QUORUM SENSING AND ITS ROLE IN ORAL BIOFILMS DEVELOPMENT

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Abstract

Quorum sensing systems has been identified as one of mechanism carried out by numerous Gram-positive and Gram-negative bacteria to coordinate virulence and biofilm development. Using quorum sensing, bacterial colonies synchronize gene expression and pheonotype change allowing them to protect their niche. The purpose of this review is to present a synopsis of the literature on bacterial quorum sensing and we highlight the role of specific signaling molecules that might be used as a target of inhibitor agent in dental preventive perspective. *Indonesian Journal of Dentistry 2006; Edisi Khusus KPPIKG XIV:87-91*

Key words:

Introduction

We used to think that microbes just float around by themselves, multiply, and die. We can see such situation in the laboratory. In the real world, for example in our mouth, bacteria form complex social lives on distinctly different surfaces, including enamel and cementum (hard tissues), as well as on epithelial cells (soft tissue). When a bacterium adheres to wet surface, such as pellicle on enamel, it senses that it is no longer free floating like living in saliva. Therefore, to survive in oral ecosystem, the changed environment has to be communicated to other microbes, thus they create a communication network by sending and receiving chemical signal. The communication system is called quorum sensing, and the communities developed by bacteria are biofilms as it contains life (bio).[1]

Biofilms are characterized by their species composition, their surface or substratum composition, and the conditioning film coating the surface on which their form. Biofilms formed on dental hard surfaces (dental plaque) are usually several bacterial cell layers thick. In contrast, bacterial colonization of the soft gingival tissue often occurs as a monolayer since the epithelial cells are constantly being replenished by host-cleansing mechanisms. There are also some evidences that gingival epithelial cells are invaded by some oral bacteria instead of merely adhere to epithelial cell surface.[2] Thus, it is clear that the bacterial communities on soft and hard surfaces are distinct.

It is now recognized that biofilm formation is an important aspect of many human diseases, including oral health problems.[3] Therefore, it is the purpose of this review to highlight the mechanism of how cell-cell communication affects biofilm structure and behavior. The unique nature of the quorum sensing mechanism might allow scientist to design an inhibitor against bacterial population.

Description and Overview of Biofilms

Biofilms are dense aggregates of surfaces-adherent microorganism embedded in an extracellular polysaccharides matrix exuded by bacteri.[4] Biofilm formation is initiated by interactions between planktonic bacteria and a surface in response to appropriate signals (Figure 2).[3] The surface may be inert, nonliving material or living tissue. A biofilm can be formed by a single

bacterial species, but in nature biofilms more often consist of many species of microorganisms. Once the bacteria from planktonic state (freely suspended) attach to any oral surfaces, they change their behavior. The most obvious change is that the bacteria begin to produce sticky matrix (slimy) material that glues them to the surface. Other bacteria that may not make much glue themselves colonize the developing biofilms.

In oral cavity, dental plaque is a unique biofilm found in oral ecosystem. Colonization of oral bacteria on a clean tooth surface is a highly specific and complex process. Once established, this oral biofilms are resistance to physical forces such as the shear forces produced by the washing action of saliva. Additionally, bacteria belong to dental plaque, for instance *Streptococcus mutans*, can withstand nutrient deprivation, pH changes, disinfectants, and antibiotics better than when living in saliva.[4,5] Dennis et al (2003)[5] also demonstrated that *S. mutans* grown in biofilm have the ability to maintain a subpopulation of competent cells, which is indicates that the biofilm environment provides condition for the bacteria to take foreign DNA produced by other neighboring cell. Another microorganism that has been implicated in root canal biofilm infection is *Enterococcus faecalis*. This bacterium not only reported as a leading cause of problems found after endodontic treatment, but also it has been associated with endocardial infection.[6,7] Enterococcal infection result from the complex interplay of multiple host and bacterial factors. One example of the bacterial virulent factor involved in such interaction is Esp, a protein found on the surface of *E. faecalis* cell.[8] This protein enhances biofilm formation *in vitro* and seems to be correlated with the biofilm formation *in vivo*.[9]

Like biofilm on supragingival area, the biofilm associated to periodontal problems is complex. In order to colonize the periodontal pocket, some bacteria needs environment prepared by others. Thus, communication between strains appears to be the key to study, how bacteria tend to be grouped in clusters (microcolonies) according to nutritional and atmospheric requirements. The communication process is referred to quorum sensing. This bacterial communication system has been found to help trigger the regulation of different set of genes, which take the responsibility for facilitating the bacteria living as a member of the biofilm.[10-13] Therefore, the nature of a biofilm may explain why periodontal diseases have been so difficult to prevent and treat. An improved understanding of biofilm will lead to new strategies for management of these widespread diseases.

