REVIEW ARTICLE

Esoteric communiqué amid microbes in an oral biofilm

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ABSTRACT

Dental biofilms are complex and multispecies ecosystems, and its formation requires coordinated chemical signaling between different micro-organisms present in the oral cavity. During the initial stages of its formation, planktonic bacterial cells directly attach to surfaces of the oral cavity or indirectly bind to other bacterial cells. This binding occurs through co-aggregation, which is critical for the temporary retention of bacteria on dental surfaces as well as bacterial colonization. It is during this colonization that the micro-organisms are able to interact with each other. In general, interspecies interactions involve communication, typically via quorum sensing, and metabolic cooperation or competition. Interactions among species within a biofilm can be antagonistic, such as competition over nutrients and growth inhibition, or synergistic. In this review, we discuss these important interactions among oral bacteria within the dental biofilm communities and novel therapies that could inhibit pathogenic micro-organisms and disrupt biofilm.

Key words
Bacteria, biofilm, communication, interspecies, quorum-sensing

INTRODUCTION

Periodontal microbiological study has evolved through the ages from the initial animalcules observed by A.V. Leeuwenhoek (1632-1723) to the "chemicoparasitic" theory of W.D. Miller (1890), followed by isolation of *Streptococcus mutans* by Clarke (1924) till the latest large scale 16SrRNA/DNA-based observations.[1] Over 700 bacterial species have been identified from the human oral cavity, making it one of the most complex microfloras of the human body. The residents in this community display extensive interactions while forming biofilm structures, carrying out physiological functions, and inducing microbial pathogenesis.[1] Thus, Communication is a key element in successful organization within this microbial biofilm population.[2] It is reasonable to assume that the interactions between the oral microbial residents may influence the properties of the whole community. In this regard, oral microbial communities (biofilms) may represent a micro example of the "Gaia" hypothesis (Lovelock J. E. 1965) i.e., biofilms are analogous to the planet Earth, where the properties of the latter as a whole are determined by the interactions of all of the residents as well as interactions of the populations with the inanimate supporting structures. Thus, a micro-Gaia community presumably exists in the dental biofilm. The interactions between the inhabitants could be envisaged as forms of "war and peace" among the bacterial residents of a biofilm.[1]

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Co-aggregation and the development of multi-species biofilms

Adherence of microbial cells to immobilized bacteria is called "co-adhesion," and binding of bacteria in suspension is called "co-aggregation."[3] The first organisms to attach are the primary (early) colonizers such as streptococci and Gram-positive rods within the first 4 hours of plaque formation. This colonization is mediated through specific or non-specific physico-chemical interactions with components of an adsorbed, organic conditioning film resulting in the formation of microcolonies. After 24 hours, however, the surface of mature plaque contains many more morphological types of bacteria, which co-aggregate to form intricate structures such as 'corncobs.' Secondary colonizers are then able to attach to the primary colonizers, and the biofilm begins to develop into a multispecies community as conditions in the biofilm change.

An important early physiological event occurring during the development of a biofilm, which leads to the adhesion of secondary colonizers, is the increased production of extracellular polymeric substances (EPS). These polymers envelop the attached cells within the biofilm, strengthen adhesion between cells within the biofilm, and can also act as receptors for co-aggregation interactions. The partnerships between dental plaque bacteria are highly specific, and primary colonizers can co-aggregate with each other but not usually with secondary colonizers.[4] *Fusobacterium nucleatum* is, therefore, proposed to be a bridge organism, because it can co-aggregate with both primary and secondary colonizers. In the absence of *Fusobacterium nucleatum*, many other secondary colonizers cannot become part of the dental plaque community. The correlation between the co-aggregation ability of plaque bacteria and the temporal sequence of bacterial integration into dental plaque very strongly implicates co-aggregation as a process closely linked with plaque development.

Mechanisms of communication

"Bacteria chatter continuously, and their words are chemical,"

"Not only can these cells talk with one another, they're multilingual."[5]

Bacteria communicate with one another using chemical signaling molecules as words. Three major bacterial languages have been extensively investigated: Those used by Gram-negative cells and mediated by Acylated Homoserine Lactone (AHL), the oligopeptides quorum-sensing (QS) molecules i.e., Competence Stimulating Peptide (CSP), and the Gram-positive microbes use a universal dialect to communicate called Auto inducer (AI-2), which is a molecular mix important for interspecies signaling- the process being called "quorum-sensing (QS)."

