorganisms.

S. mutans strains produce a number of distinct bacteriocins, termed mutacins. Bacteriocins may also affect interspecies interactions by acting as analogues of signalling molecules.

The lantibiotic bacteriocins produced by Streptococcus pyogenes and Streptococcus salivarius are structurally similar and can interact with the signaling systems of each other¹⁵.

The genetic determinant for nigrescin, produced by P. nigrescens was identified by Teanpaisan et al (1998)¹⁶ which displayed a bactericidal effect against P. gingivalis, P. intermedia, T. forsythia, and Actinomyces sp.

Interactions Mediated by Signaling Molecules:

Communication is the process by which organisms pass information in the form of signals and induce responses such as behavioural change or altered gene expression in the receiver (Keller & Surette, 2006)¹⁴. Bacteria also sense changes in the local environment (cues) that are caused by neighbouring cells.

Two classes of molecules produced by oral bacteria have been implicated as true signals, produced specifically for the purposes of cell-to-cell communication. These are competence-stimulating peptides (CSPs) and autoinducer-2 (AI-2)^{17,18}.

CSPs are short peptides, approximately 17–21 amino acids, produced by many Streptococci from proteolytic digestion of the comC gene product. *S. mutans* CSP inhibits the formation of hyphae in *Candida albicans* (Jarosz et al., 2009)¹⁹ indicating that the ability to respond to CSP may not be confined to the domain bacteria. CSPs have diverse effects on oral streptococci, including promoting competence, biofilm formation and DNA release (Perry et al., 2009)²⁰. The CSP sensing pathway in *S. mutans* is linked to the production of mutacins, (Wang & Kuramitsu, 2005)²¹.

The molecule AI-2 has so far the only signalling molecule found to be widespread among both gram-positive and gram-negative bacteria (Federle & Bassler, 2003)²². AI-2 is a product of the activated methyl cycle, generated by LuxS-mediated cleavage of the intermediate S-ribosylhomocysteine to homocysteine and 4, 5-dihydroxy-2, 3-pentanedione (DPD) (Chen et al., 2002)²³. The luxS gene, encoding S-adenosylhomocysteinase (LuxS) is present in the genome sequences of many oral bacteria. The highest levels of AI-2 were produced by periodontal pathogens such as *P. gingivalis*, *Prevotella intermedia* and *F. nucleatum* (Frias et al 2001)²⁴. *A. Actinomycetemcomitans* was shown to possess a luxS gene and produce AI-2 (Fong et al., 2001)²⁵. An important function for AI-2 in mutualistic associations was demonstrated in studies of biofilms formed with *A. oris* and *S. oralis* (Rickard et al 2006, 2008)²⁶.

Genetic Exchange between Biofilm Residents:

Mechanisms mediating genetic exchange in biofilms include conjugation, transformation, and transduction. It has been shown that a shuttle plasmid present in *T. denticola* could be transformed into *S. gordonii*. In the study by Kreth et al(2005)²⁷ it was observed that *S. mutans* CSP induced coordinated expression of competence and mutacin production genes. On this basis it was proposed that *S. mutans*, may acquire transforming DNA from other species living in the same ecological niche.

New Techniques For Studying Oral Biofilm:

1. **16S rRNA Gene Sequencing Approaches:** (Woese et al 1985)²⁸
Variation within the sequences of 16S rRNA-encoding genes are studied.
2. **PCR-Based High-Throughput Approaches:**
This includes denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis , terminal restriction fragment length polymorphism (T-RFLP) , and denaturing high-performance liquid chromatography (DHPLC)².
3. **Ibis T5000:**
A high-throughput tool for analyzing microbial communities based on the use of broad-range primers to amplify PCR products.
4. **Checkerboard Approaches: (Socransky et al.)** ²⁹
Enables simultaneous profiling of multiple species within the same plaque sample in a semiquantitative manner.
5. **Genomic and Metagenomic Approaches;**
Accomplished for deep-sea samples as well as for samples from beneath the earth's surface.

New Approaches For Controlling Oral Microbial Pathogenesis:

1. Inhibiting Adherence with Antagonists:

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. A dodecapeptide analogue of the active binding site of SpaP which inhibits attachment of *S. mutans* to teeth has also been identified.

2. Replacement Therapy:

Developed by J. Hillman and colleagues (2002), involves the introduction of a noncariogenic *S. mutans* strain which produces a bacteriocin active against other *S. mutans* strains into the oral cavity to replace the naturally occurring cariogenic strains³⁰.

3. Regulating the Levels of Nonpathogenic Bacteria to Influence Virulence

Involves the introduction of a noncariogenic *S. mutans* strain which produces a bacteriocin active against other *S. mutans* strains into the oral cavity to replace the naturally occurring cariogenic strains.

4. Probiotic Approaches:

It involves the utilization of oral Streptococci which are able to metabolize arginine or urea to ammonia

5. Interference with Signaling Mechanisms:

This employs mechanisms by which *S.gordonii* interferes with the virulence of *S. mutans* in animal models.

Targeted Antimicrobial Therapy via a Novel STAMP Technology: (Eckert et al.2006)³⁰

A fusion peptide with a killing moiety made of a nonspecific antimicrobial peptide and a targeting moiety made of a species-specific binding peptide is used.

Future Perspectives:

Signalling between bacteria may have important implications for the virulence of oral pathogens. *Streptococcus cristatus* downregulates the expression of the *P. gingivalis* major fimbria gene *fimA* and inhibits biofilm formation by *P. gingivalis* (Xie et al., 2007)³¹. *S. cristatus* also modulates the ability of oral epithelial cells to respond to *F. nucleatum* (Zhang et al., 2008)³². Therefore, when assessing the ability of oral bacteria to cause disease it is essential to consider the community in its entirety rather than relying solely on observations of individual components. The ongoing development of high throughput techniques such as DNA microarrays and massively parallel sequencing is greatly enhancing studies of gene expression in mixed species communities. It remains to be seen whether these approaches will lead to new interventions that can change the course of oral microbial diseases which could then serve as a basis for exogenously modulating the interactions between biofilm constituents, resulting in novel approaches for controlling biofilm activities.

II. Conclusion

Many of the specific bacteria which are of medical and environmental concern reside in similar multispecies biofilm structures. Since most of these are present in heterogeneous biofilms, of which oral biofilms appear to be among the most complex, it is likely that these micro-communities exhibit properties which are dependent upon how the resident organisms interact. Interactions involving potential human pathogens as well as environmentally significant organisms are therefore of prime importance in this regard. Nevertheless, it is recognized that our understanding of the interactions which occur between biofilm residents is still in its infancy.

Conflict of interest: NONE

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