ECOLOGY OF ORAL BIO-FILM

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Abstract

Most microbial ecosystems contain large numbers of genetically distinct microorganisms, and the human mouth is not different. The biofilm communities are complex and dynamic structures that accumulate through the sequential and ordered colonization of multiple oral bacteria. Oral biofilms are characterized by surface attachment, structural heterogeneity, complex interspecies interactions, and an extracellular matrix of polymeric substances, and are high-density micro-niches that differ dramatically from surrounding conditions. Here, it is an attempt to discuss the ecology of oral biofilm.

Key Words Microbial ecosystem, biofilm, quorum sensing

INTRODUCTION

The biofilm communities are complex and dynamic structures that accumulate through the sequential and ordered colonization of multiple oral bacteria (Kolenbrander et al., 2002). One of the most notable features of oral biofilms is that oral bacteria growing in the biofilms frequently express phenotypes that are different from those of planktonic bacteria. Oral biofilms are characterized by surface attachment, structural heterogeneity, complex interspecies interactions, and an extracellular matrix of polymeric substances, and are high-density micro-niches that differ dramatically from surrounding conditions. Bacterial species present in the oral biofilm communities interact cooperatively or competitively with other members. It has been shown that the bacterial interactions that influence biofilm formation, metabolic change, and physiological function involve various different mechanisms (Fig. 1.). From a physical aspect, planktonic bacterial cells attach directly to surfaces of the oral cavity or bind indirectly to other bacterial cells that have already colonized (Kolenbrander et al., 2002). From metabolic and physiological points of view, avenues of communication within oral biofilms are likely to include metabolic communication, genetic exchange, quorum-sensing, etc. (Chalmers et al., 2008; Sedgley et al., 2008).

ADHESION TO TOOTH SURFACES:

Bacterial adhesion to and subsequent colonization of the surfaces of teeth and tissue are the first steps toward the formation of oral biofilms (Fig. 1). Tooth pellicle is a thin film that covers the tooth soon after the tooth is thoroughly cleansed, and it originates from salivary proteins. Oral bacteria such as Streptococci viridanscan colonize the tooth surface by binding to the complex proteinaceous pellicle (Rogers et al., 2001; Kolenbrander et al., 2002). Many oral streptococci have the ability to bind to proteins such as alpha-amylase, proline-rich proteins, and proline-rich glycoproteins, and are recognized as early colonizers. Streptococcus gordonii is one of the early colonizers in dental biofilms. This species binds to acidic proline-rich proteins that account for 25-30% of the total

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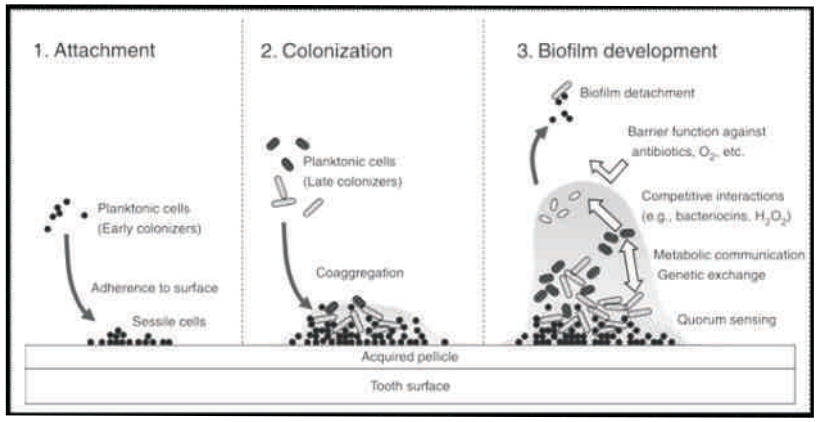

FIG 1: A diagrammatic representation of biofilm formation on the tooth surface and the potential roles of bacterial interactions. The tooth pellicle is generally colonized by early colonizers. Co-aggregation contributes to sequential binding and colonization. Bacterial interactions include metabolic communication and genetic exchange. The development of a biofilm having a high bacterial cell density increases the concentration of signaling molecules.

proteins in saliva. The alpha-amylasebinding protein A of S.gordonii interacts with salivary amylase, which suggests that this interaction contributes to the attachment of S. gordonii to the tooth surface (Rogers et al., 2001). S. sanguinis is thought to be one of the first bacterial species to adhere selectively to and colonize saliva-coated teeth. This species generally appears in the human oral cavity after tooth eruption, and it becomes a normal inhabitant of the human mouth.

