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REVIEW ARTICLE

# Interspecies communication in oral biofilm

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## Abstract

Oral biofilms are composed of >700 bacterial strains in a matrix of salivary glycoprotein and extracellular polysaccharides which display extensive interactions while forming organized structures in a biofilm to carry out physiological functions and induce pathogenesis in microbes. Interactions among oral bacteria in humans are essential to the development and maturation of the plaque. These interactions can occur at several levels which include physical contact, metabolic exchange, small-signal molecule-mediated communication, and exchange of genetic material. These levels of interspecies interaction benefit the microorganism by providing a broader habitat range, effective metabolism, increasing the resistance to host defense, and enhancing their virulence. This generally has a deleterious effect on the host and is attributed to many chronic infections which possess a curative challenge. This review summarizes the interaction between different microbial species present within a biofilm and how they communicate with each other.

## Introduction

The term biofilm describes the relatively undefinable microbial community associated with a tooth surface or any other hard, non-shedding material.[1] Biofilms are comprised one or more communities of microorganisms, planted in a glycocalyx, that are attached to a solid surface. Dental biofilms are awfully complex, multispecies ecosystems, where oral bacteria interact harmoniously or competitively with other members at various levels including physical contact, metabolic exchange, small-signal molecule-mediated communication, and exchange of genetic information.

For the biofilm to form initially, planktonic bacterial cells either directly attach to surfaces of the oral cavity or indirectly bind to other bacterial cells that have already colonized. For the interim retention of bacteria on dental surfaces, adherence is essential through coaggregation which further facilitates colonization of bacteria. Plausibly the crucial regulatory factors that dictate the bacterial composition and/or metabolism are metabolic communication, genetic exchange, production of inhibitory factors, and quorum sensing. Metabolic communications may occur habitually in oral biofilms as each bacterium can easily access a neighboring bacterial cell and its metabolites.

The unearthing fact that cells in biofilm communities interact explains how dental plaque acts as a mono unit. Socransky and Haffajee through their research work on cluster analysis have explained much of the distinctive colonization patterns and positive cooperation seen in oral biofilms, wherein each cluster of bacteria is created based on similarities and differences in nutritional and atmospheric environments.[2] These associations provided further knowledge to the concept that the microbial communities in oral biofilms were extensively involved in interspecies communication.

Microbial interactions are integral for primary colonization on tooth surfaces. For the biofilm to form bacteria should contact physically not only the surface of the tooth but the tissues of the host also. Lack of adherence on teeth leads to swallowing of bacteria with saliva. Through proper adherence, bacteria can build structured, closely knit, complex polyspecies communities known as dental plaque.

With adhesion of every new type of cell, an inceptive surface is created for the linking of different strains of bacterial species, leading to increased number of such surfaces and changes in species diversity eventually. These collaborations are proof of bacterial communications, the absence of which cannot create an organized plaque matrix. It is doubtful that different species in oral biofilms are self-sufficient, distinct constituents; rather, these microorganisms through intra - and inter-species communication function as a harmonious and coordinated community.

On the basis of culture-dependent and -independent methods, estimation of the diversity in bacterial species has revealed about 500 species.[3] Tests conducted to assess oral bacterial adherence, physical interactions in intra- and inter-species revealed specific patterns of recognition to their partner cells. This bacterial recognition in suspension between cells which are genetically distinct and resultant clumping is called coaggregation.[4]

## Quorum Sensing and Exchange of Genetic Information

Quorum sensing in bacteria "involves the regulation of expression of specific genes through the accumulation of signaling compounds that mediate intercellular communication."[5]

The acquired pellicle which forms on the surfaces of teeth within no time after teeth cleaning consists of molecules derived from host and is a great source of receptors which are identified by the initial colonizers in the oral biofilm. These receptors include varying enzymes such as mucins, agglutinins, proline-rich proteins, phosphate-rich proteins such as statherin, and alpha-amylase.[6] Each is a known receptor for particular oral species.

Streptococci comprise 70%–90% of the overall bacteria that inhabit the teeth in the first 3–4 h after professional teeth cleaning. Other primary colonizers include *Actinomyces*, *Capnocytophaga*, *Eikenella*, *Haemophilus*, *Prevotella*, *Propionibacterium*, and *Veillonella*. The bridging organism in oral biofilms between early and late colonizers is *Fusobacterium nucleatum*, which to some extent explains the fact that why they are so extensively found in plaque samples from both healthy and diseased sites. Other than Fusobacteria which act as the predominant bridge between primary and secondary colonizers, there is a possibility of bridging among primary colonizers as well.

