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Review Article

Quorum Sensing - Polymicrobial Challenge in Periodontics

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Received: December 16, 2019; **Published:** January 07, 2020

Abstract

Periodontal disease is a multifactorial disease caused by imbalance between complex microbial community and the host immune response. Plaque provides favorable environment for the survival and growth of microorganisms inducing periodontal disease. The current concept of biofilm formation and cell to cell communication of microorganisms present in the biofilm occurs by the production of chemical signaling molecules which has become the niche for molecular level research projects. This biological process is called quorum sensing which modulates the expression of genes involved in survival, biofilm formation, virulence and pathogenicity of microorganisms. To eradicate this communication, quorum quenchers play a role in the inhibition of signals of quorum sensing. This review article enlightens the quorum sensing signaling and the magical action of quorum quenchers from the data collected by different analysis.

Keywords: *Quorum Sensing; Quorum Quenching; Bacterial Communication*

Introduction

Periodontal disease is an inflammatory disease which is multifactorial in origin causing destruction of the periodontium and also acts as a risk factor for systemic diseases such as cardiovascular diseases, endocrine disorders, hemorrhagic disorders, renal diseases, liver diseases, pulmonary diseases and pre-term low birth weight infants [1]. Throughout life, body interface surfaces are exposed to microbial colonization resulting in establishment of microbiota lives in harmony with the host. As teeth provide hard, non-shedding surfaces there is a development of extensive bacterial deposits. The accumulation and metabolism induced growth of bacteria on tooth surfaces is the influencing factor for initiation of gingivitis and periodontitis.

On mechanical cleaning of teeth, hydrophobic macromolecules begin to adsorb to the tooth surface forming conditioning film known as acquired pellicle which consists of various salivary glycoproteins and antibodies. This results in alteration of the surface free energy which increases the efficiency of bacterial adhesion. Bacteria adhere to these coated surfaces leading to formation of a biofilm. In 1 mm^3 of dental plaque weighing approximately 1 mg contains 200 million bacteria.

Plaque formation

The oropharynx is an open ecosystem wherein bacteria are always present. Approximately 700 species of microorganisms colonize the human oral cavity which includes commensals and sparse population of pathogenic bacteria. Bacteria attempt to colonize in all favorable

locations. Most bacteria, however, can only persist after the formation of a biofilm upon desquamation-free surfaces, i.e., hard substances (tooth and root surfaces, restorative materials, implants, prostheses etc.). In the presence of healthy dental and gingival relationships, there is a balance between the additive and retentive mechanisms of biofilms vis-à -vis the abrasive forces that tend to reduce biofilm formation, e. g., self-cleansing by the cheeks and tongue, diet and mechanical oral hygiene measures. Primary colonization of bacteria occurs by adsorbing on to the pellicle coated surface. The first bacteria that accumulate supragingivally on the tooth surface are mostly gram-positive. At 24 hours plaque mainly shows streptococci, of which Streptococcus sanguis is predominant. In the course of the following days, gram negative cocci as well as gram-positive and gram-negative rods and the first filamentous forms begin to colonize (Listgarten et al. 1975, Listgarten 1976). By means of a variety of metabolic products, the bacterial flora provokes the tissue to increased exudation and migration of PMN leukocytes into the sulcus ("leukocyte walls" against the bacteria). The increase in PMN diapedesis and the flow of sulcus fluid lead to initial disintegration of the junctional epithelium. This makes it possible for bacteria to more easily invade between the tooth and the junctional epithelium, and invade the subgingival area. In the total absence of oral hygiene, plaque formation and an initial host defensive response within gingival tissue occur.

Extending apically from the supragingival region, a subgingival plaque biofilm will often form within the existing gingival sulcus/pocket, previously called the "adherent" plaque. In addition to gram-positive bacteria such as streptococci, actinomyces, etc., as the probing depth increases so does the number of anaerobic gram-negative bacteria increases. This subgingival biofilm gets calcified and forms calculus. In addition, the gingival pocket also contains loose agglomerates of non-adherent, often mobile bacteria (with a high concentration of gram-negative anaerobes and spirochetes). In acute phases, periodontopathic bacteria often increase dramatically. These include Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, spirochetes and other species. Despite these alterations in the subgingival plaque, periodontitis, even in the acute stage, cannot be characterized as a "highly specific" infection because large differences have been reported in the bacterial composition between patients and even within different pocket locations in the same patient (Dzink *et al*. 1988, Slots & Taubmann 1992, Lindhe 1997).