CO-AGGREGATION AMONG ORAL BACTERIA:

Planktonic bacterial cells that cannot directly colonize the tooth surface may bind via receptors to the cell surfaces of early colonizers that adhere to the surfaces. Co-aggregation is a specific cell-to-cell reaction that occurs between distinct bacterial cells and is one of the most important mechanisms underlying oral bacterial colonization and oral biofilm formation. Secondary colonizers bind to bacteria that are previously bound to the teeth. Sequential binding results in the appearance of a nascent surface that forms a bridge with the adjacent co-aggregating partner cells. For example, a typical periodontal pathogen, namely, Porphyromonas gingivalis, can bind to early colonizers. Co-adhesion between P. gingivalis and S. gordonii ismediated by two sets of adhesion–receptor pairs: thelong (major) and short (minor) fimbrial subunitproteins of P. gingivalis that interact with streptococcalglyceraldehyde-3-phosphate dehydrogenaseand Ssp surface proteins, respectively. The long (major) fimbriae of P. gingivalis are composed of the FimA protein, which binds to the glyceraldehyde-3-phosphate dehydrogenase present on the surface of S. oralis (Maeda et al., 2004). The short fimbriae of P. gingivalis are approximately 6.5nm wide and 103 nm long, and are composed of the Mfa structural subunit protein.The Mfa protein engages the Ssp proteins on the streptococcal cell surface and increases the avidity of binding to be more resistant to shear forces (Fig 2).The process of bridging between a co-aggregation of cells consisting of more than 3 bacterial species is very important, because it connects a few species that are not co-aggregation partners. Fusobacterium nucleatum can co-aggregate with many oral bacteria, including streptococci and

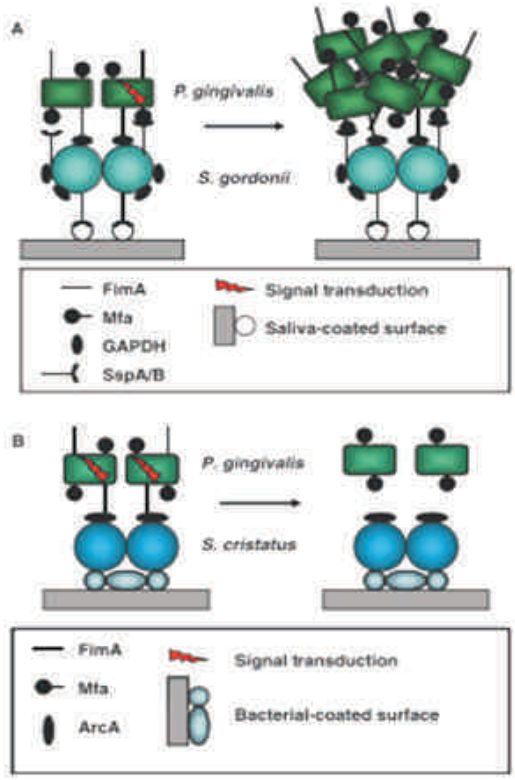

FIG 2: Schematic representation of differing community-relevant events that occur following the binding of Porphyromonas gingivalis to Streptococcus gordonii or to Streptococcus cristatus.