Quorum sensing is required in the regulation of genetic competence, mating, bacteriocin production, sporulation, stress responses, virulence expression, biofilm formation, and bioluminescence.

## Metabolic Communication

Bacterial communications are instrumental as the evacuation products of one organism, can be of nutritive value to another. Similarly, the lytic products of a substrate create biologically available substrates for another organism.

In the biofilm, bacteria form polyspecies communities which are discriminated basically by their location, coronal or apical to gingival margin, or within the epithelium. Greater number of periodontal pathogens has been found subgingivally which are known to cause periodontal tissue destruction. Research has proven that cocultures of putative periodontal pathogens produce more biomass than their respective monocultures.

Cell-free supernatants from monocultures of *Porphyromonas gingivalis* and *Treponema denticola* were analyzed to detect whether the growth of the companion organism can be stimulated by them. It was found that the supernatant from cultures of one species could stimulate the growth of another.[7] This implies that cross-feeding between two species occurs. Further, research on biofilm architecture together with their physiological consequences at these sites is imperative to sort out the incidence and character of metabolic communication.

## Soluble signal Communication within Bacteria in Biofilm

One of the multiple ways by which oral bacteria communicate is the competence-inducing pathways gene transfer, which is one of the commonly studied ways of oral bacterial communications. This is mediated by competence-stimulating peptides (CSPs), which are small, cationic peptides of 14–23 amino acid residues that are produced by at least 10 species of oral streptococci.[8]

Competence refers to a physiological state in bacteria where the bacteria develop a capacity to take up exogenous DNA. It is an intricate process which involves multiple protein components and sophisticated regulatory networks. One of the major regulatory factors is the availability of a DNA pool when the cells become competent. In *Streptococcus mutans*, quorum sensing is mediated by a CSP which also induces genetic competence which leads to increased transformation frequency of biofilm grown *S. mutans* which is 10–600 times greater than for planktonic cells.

## Oral Bacterial Mixed-species Communication by Autoinducer-2 (AI-2)

AI-2 is a comprehensive signal (quorum sensing molecule) that may foster the growth of certain species in a mixed-species community. The genes that code for AI-2 are LuxS and they have been detected in various strains of bacteria.[9]

Low levels of AI-2 stimulate commensals, whereas higher levels are required for periodontopathogenic bacteria. AI-2 is suggested to be a signal which conciliates messages among different species in a polyspecies community, which distinguishes it from the family of acyl homoserine lactones, typified by AI-1 that helps in regulation of expression of genes in genetically identical cells.

It is demanding to hypothesize the intricate mechanisms of communication in mixed species by the proposed interspecies signal AI-2. Presuming that all the species in a mixed-species community such as oral biofilm are producing pro-AI-2, the species-specific mode of communication may be based simply on exactly detecting the proper isomer in the pro-AI-2 signal blend, causing an advantageous gene regulation response.

## Horizontal Gene Transfer (HGT) within Oral Bacteria

HGT between various oral bacteria is carried out in the form of conjugation, transformation, and transduction. This is possible due to the closely knit structure of various bacteria in oral

biofilms and the accessibility of exogenous DNA to pass through the oral cavity.

HGT results in transfer of virulence factors. Bacteriophages are the main culprits who convert many non-pathogenic bacteria to pathogens. The property of increased resistance to antibiotics shown by certain bacteria is caused by HGT through plasmids and conjugative transposons.[10]

## Direct Contact Signal: Antigens I and II

Many species of streptococci in humans exhibit surface proteins such as the antigen I or II. These multifunctional proteins adhere to other bacteria, tissues of the host, pellicle on teeth, and other soluble molecules. Thus, the Ag I or II polypeptides are superb agents which can facilitate streptococcal adhesion to oral surfaces and provide the right set of circumstances for other bacteria for interspecies communication in the oral environment.