Streptococci and other precursor organisms provide unique receptor sites for later, more pathogenic colonizers such as *Fusobacterium nucleatum* which acts as bridging bacteria, *Tannerella forsythia, Treponema denticola* and *Porphyromonas gingivalis* [2], which are closely associated with the development of periodontitis [3].

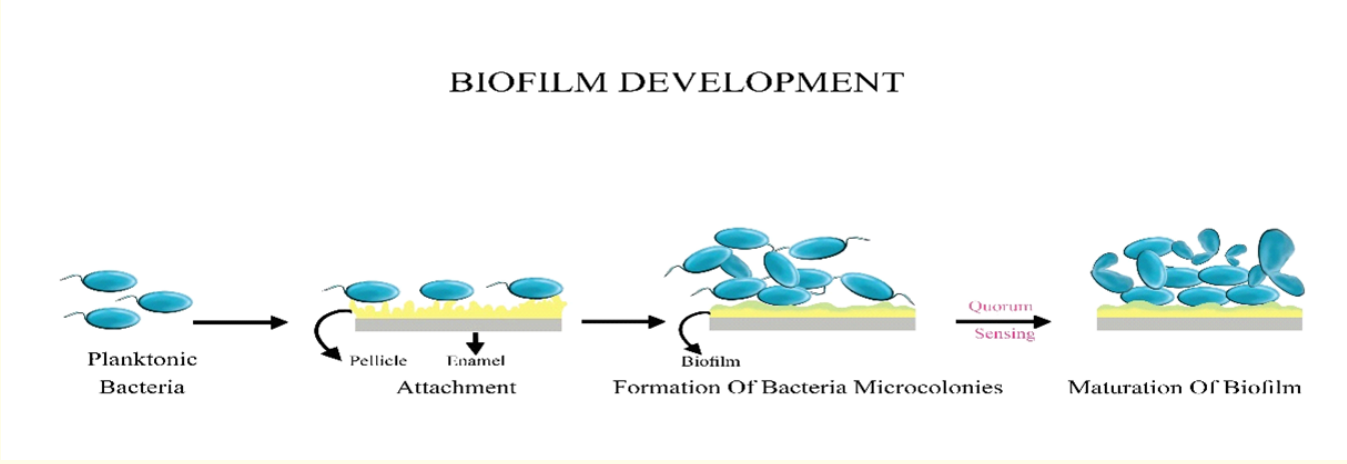

Figure 1: *Biofilm formation.*

F. nucleatum, P. intermedia, and *P. gingivalis* are the three very important microorganisms in the development of periodontal disease. *Fusobacterium nucleatum* plays an important role in the subgingival microbiota. It has ability to coaggregate with most of the microorganisms in plaque and with periodontal pathogens. *P. intermedia* is elevated in acute necrotizing ulcerative gingivitis. *P. gingivalis* plays an important role in bone and tissue destruction.

Micro-organisms are mostly organized in communities and they assemble on substrates known as biofilm. A typical biofilm forms at the interface and comprises of microbial cells enclosed within a matrix consisting of polysaccharides, proteins, nucleic acid and lipids, derived from microbial and environmental sources [4]. Plaque biofilm is the etiologic factor for periodontitis. This biofilm is stabilized by extracellular DNA (eDNA) [5]. In addition, this eDNA may be a source for potential transfer of antibiotic resistance or virulence genes between species within the communities [6]. The creation of bio-films is regulated by signaling process called quorum sensing.

Quorum sensing

Quorum is a Latin word. Bacteria produce chemical signals and other bacteria can respond to them by a mechanism through which they communicate population behavior and phenotype known as cell-cell communication or cell-cell Signaling or quorum sensing [7,8]. As communication is the resolving factor to interpret discrepancies, biofilms forming bacteria adopt specialized roles and communicate with one another [9].

To initialize communication between them, Autoinducers or pheromones which are small hormone like chemical signaling molecules play an important role. in formation and maturation of plaque. It either diffuses freely across the cell membranes or is actively transported out of the cell [10]. Autoinducer 2 is described as a global signal molecule for interspecies communication, as it is made by gram-positive as well as gram-negative bacteria [10]. When the population of Quorum Sensing producing bacteria leaps, the individual organisms engenders and secrete the autoinducers into the extracellular environment. Thus, the concentration of external autoinducer is congruent with cell population density. By monitoring the extracellular autoinducer concentration, the bacteria can count one another and alter target gene expression [11]. Though planktonic cells secrete chemical signals at low concentration, there is no change in genetic expression. When Biofilm cells held together in dense populations, the secreted chemical signals attain at higher concentrations. These molecules then re-cross the cell membranes and trigger changes in genetic activity leading to the production of various products which increase bacterial pathogenicity [9] (Table 1).