"Quorum-sensing" is the term given, because the frequent observations of signals are seen only to accumulate in environments that support a sufficiently dense population - A quorum of such signal-generating bacteria. When a QS signal molecule reaches a critical level, the population at large responds, usually through the co-ordinated expression of specific target genes.[6] Gram-positive and Gram-negative bacteria use QS communication circuits to regulate a diverse array of physiological activities. These processes include symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation.[7]

Signaling molecules (Bacterial dialects)

Acylated homorserine lactone

Acylated Homorserine Lactone (AHL) is the signal molecule used by gram-negative bacteria. On reaching a threshold concentration, it binds to and activates a regulatory protein, which then binds to a specific site on the DNA. This binding of the regulatory protein transcription activator results in production of the specific quorum-dependent protein as well as more enzymes to make the acyl homoserine lactone.[7]

Competent-stimulating peptide

Competent-Stimulating Peptide (CSPs) are short peptides, approximately 17-21 amino acids, produced by many streptococci from proteolytic digestion of the comC gene product. CSPs have diverse effects including promoting competence, biofilm formation, and DNA release.

In addition, the CSP-sensing pathway is linked to the production of mutacins, bacteriocins with antimicrobial activity against a range of oral bacteria.[8]

Autoinducer-2 (AI-2)

Autoinducer-2 (AI-2), a furanosyl borate diester, is made by many species of Gram-negative and Gram-positive bacteria. In every case, production of AI-2 is dependent on the *LuxS* autoinducer synthase. AI-2 is produced by many periodontal pathogens such as *Porphyromos gingivalis, Prevotella intermedia*, *Fusobacterium nucleatum,* and *Aggregatibacter actinomycetemcomitans.* AI-2 regulates iron uptake in *Aggregatibacter actinomycetemcomitans,* which exhibit a growth defect under iron limitation.[9]

Recent advances in the field indicate cell-cell communication via AI between

A. Different bacterial species
B. Between bacteria and non-bacterial organisms
C. Within bacterial cell.

Interaction between different bacterial species

Cooperation in biofilm

Metabolic co-operation

EPS is the major component of biofilm of almost all bacterial species. Cross-linking in EPS can provide shelter to the bacteria by blocking harmful agents outside and trap nutrients from the environment. It also influences iron exchange within the biofilm.

Oxygen metabolism and exchange within biofilm between different aerobic and anerobic species had a special significant role for the survival of obligate anerobes. The aerobic species consume Oxygen in the environment, which results in production of a local redox potential gradation that provides local anerobic conditions. This local anaerobic environment is the foundation of obligate anaerobic species survival.[10]

Resistance to antibiotics

The antigens of biofilm bacteria are hidden in the biofilm matrix and become less susceptible to the host immune system or applied antibiotics. Antibiotic resistance

genes can be transferred between bacterial cells within biofilm. The common carriers of resistance genes are plasmids. The replication of plasmids is independent of chromosomal DNA replication, and the number of plasmids in a cell varies widely. Plasmids with antibiotic resistance genes are gained by bacterial conjugation. The antibiotic resistance of bacterial cells in biofilm was reported to be 1,000 to 1,500 times greater than the resistance of planktonic cells and has become a rising problem in recent years.[10]

Competition in biofilm

The bacterial species compete for nutrients, binding sites, and the chance to survive. Several major competitive mechanisms are widely adopted by bacteria, i.e., "bacteriocin synthesis," QS, and hydrogen peroxide excretion.[10] Bacteriocin production is a spiteful behavior of bacteria that is central to the competitive dynamics of many pathogens present in biofilm.[11] Streptococci possess the strongest ability of producing bacteriocins among all oral bacteria. Bacteriocins produced by *Streptococcus mutans* are termed as "mutacins," which inhibit various gram-positive bacteria. *Streptococcus salivarius* also produces bacteriocins, such as "salivaricin A" (SalA), which strongly inhibits *Streptococcus pyogenes*. Therefore, the bacteriocins expressed by some strains greatly affect other strains living in biofilm. *Streptococcus salivarius*, *Streptococcus gordonii, Streptococcus sanguinis, Streptococcus mitis,* and *Streptococcus oralis* were shown to inhibit mutacin production by degrading *Streptococcus mutans* CSP. *Streptococcus mutans* and *Streptococcus salivarius* are not usually found in close proximity in the oral cavity, because *Streptococcus mutans* colonizes tooth surfaces, whereas *Streptococcus salivarius* is almost exclusively localized to soft tissues. However, it is possible that interactions between these organisms occur at a distance.[8] Also, *Streptococcus mutans* has inhibitory effect on *Streptococcus sanguinis* by production of large amount of organic acid. Although both *Streptococcus mutans* and *Streptococcus sanguinis* can metabolize glucose and produce lactate when incubated with excess glucose, *Streptococcus mutans* produces more acid than *Streptococcus sanguinis* due to the higher ATP-glucose phosphotransferase activity of *Streptococcus mutans* than *Streptococcus sanguinis*. *Streptococcus mutans* inhibits the ability of *S. sanguinis* to produce hydrogen peroxide (H_2O_2).[10] The H_2O_2 production of *Streptococcus sanguinis* is shown to be 66% reduced when *Streptococcus sanguinis* is cultivated along with *Streptococcus mutans*, compared to being cultivated alone.[10]