Quorum Sensing

Quorum sensing was first described in marine bioluminescent bacteria, *Vibrio fischeri*. When this species colonize squid, they produce a glowing substance. However, in order to make a visible light, the bacteria numbers have to be enough.[14] This concept explains why bacterial activities are only productive when carried out in unison by a community of bacteria.

Quorum sensing (QS) can be defined as a form of bacterial communication that helps regulate group behavior.[15] However, this system needs the presence of a critical number (a quorum) of individual's bacteria cell before they can engage in particular activities. The question is how individual bacteria know how many neighbors they have before they deciding to carry out a particular function to contribute to the colony. Now, scientist realized that the acceptor bacteria sense signal relayed by their neighbors. This net work system would run because each bacterium has protein on its cell surface that act as receptors to sense signals, which is a small chemical substance called autoinducer (AI), released by other bacteria (the donor bacteria). The receptors do not trigger any behavioral change of the recipient bacteria until there are enough donor bacteria to allow the signal concentration reaches a critical (threshold) level.[16] Once this occurs, the recipient bacteria know they have a quorum, which is possible for the AI causes a series of gene activation leading to phenotype changes and thus adopting communal behavior, such as forming biofilm.[17] QS network has subsequently been known to be a universal process and is found in wide spread among Gram-positive and Gram-negative bacteria.[14] This is because beside the biofilm formation, several important activities, such as releasing toxins, or expression other virulent factors, are now having been known to depend on a quorum sensing mechanisms.[10]

In Gram-positive bacteria, the autoinducers are post-translationally modified peptides (AIPs) (Fig. 2). In this group, the quorum signals are exported to extra cellular milleu via a specific transporter (ATP-binding cassette) and are transduced by two-component signal transduction systems.[18] When the AIPs released by the respected bacteria, it will bind to the cell surface-bound histidine protein kinase, which autophosporylates, and at the same time phosphorylates a response regulator that activates transcription of one or more target genes.[4] In

contrast, the mode of quorum sensing in Gram-negative bacteria is mediated by proteins of acylated homoserine lactone (AHL) (Fig.2). AHL, some times are called AH-1 (N-3-hydroxybutanolyl-L-homoserine lactone). This protein is produced by the LuxI family of AHL synthases.[19] These proteins are diffuse away from the cell of Gram-negative bacteria and then are sensed by proteins belonging to LuxR family of response regulators. This LuxR contains two domains (Figure. 2), the AHL binding domain and a DNA binding domain. When AHL is bound, it alters the configuration of the LuxR, enabling it to interact with DNA and act as a transcriptional activator to interact with the same or other bacteria cells by attaching to and activating specific cell surface-associated or intracellular receptors.[20] The two proteins, LuxI and LuxR, are coded by *luxI* and *luxR*, respectively. They are often linked genes, whereas the QS target genes are localized elsewhere on the bacteria genome (Figure 1).

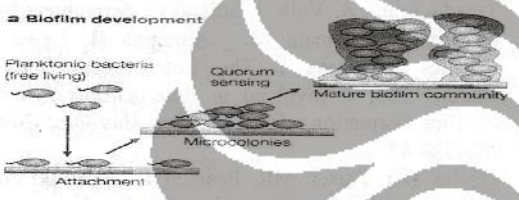

Figure 1. A schematic representation of the steps a bacterial species takes in forming a biofilm. The incoming planktonic bacteria attach to a surface, sending out chemical signals. The mature biofilm are arranged in microcolonies that are surrounded by protective matrix (polymer). www.erc.montana.edu (10-03-2006).