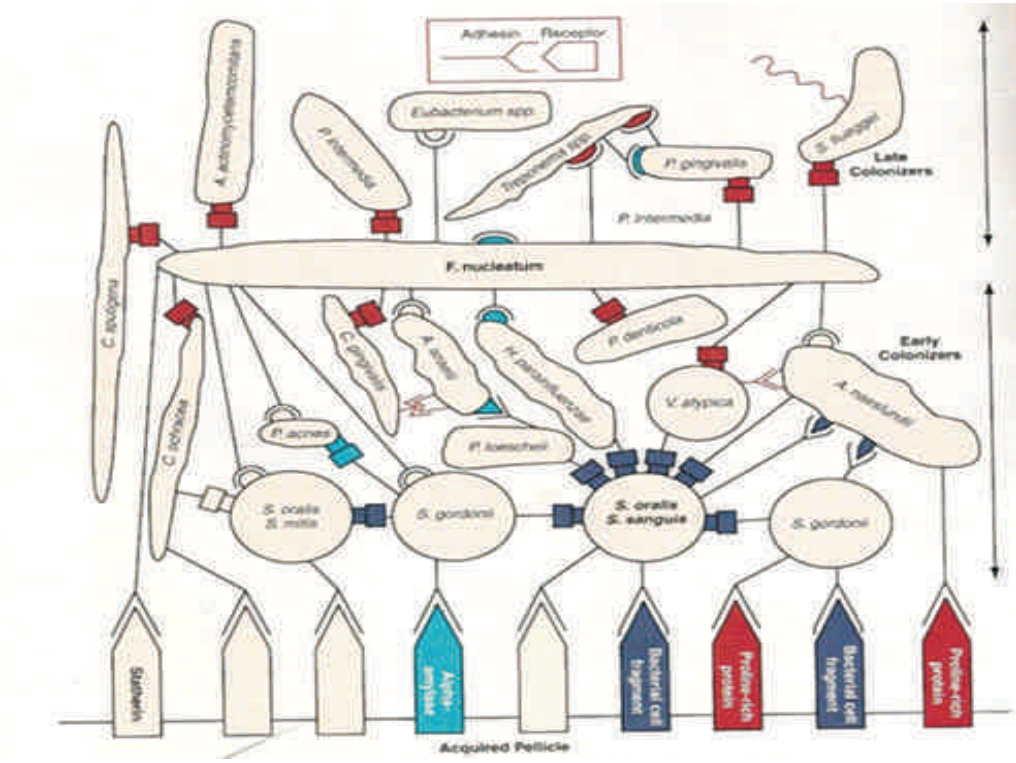

FIG 3: Schematic representation of co-aggregation of primary and secondary colonizers and bridging action of F. nucleatum.

obligate anaerobes. Therefore, this species is a key component of dental biofilms and serves as a coordinator that bridge the late and early colonizers (Kolenbrander et al., 2002) (Fig 3). Co-aggregation between F.nucleatum and other bacteria is a highly specific process involving interaction among the surface molecules of bacterial cells. The co-aggregation reactions between F. nucleatum and Gram-negative bacteria are mediated by lectin-carbohydrate interactions. For instance, the co-aggregation between P. gingivalis and F. nucleatum is mediated by a galactoside moiety on the surface of P. gingivalis and a lectin moiety on that of F. nucleatum, which is inhibited by lactose. Capsular polysaccharides and lipopolysaccharides of the P. gingivalis sero type K5 act as receptors mediating the coaggregation between oral bacteria (Rosen and Sela, 2006). In contrast, although coaggregation between F. nucleatum and many other Gram-positive bacteria has been observed, co-aggregation is rarely inhibited by sugars (Kolenbrander et al., 1989; Kang et al., 2005; Nagaoka et al.,2008). Thus, intergeneric co-aggregation between F. nucleatum and Gramnegative cells is quite different from that with Gram-positive bacteria.

Co-aggregation among oral bacteria is thought to contribute to not only bacterial colonization through physico-chemical mechanisms, but also to metabolic communication and genetic exchange, because each bacterium can easily access a neighbouring bacterial cell and its metabolites.