## Can Alterations in Oral Bacteria Cause Oral Cancer?

Oral squamous cell carcinoma (OSCC) is considered the most common malignant neoplasm of the oral cavity. Late detection of these carcinomas at advanced stages results in poor prognosis. Hence, improved detection of these disorders is crucial. Role of oral bacteria in inflammation is an undisputed fact and their association with progression of OSCC provides a realistic target for diagnosis. The diagnosis of epithelial precursor lesions is relatively easy compared with that of other types of carcinomas due to the innate nature of these oral neoplasms. However, this slow transition from an epithelial precursor lesion to cancer requires further and continuous long-term follow-ups. The plausible mechanisms by which alterations in oral bacteria lead to oral carcinogenesis are through inhibition of apoptosis, activation of cell proliferation, promotion of cellular invasion, induction of chronic inflammation, and production of carcinogens.[11] Focused research on compositional changes in oral microflora has great potential to formulate a biomarker which can help in monitoring the stages of transition from epithelial precursor lesion to cancer in oral carcinogenesis.

## Newer Technologies for Detecting or Studying Oral Biofilms

1. 16S rRNA gene sequencing approaches: [12]
   Discrepancy within the sequences of 16S rRNA-encoding genes is studied through this approach.
2. PCR-based high-throughput approaches: [13]
   This includes temperature gradient gel electrophoresis or denaturing gradient gel electrophoresis, denaturing high-performance liquid chromatography, and terminal restriction fragment length polymorphism.
3. Ibis T5000:[14]
   It is a high-throughput tool for examining various communities of microbes based on the use of broad-range primers to amplify PCR products.
4. Checkerboard approaches: [15]
   Given by Socransky which enables concomitant profiling of multiple species within the same biofilm sample in a semi-quantitative manner.
5. Genomic and metagenomic approaches.

### Future directions

Spatial heterogeneity in oral biofilms prevails in relation to location of species in the lateral and axial dimensions, which changes over a period of time due to communication of bacteria of different species and gene expression. Explaining the complex structure of oral biofilm is possible at the species level with the use of confocal microscopy along with suitable probes based on 16SrDNA sequences and antibodies that identify epitopes on cell surface of oral bacteria. Explicitness of probes can be checked in biofilm models *in vitro*. *In situ* temporal oral biofilm research can be conducted. The innovation of reporters based on fluorescence which can work in an oxygen-free environment should be entertained. With the availability of these probes in future, the study of the regulation of genes in periodontal biofilms can be promoted.

Regulation of genes instigated by cell-to-cell contact is underinvestigated. Future studies can be facilitated by the invent of reporters based on green fluorescent protein. For analysis of gene expression, the present-day approaches in use are DNA microarrays, *in vivo* expression technology, and proteomics.

Sequencing of the genomes of *F. nucleatum*, *S. mutans*, *P. gingivalis*, and *Aggregatibacter actinomycetemcomitans* has been done. Investigation *in silico* will offer golden opportunities to detect particular genes (comC and luxS) hypothesized to be relevant to intra- and inter-species communication. Indeed, searching *in silico* yielded comC of *S. mutans* and luxS of *Streptococcus pyogenes*, *A. actinomycetemcomitans*, and *P. gingivalis*.

It is an established fact that even a minute regulation of the expression of genes in any organism within a community may lead to drastic changes in the organism's capacity to involve in the community activities. Gradual changes in composition of species in natural communities with the passage of time signal us to consider that even a small change in sequence of a relevant gene may cause only an ultrafine change but that is crucial in attributing to microorganism's succession in community. These ultrafine changes may be regulated by multiple integrated pathways. Outcomes of research in future will help us in drawing an effective comprehension of how microscopic changes can lead to macroscopic shifts in composition of population and metabolic output of polyspecies communities.

## Conclusion

Biofilms are spatially and functionally organized metabolically interconnected microbial ecosystems. Key to successful organization is communication. Oral bacteria are able to form successful complex organizations as they have developed various

means to communicate within themselves. Homeostasis is maintained in the host as these bacteria both commensals and pathogens coevolve together with their host to attain a highly intricate relationship for their existence.

Two different species that otherwise do not connect are brought together by coaggregation bridges. Such bridges are crucial for temporary bacterial retention on a nascent surface as they might, in turn, favor gradual colonization of various bacteria in the biofilm. For successful colonization of mixed species on teeth surfaces as well as genera, succession competitive and cooperative mechanisms play a pivotal role in both health and disease.

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