Interaction between bacterial cells and other non-bacterial species

Co-operation

Co-aggregation of the *Streptococci gordonii* and *Candida albicans* contributes to biofilm formation and results in closer proximity for cell-cell communication. Through a diffusible signal (DS) molecule, *Streptococci gordonii* suppresses farnesol-mediated inhibition of hyphae formation, thereby enhancing the potential for *Candida albicans* to form biofilms and its ability to invade tissue. In addition, *Streptococcus* promotes fungal growth by secreting metabolic products that can be used as a carbon source by *Candida albicans*. Likewise, *Candida albicans* enhances the survival and colonization of *Streptococci gordonii* by reducing the oxygen tension to levels preferred by *Streptococci gordonii*, thus providing bacterial growth stimulatory factors as a result of nutrient metabolism. These favorable conditions promote the formation of mature fungal-bacterial biofilms surrounded by an extracellular matrix, to which other bacterial or fungal species can bind. These interactions may make oral infections more persistent and harder to treat.[12]

Also, bacteria may be infected by viruses without being harmed. These bacteria have a virus-derived molecular genetic identity providing immunity to them.

Competition

Lactobacillus species defends the host against colonization of pathogens such as *Candida albicans*. Evidence suggests that the bacterium reduces the adhesion of *Candida albicans* to epithelial cells either by (a) outcompeting fungal cells for adhesion sites or (b) by secreting biosurfactants such as surlactin that physically decrease fungal binding. Most *Lactobacillus* strains release (c) hydrogen peroxide (H_2O_2) and (d) lactic acid or other fatty acids that inhibit *Candida albicans* proliferation and invasive hypha formation. (e) Bacteriocin-like substances produced by *Lactobacillus* suppress the fungal growth to directly decrease its load.[12]

Bacteria are important hosts for multiviral colonization. Virus replicates through the lytic or lysogenic pathway. In the lytic pathway, the virus utilizes bacterial resources to replicate and then destroys the host cell, releasing new viruses which infect other cells. In the lysogenic pathway, the viral genome inserts itself into the bacterial genome and replicates along with it, while repressing viral genes leading to lysis.

Communication within bacterial cell

Intra-organismic communications within the bacterial cells include generation, modifications, regulation of prokaryotic gene word order, and its evolutionary roots. Prokaryotic gene order is not as well-preserved as the sequences, which code for proteins. Only some higher order regulations (operons) that code for physically interacting proteins are found in almost all bacterial (archaeal) genomes. Exchange of whole genes or gene-blocks enables bacterial lifestyles to combine several bacterial competences, i.e., phenotypes. The transformation process includes the release of naked DNA, followed by the uptake and recombination, i.e., the

integration. Horizontal gene transfer is a main resource for integrating newly evolved genes into existing genomes and does not need the slow steps of chance mutations to alter the genomes but accelerated genome innovations in both bacteria and archaea.[12] The phenomenon of horizontal gene transfer is driven by viral competences inherent in bacterial settlers such as phages, plasmids, retroplasmids, and transposons.

Novel therapies

The recent emergence and spread of multi-resistant micro-organisms and refractory biofilm-induced infections have prompted an intense search for novel therapies that could inhibit pathogenic micro-organisms and disrupt biofilm.