METABOLIC COMMUNICATION AMONG ORAL BACTERIA:

For oral bacteria, nutrients are available from saliva, gingival crevicular fluid, food containing sugars, food debris, and metabolic products of other bacteria (Fig. 4). Metabolic communications among oral bacteria may occur through the excretion of a metabolite by one organism that can be used as a nutrient by a different organism, or through the breakdown of a substrate by the extracellular enzymatic activity of one organism that creates biologically available substrates for different organisms (Kolenbrander et al., 2002). As an example, Byers et al. (1999) proposed that the hydrolysis of host glycoproteins by S. oralis and the subsequent utilization of released monosaccharides are important in the survival and persistence of this species and other oral bacteria. Similarly, the desialylation of immunoglobulin A1, the dominant isotype of antibody in the oral cavity, by oral Gram-positive rods may facilitate the proteolytic activities of other oral bacteria, and the concerted action may positively influence the survival of the bacteria in the oral community (Frandsen, 1994). Oral bacteria present in dental biofilms provide their metabolites as energy sources for other members. Short-chain fatty acids produced by oral bacteria are thought to be an essential carbon source for certain oral bacteria. Several studies have suggested a symbiotic association between Streptococcus and Veillonella species via lactic acid produced by the former

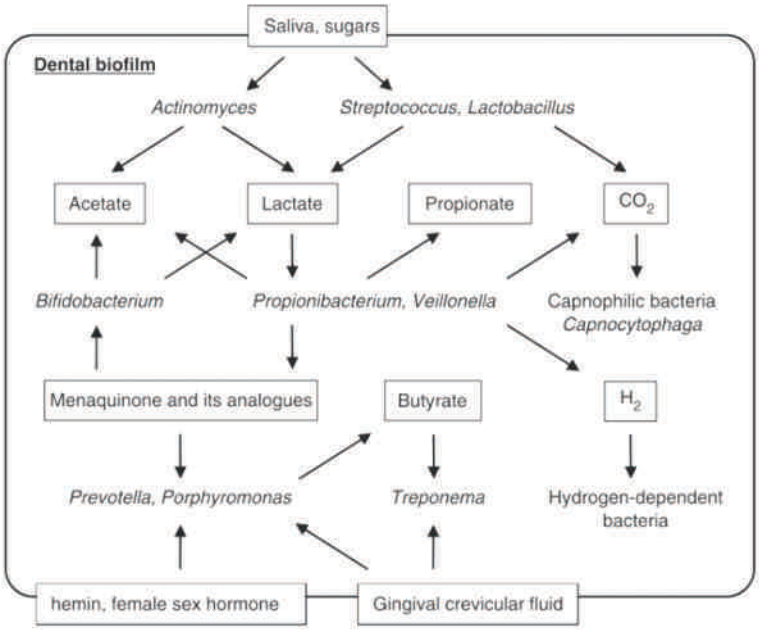

FIG 4 : Illustration of representative metabolic relationships among oral bacteria within the dentalbiofilm communities. Streptococcus, Lactobacillus, and Actinomyces secrete lactate, and it is utilized by Veillonella and Propionibacterium as a carbon source (Marcotte and Lavoie, 1998; Chalmers et al., 2008). Veillonella and Propionibacterium produce menaquinone and its analogues, which promote the growth of vitamin K-auxotrophic bacteria such as Porphyromonas, Prevotella, and Bifidobacterium (Marcotte and Lavoie, 1998; Hojo et al., 2007). Carbon dioxide, produced by aerotolerant Propionibacterium, hetero-fermentative Lactobacillus, and Veillonella, is utilized by Capnophilic bacteria such as Capnocytophaga (Kapke et al., 1980).