Acylated hemoserine lactone	Gram negative and Intraspecies
Peptides	Gram positive and Intraspecies
Furanosyl borate diester	Gram positive and gram negative, Interspecies

Table 1: *Autoinducers.*

At this instance, bacteria produce a light inducing property to attract one another to increase dense population known as bioluminescence. The bacterial signaling was first described in the bioluminescent marine organism *Vibrio fischeri* [Nealson and Hastings, 1979] where a diffusible signal N-acyl homoserine lactone (AHL) was responsible for the induction of bioluminescence [12]. In the light organ of the Hawaiian squid Euprymnascolopes, *V. fischeri* colonizes and induce the expression of genes required for bioluminescence. This bioluminescence increases the density of microorganism present in a particular area and makes cell to cell communication effective. When they are at high cell concentrations, the level of the autoinducer becomes adequate to induce transcription of the genes that produce the enzyme luciferase [12]. This enzyme causes the oxidation of the reduced flavin mononucleotide to produce a long-chain fatty acid, water and flavin mononucleotide. This reaction causes the emission of blue-green light along with the oxidation reaction and therefore is termed as bioluminescence. Different luminescent bacteria may show different luminescence spectrum and colour of the emitted light due to the shift in wavelength caused because of the sensitizer proteins [13].

Quorum Sensing is believed to regulate and control the traits of micro-organism such as competence development, sporulation, Antibiotic resistance, virulence factor, induction, Cell differentiation and nutrient flux along with other physiological events in pathogenic bacterial infections [11,14].

Finally, when the threshold concentration is reached, the population is considered to be "quorate" [14]. This causes the binding of the autoinducer to the cognate receptors present within the bacteria. This then further triggers a signal transduction cascade that results in wide-spread changes in the gene expression [15].

Types of quorum sensing systems

Gram-negative bacteria: uses 2 proteins which controls the expression of luciferase operon (luxICDABE) required for light production:

- The LuxI-type (auto inducer synthase) -involves in synthesis and emission of acyl homoserine lactone.
- The LuxR type (cytoplasmic autoinducer receptor)- binds to received acyl homoserine lactone [16,17].

After production, the AHL freely diffuses inner and outer membrane of the cell and increases its concentration in response to increasing cell density [18]. When the signal reaches a critical, threshold concentration, it is bound by LuxR and this complex activates transcription of the operon encoding luciferase [19]. LuxR- AHL complex also acts as positive feedback activator, leading to high expression of luxI gene and production of LuxI protein. This regulation induces the environment with the signal production and produce light [20]. A large number of other gram-negative proteobacteria possess LuxIR-type proteins and communicate with AHL signals [21]. These systems are used predominantly for intra-species communication as extreme specificity exists between the LuxR proteins and their cognate AHL signals [17]. The following figure shows the cell density dependent action of AHL.

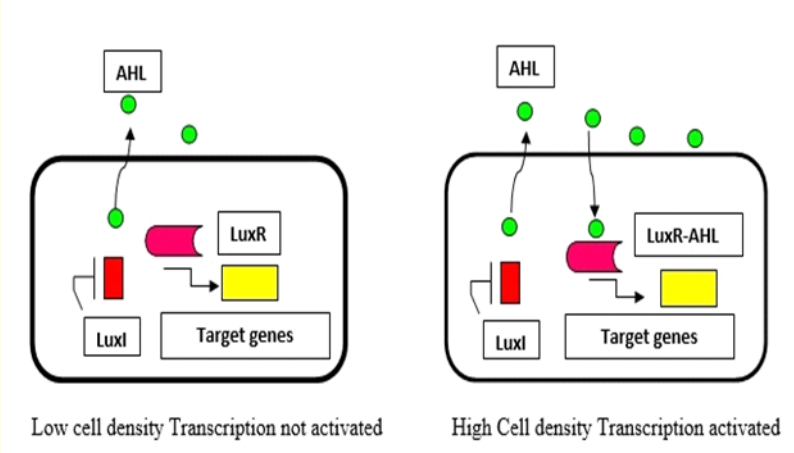

Figure 2: *Action of autoinducers.*

Gram-positive bacteria use oligopeptides processed from precursors as autoinducers. These are auto inducing peptides [22]; Gram-positive bacteria use two processes in quorum sensing [16]. They are:

1. A two component signal transduction system
2. Internalization

Need for eradicating the quorum sensing process

Biofilms are more resilient to mechanical removal and to killing by the host immune system because the matrix within the biofilm protects bacteria from exposure to innate immune defenses and antibiotic treatments [23]. Hence a new era of therapeutics have evolved which turned into a promising potential tool for abolition of infectious diseases caused by bacteria. Thus, Quorum sensing plays a key role in pathogenesis of disease causing by bacteria with its influence at the genetic level leading to regulation of various factors involved in the process.

Mode of eradicating

Several ways are there for disrupting the quorum sensing as quorum sensing forms the basis of infection. Following ways are enlisted below:

A) Early colonizers are responsible for creating the pathogenic state. The presence of periodontal pathogens in dental plaque could modulate the virulence properties of *S. mutans* by interfering with its com quorum sensing system. Since the com quorum sensing system exists in many species of earlier dental plaque colonizers. This interference of quorum sensing by periodontal pathogens such as *P. gingivalis* and *T. denticola* could, at least in part, be a mechanism of bacterial antagonism in periodontal diseases [1].

B) Quorum quenchers - synthetic and naturally occurring compounds.

Quorum quenchers-action of magic bullets

Prevention and control of bacterial infection by quorum quenching, which is an antipathogenic or signal interference of quorum sensing has been explored [24]. It retards the action of quorum sensing by enzymatic hydrolysis of AHL autoinducers. Quorum sensing blockage are at different site:

- QQ by small quorum-sensing inhibitors (QSI).
- QQ by AHL-lactonase.
- QQ by AHL-acylase.
- QQ by paraoxonase enzymes [20].

Natural quorum sensing inhibitors

Algae: Anti QS agents were first characterized in the marine red alga, *Delsia puchra*. Halogenated furanones produced by *Delsia puchra* could inhibit QS in a number of bacteria.

Fungi: Patulin and penicillin acid produced by *Penicillium coprobium* and *P. radicicola*, respectively, as inhibitors of quorum sensing in *P. aeruginosa*. These two compounds target the Las R and Rh1R QS regulators [25].

Insects: A venom alkaloid from the fire ant *Solenopsis invicta*, solenopsin A - inhibits quorum sensing in *P. aeruginosa* [26].

Honey: Had QSI activity against *Erwinia carotovora*, *Yersinia enterocolitica*, *Aeromonas hydrophilia* [27]

Garlic	Ajoene, Disulphides, Trisulphides [28]
Turmeric	Curcumin [28]
Citrus flavonoids	Flavonine naringenin [28]
Horseradish	Iberin [28]
Red Marine Alage (Dalea Pulchra)	Halogenated Furanones [29]
Grape Fruit Extract	Furocoumarins, Carotenoids, Limonoids, Pectin [29]
Clove Extract	Eugenol, Hexane, Menthol [30]
Coffee Extract	Caffeine [31]

Table 2: *Quorum Quenching compounds in plants.*

Synthetic quorum sensing inhibitors

There are basically three ways to block QS by developing on the AHL scaffold [20]:

- Introduction of substitutions in the acyl side chain without any change in the lactone ring.
- Introduction of substitutions and alterations in the lactone ring with unchanged acyl side chain.
- Extensive modifications in both the acyl side chain and the lactone ring.

The synthetic drugs which act as quorum sensing inhibitors are Macrolides**,** RNA III- related compounds, Halogenated furanones, Homoserine lactone analogue - Antagonism of Homoserine lactone activity and can be prepared as homoserine lactone vaccine [32].

Discussion

The biofilm formation can be disrupted by the naturally occurring quorum sensing inhibitors which plays pivotal as quorum quenchers in inhibiting the formation of dental plaque and further progression of periodontal disease. The discovery and further research of cell signaling among microbes results as a new milestone in eradication of polymicrobial disease.

Conclusion

Several studies are being done on the benefits of a quorum sensing inhibition. Understanding the extent and significance of bacterial intercellular communication is still in its early stages and more discoveries are required to know the real extent and significance of bacterial cell-cell communication in the environment. Phytochemical quorum sensing inhibitor compounds may serve as a panacea in dentistry. Further research should be carried out to study the ability of quorum quenchers.

Acknowledgment and Conflict of Interest

Nil.

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Volume 19 Issue 2 February 2020