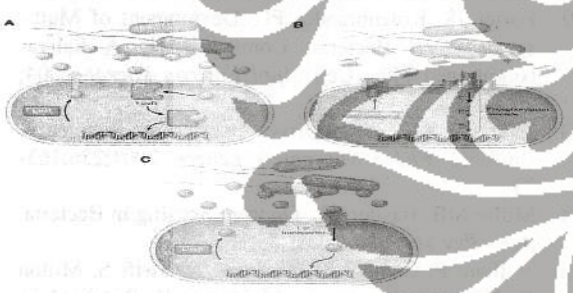

Figure 2. The three known quorum sensing pathways. A, in a Gram-negative bacteria, the sinthesized AHL (spheres) is released, which involving LuxI, then reenters the bacteria cell and bind to LuxR prior to interact with the DNA. B, in Gram-positive bacteria, upon AIP (linked shapes) binding, the receptor kinase is activated, leading to its autophosphorylation, which in turn activate several genes. In both Gram-negative and Gram-positive bacteria, the autoinducer (AI-2) (spheres) is synthesized involving LuxS. When released, it reenters bacteria trough a Lsr transporter and acts on AI-2 regulated genes. Based on Xavier and Bassler (2003).

A part from AIPs and AH-1, there is another autoinducer (AI-2) that common to both Gram positive and Gram negative bacteria (Figure 2), thus it allows for interspecies communication.[21] AI-2 has been demonstrated to have a role in communication between some oral bacteria, such as *Porphyromonas gingivalis* and *Streptococcus gordonii*. As reported by McNab et al.,[22] the inactivation of *lux*S (the gene encoded for AI-2) in genome of those bacteria has lead to impaired interaction of both strains in biofilm. Two other periodontopathogenic bacteria, *Prevotella intermedia* and *Fusobacterium nucleatum* was also possses autoinducer-like activitie.[23] However, other reports showed contradictive result.[24,25] Inactivation of the AI-2 synthase gene in *S. mutans* and *S. gordonii*, did not seem to have any effect in the amount of single-species biofilm. Therefore, the exactly role of this signal molecule in controlling oral bacteria phenotypes remains to be clarified.

Significance of Quorum Sensing Signal in Oral Biofilm Development

The significance of QS behavior is now known to be widespread in oral bacteria, particularly those related to dental plaque. In dental plaque, bacteria distribute themselves according to who can survive best in.[26] Thus, the bacteria in dental plaque or other oral surfaces are not randomly distributed, but rather organized. Moreover, cell of oral bacteria in dental plaque also communicate with one another via horizontal gene transfer beside of quorum sensing signal as stated above. When growing in biofilm, *S. mutans* are not only sense the quorum sensing signal molecule, but they also able to incorporate foreign DNA more efficiently than their planktonic counterpart.[27] This means, oral biofilm can function as a genotypic reservoir by harboring transferable mobile elements and genes.

Studies using molecular approaches to investigate the microbial gene expression and regulation have revealed that formation of dental plaque involves multiple, convergent signaling pathway for the growth of bacteria, from planktonic state to the biofilm mode of growth.[28] Consequently, oral bacteria belong to dental plaque and their planktonic counterparts are different phenotypically. This is the case when comparing the level of protein secreted by planktonic bacterium of *Actinobacillus actinomycetemcomitance* with it's counterparts in biofilm. Proteins that are virulent factor were

secreted more frequently by the later.[29] This is only one reason why oral bacteria within biofilm matrix are protected, not only by the mechanically action of saliva, but also from host defense mechanism and antimicrobial agents. The clinical relevance of such genetic exchange is the ability of some subpopulations of oral bacteria to adopt and survive in specific niche, while they are continuously exposed to various stresses, such as low pH, high osmolarity, oxidation and antimicrobial agent, including those used in mouth rinses.[30]

There are also many potential benefits to study the oral biofilm mechanisms. Preventive purposes are one example. It seems reasonable and would be a more logical option to target processes involved in the oral biofilm formation of single or mixed-bacterial communities that have the potential to cause oral diseases. Although there are numerous products currently available in the market, as repealing the adhered bacteria on to a clean tooth surface, but they usability has met with limitations. The problem a part due to the formation of conditioning film (pellicle) that rapidly adsorbed on to a clean enamel surfaces, or even to specially treated tooth surfaces that are intended to prevent bacteria adhesion. Other reason is oral bacteria generally posses more than one type of adhesion factors on their cell surface. These molecules not only participate in interacting with host receptors, but the similar molecule is also involved in adhesion process to other bacteria.[12]

To conclude, the nature of a biofilm may explain why oral problems, such as caries and periodontal diseases have been so difficult to prevent and treat. One of the major reasons that biofilm research has progress rapidly in recent years is advent of molecular approaches for their study. Therefore, a greater understanding of the significance of oral biofilm as a mixed population will have the potential to impact significantly on dental practice. However, much works remain to be done including interference with oral bacteria communication networks, which coordinate or regulate activities within oral biofilm, to provide practical benefits in dentistry.

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