(Kumar et al., 2005). In human studies, streptococci and veillonellae often occur in the same site of the oral cavity (Haffajee et al., 1998; Kumar et al., 2005). Moreover, interestingly, these human clinical studies suggested that these genera are associated with periodontal health. Kumar et al. (2005) stated that the parallel relationship is not surprising in view of the fact that veillonellaeutilize short-chain acids such as lactates that are secreted by Gram-positive bacteria such as streptococci. From both in vitro and in vivo studies, it is probable that the growth of veillonellae present in the oral cavity depends on lactate produced by other oral bacteria. Likewise, P. gingivalis and Treponema denticola are frequently detected together in the dental biofilms of persons with periodontitis. Growth enhancement due to the mutual symbiotic relationship between P. gingivalis and T. denticola has been described in previous studies (e.g., Grenier, 1992). The growth factors produced by P. gingivalis and T. denticola were identified to be short-chain fatty acids. The growth factor produced by T. denticola is thought to be succinic acid, which appears to be incorporated into the lipids and phospholipids present on the cellenvelope of P. gingivalis (Lev and Milford, 1971; Lev, 1979). Moreover, the growth of T. denticola is stimulated by the isobutyric acid that is produced by P. gingivalis (Grenier, 1992). Because these 2 species can co-aggregate, their metabolites are easily accessible to each other, since they are not diluted. Several exogenous quinones influence bacterial growth and metabolism. Vitamin K often has a highly stimulatory effect or is required for most Prevotella and Porphyromonas strains. Vitamin K is not synthesized in humans; therefore, it is known that auxotrophicmicro-organisms that require vitamin K acquire it from the bacteria present in dental biofilm. Veillonella species—a ubiquitous component of dental biofilms—is thought to produce menaquinone (vitamin K2), which is then utilized by Prevotella and Porphyromonas (Marcotte and Lavoie, 1998). Similarly, quinines and their related compounds stimulate the growth of Bifidobacterium (Isawa et al., 2002).

INHIBITORY METABOLITES:

While cooperative interactions of nutrients and colonization exist in dental biofilm communication, close competition with antagonists also occur. Proteinaceous bactericidal substances like bacteriocin are produced by bacteria to inhibit the growth of closely related bacterial species orstrains. For instance, S. mutans is able to produce several kindsof bacteriocins called mutacins, including lantibiotics and nonlantibiotics. Bacteriocins are typically thought to have a narrow spectrum; however, some of them produced by oral bacteria apparently have a relatively broad spectrum, such as

the lantibiotics mentioned above. It has been reported that a 56-kDa novel bacterioc in produced by Lactobacillus paracasei HL32 inhibits the growth of P. gingivalis, Prevotella intermedia, Tannerella for sythensis,S. salivarius, and S. sanguinis (Pangsomboon et al.,2006, 2009).

Bacteriocin or bacteriocin-like activities have been documented for many other oral bacteria. Based on many in vitro studies, other bacterial metabolites, such as hydrogen peroxide and short-chain fatty acids, are strongly suggested to be competitive factors in oral biofilms. It is suggested that hydrogen peroxide produced by S. sanguinis contributes to the antagonism against S. mutans in an oral biofilm model (Krethet al., 2005a, 2008). Many oral bacteria produce large quantities of short-chain fatty acids as the end-products of carbohydrate fermentation. The production of lactic acid lowers the pH in dental biofilm, thereby having a disadvantageous effect on less aciduric oral bacteria (Dashper and Reynolds, 2000).

QUORUM SENSING:

Quorum-sensing is a process of chemical communication among bacteria; it is defined as gene regulation in response to cell density, which influences various functions, viz., virulence, acid tolerance, and biofilm formation. Because bacteria within biofilms reach a high cell density, quorum-sensing is considered one of the important bacterial functions. Auto inducer-2(AI-2) is one of the most well-known signaling molecules associated with quorum-sensing. It was initially identified in Vibrio harveyi (Bassler et al., 1994) and subsequently in several bacterial species. The synthesis of AI-2 is catalyzed by LuxS, anenzyme encoded by the luxS gene. The luxS gene is highly conserved in the genome of a wide range of Gram-positive and Gram-negative bacteria. Many studies suggest that oral bacteria have a quorum-sensing system that depends on LuxS/AI-2(Table).

Competence-stimulating peptide (CSP) mediates bacterial cell-to-cell signal communication (Table). CSP is a small soluble peptide having from 14 to 23 amino acid residues and is potentially produced by many species of oral streptococci(Li et al., 2001a). CSP is implicated in bacteriocin production, virulence, and biofilm formation. Moreover, CSPenhances genetic competence, which allows for the transport of exogenous DNA into cells (Dubnau, 1991). CSP derived from S. mutans has been reported to induce the bacteriocin (mutacinIV) gene, and it has been shown that S. mutans possibly utilizes the bacteriocin to acquire the ability to transform DNA from other streptococcal species, such as S. gordonii, living in the same ecological niche (Kreth et al., 2005b).