Anti-microbial peptide

AMPs are genetically common molecules of innate immunity that have been discovered in single-cell and multicellular forms of life. Their mode of action often involves binding to the negatively charged moieties, e.g., lipopolysaccharide (LPS), on the microbial membrane. Once bound to the microbial surface, the peptides are predicted to lead to membrane disruption by insertion, but may also translocate into the microbe and kill by intracellular mechanisms. Due to their attraction to negatively charged structural molecules on the bacterial membrane, development of resistance to these peptides is rare.[13]

Eckert *et al.* in 2006 has developed a specific, highly novel anti-bacterial agent using the species-specific nature of CSP, called specifically targeted anti-microbial peptides (STAMPs). It recognizes *Streptococcus mutans* and kills it, but there is no effect on other types of non-cariogenic bacteria.[14]

Vaccines

Immunization against dental caries, and also periodontal disease—has been a central research topic in recent decades. The aim of providing immunization is to inhibit adhesion or to reduce the virulence of putative microbial etiologic agents. Micro-organisms could, for instance, be cleared from the oral cavity by antibodies prior to colonization, antibodies could block adhesins or receptors involved in adhesion, or modify metabolically important functions or virulence factors. Vaccination accomplished can be active immunization, passive immunization, or DNA vaccination, made from the antigenic epitopes in periodontopathic bacteria.[15,16]

Animal studies using either active or passive immunization approaches have been successful. There are also data to show that passive immunization in humans impedes re-colonization of selected target micro-organisms in both caries (Koga *et al.*, 2002; Smith, 2002) and periodontal disease (Booth *et al.*, 1996). As vehicles for passive immunization, both milk from immunized cows (Shimazaki *et al.*, 2001) and transgenic plants (Ma *et al.*, 1998) have been tested with encouraging results. Likewise, chimeric recombinant microbial vectors, which are avirulent but express antigens from *Streptococcus mutans* (Huang *et al.*, 2001; Taubman *et al.*, 2001) or *Porphyromonas gingivalis* (Sharma *et al.*, 2001), have been shown to provide protection against dental caries and alveolar bone loss, respectively, in experimental animals.[16]

A major problem is that immunization approaches are generally directed against single bacterial species epitopes, whereas both dental caries and periodontal disease are ecologically driven multi-microbial diseases (Marsh, 1994). Furthermore, since micro-organisms have the ability to form biofilms and to adapt and undergo transformation that may lead to altered anti-genicity; it is still questionable whether immunization can provide lasting protection.[16]

Probiotics and replacement therapy

Probiotic approaches include replacement of pathogenic bacteria by using harmless bacteria. Probiotic species mostly belong to the genera *Lactobacillus* and *Bifidobacterium.* Three main modes of action have been proposed to contribute to the effects of probiotics: 1) Production of anti-microbial substances against pathogens that inhibit oral bacteria, such as an organic acids, hydrogen peroxide, low-molecular-weight anti-microbial compounds, bacteriocins, and adhesion inhibitors produced by lactic acid bacteria, 2) Competitive exclusion mechanisms, and 3) Modulation of host defense systems.[13]

Photodynamic therapy

It involves the use of a photoactive dye (photosensitizer) that is activated by exposure to light of a specific wavelength in the presence of oxygen. The anti-microbial activity of photosensitizers of Poly-L-lysine-chlorin (e6) conjugate (pLCe6), Zn(II)-phthalocyanine, Toluidine Blue O (TBO) is mediated by singlet oxygen, which, because of its high chemical reactivity, has a direct effect on extracellular molecules. Thus, the polysaccharides present in EPS of a bacterial biofilm are also susceptible to photodamage. Such dual activity, not exhibited by antibiotics, represents a significant advantage of photodynamic anti-microbial chemotherapy. Breaking down biofilms may inhibit plasmid exchange involved in the transfer of antibiotic resistance, and disrupt colonization.[17] Biofilms of the oral pathogen *Actinomyces viscosus* have been exposed to red light in the presence of pLCe6. Confocal microscopy revealed that a photochemical wave increased the penetration of the pLCe6 conjugate by 50% and caused killing of 99% of biofilm bacteria (Soukos *et al.*, 2003).[18] Over 97% of oral bacteria were killed in multispecies biofilms irradiated with light from a helium/neon laser in the presence of TBO (O'Neill *et al.*, 2002).[19]

CONCLUSION

For hundreds of years, scientists and dentists have been looking for efficient methods to control oral diseases. Since the oral ecosystem view has become more widely accepted and oral bacterial interactions have been more elucidated, in the future, there may be medications to take advantage of inter-bacterial antagonism to control and prevent those diseases. Developing oral prophylactic strategies through interference with two-component systems or QS of biofilm micro-organisms represents an interesting future challenge. Unlike strategies that target microbial viability, such approaches may interfere with microbial adaptive pathways without killing the micro-organisms. Therefore, resistance development would probably represent a minor problem. However, future research must address the interactions within biofilm, which are not fully understood today. Because of the complex and variable nature of biofilm, this goal can only be fulfilled with future investigations focused on the model of oral ecosystems and relative factors, with advanced understanding of the mechanisms of bacterial biofilm interaction mechanisms.

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