A new class of specifically targeted antimicrobial peptides (STAMPs) has recently been reported for use in a unique strategy (Eckert et al., 2006).The STAMPs have a two-sided structure. The first is a shorthoming sequence of CPS that can be as unique to a bacterium as a fingerprint and ensures that the STAMPs will find their target. The second is a non-specific antibacterial peptide that is linked chemically to the homing sequence and kills the targeted bacterium on delivery. It has been suggested that STAMPs, which were designed based on the CSP of S. mutans, are potentially capable of eliminating S. mutans from multispecies biofilms without affecting the closely related oral streptococci such as S. gordoniiand S. sanguinis (Eckert et al., 2006).

GENETIC EXCHANGE WITHIN COMMUNITIES:

Horizontal gene transfer by transformation, conjugation or transduction is a principal driver of bacterial evolution. The closely packed environment inbiofilm communities facilitates genetic exchange among constituent cells (Nadell, 2009). The

Table. Signaling Molecules and Functions in Oral Bacteria

Signals	Bacteria	Functions	References
AI-2	*Porphyromonas gingivalis*	Hemin and iron uptake	Chung *et al.* (2001), James *et al.* (2006)
		Protease and hemoggulutinin activities	Burgess *et al.* (2002)
		Stress gene response	Yuan *et al.* (2005)
	Streptococcus mutans	Biofilm formation	Yoshida *et al.* (2005)
	S. gordonii	Carbohydorate metabolism	McNab *et al.* (2003)
	S. anginosus	Biofilm formation	Petersen *et al.* (2006)
	S. intermedius	Virulence factors	Pecharki *et al.* (2008)
	Aggregatibacter actinomycetemcomitans	Biofilm formation	Shao *et al.* (2007)
	Eikenella corrodens	Biofilm formation	Azakami *et al.* (2006)
	P. gingivalis - S. gordonii	Biofilm formation	McNab *et al.* (2003)
	S. oralis - Actinomyces naeslundii	Biofilm formation	Rickard *et al.* (2006)
CSP	*S. mutans*	Bacteriocin production, competence	van der Ploeg (2005), Kreth *et al.* (2005b)
		Biofilm formation	Li *et al.* (2001a), Aspiras *et al.* (2004)
		Acid tolerance	Li *et al.* (2001b)
	S. gordonii	Biofilm formation	Loo *et al.* (2000)
	S. intermedius	Biofilm formation	Petersen *et al.* (2004)

opportunisticpathogen Pseudomonas aeruginosa, for example, can undergo extensive genetic diversification during short-term growth in biofilm communities (Boles et al., 2004). Furthermore, conjugative plasmids themselves express factors that induce their planktonic bacterial hosts to form or enter biofilm communities, which then favors the transfer of the plasmid (Ghigo, 2001). The diversity and adaptability produced by horizontal gene transfer provide a form of biological insurance that can help biofilm communities to survive in harsh environments. There are several mechanisms by which horizontal gene transfer maybe operational. Mobile genetic elements like insertion sequences, transposons, integrons, bacteriophages, genomic islands, plasmids and combinations of these elements can be exchanged promiscuously between a broad spectrum of bacteria and contribute to bacterial genomeplasticity. Conjugation, transformation and transduction are the other mechanism by which genetic materials are exchanged between the organisms.

CONCLUSION

The oral biofilm is sophisticated social networking, based initially on very specific recognition of surface characteristics, which provides the discrimination necessary for the formation of metabolically compatible, physiologically integrated communities. Community development is controlled by programmed patterns of gene expression and multilevel regulation of protein expression and activity. Organisms within these communities continually monitor the host environment and the nature and intentions of other organisms that may seek to participate in community affairs. Interspecies communication, thus maintain a dynamic and stable environment. Once a degree of stability or maturity is reached, organisms can begin the process of genetic exchange and the production of genetically diverse daughter cells, some of which will exhibit increased fitness. This phenomenon makes oral biofilmto survive throughout life since its inception.

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