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Interspecies Communication in Oral Biofilm: A Conscious Need Based Collaboration

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Abstract

The human body contains numerous different ecosystems that provide a unique environment for colonizing microorganisms. One such habitat is oral cavity. As many as 700 bacterial species may colonize the surfaces of the oral cavity. Interspecies adherence interactions help to shape the temporal and spatial development of the complex bacterial consortia in the gingival crevice. Bacterial inhabitants of oral biofilms are known to both collaborate and compete as they strive to optimize their adaptation to these environmental constraints. An under- standing of the mechanisms of subgingival biofilm formation and development needs, therefore, to accommodate the multiple interspecies interactions that occur in polymicrobial communities.

Key Words

Oral biofilm; microorganism; bacterial species

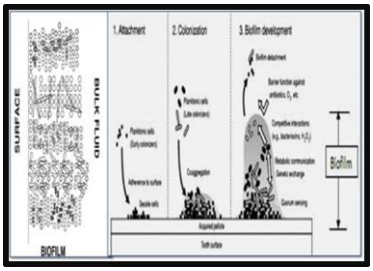
INTRODUCTION

Nothing in the universe exists alone. Every drop of water, every human being, all creatures in the web of life, and all ideas in the web of knowledge are part of an immense, evolving, dynamic whole as old, and as young, as the universe itself (Symbiosis 1982). The human body contains numerous different ecosystems that provide a unique environment for colonizing microorganisms. One such habitat is oral cavity. The 215 cm^2 surface area of the oral cavity presents numerous surfaces for microbial colonization. Dental plaque, an adherent, bacterial biofilm that forms on all hard and soft tissue, is the principal aetiologic agent in caries and periodontal diseases.[1] As many as 700 bacterial species may colonize the surfaces of the oral cavity.[2] Interspecies adherence interactions help to shape the temporal and spatial development of the complex bacterial consortia in the gingival crevice. Bacteria within these communities encounter high cell densities and, in consequence, community living involves adaptation to higher (and unevenly distributed) levels of metabolic by-products, secondary metabolities and other secreted molecules, and to the sporadic availability of nutrients and oxygen.[3] Bacterial inhabitants of biofilms are known to both collaborate (e.g. through nutritional cross-feeding) and compete (e.g. through production of bacteriocins) as they strive to optimize their adaptation to these environmental constraints. Bacteria can also communicate with one another through a variety of sensing and response systems based on either cell-to-cell contact or detection of soluble mediators. The signaling molecules are processed through transcriptional and post-transcriptional networks and they allow bacterial inhabitants of biofilms to coordinate activities at a group or community level.[3]

DENTAL PLAQUE AS A BIOFILM

All biofilms consist of three components: the surface needed for the attachment of the biofilm, the biofilm community itself, and the bulk fluid that passes over the biofilm, providing nutrients to the colonizing organisms, removal of waste products and transport of cells to new colonization sites (Fig. 1). Today dental plaque can be regarded as a specialized example of microbial biofilms (McHugh 1999). The stages involved in the development of supra gingival plaque are:[4]

- Step 1: the evolution of plaque biofilm begins



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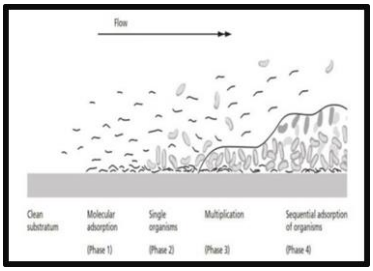

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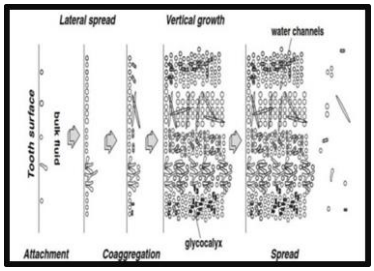

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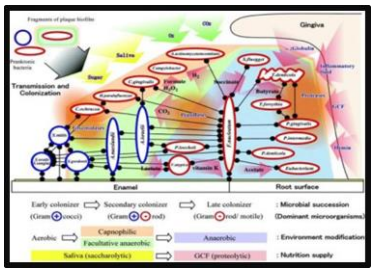

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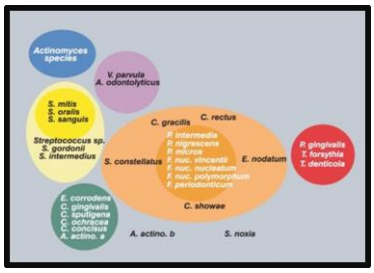

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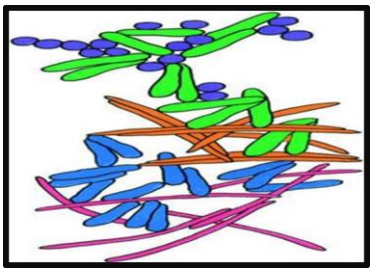

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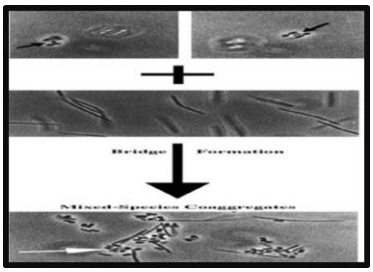

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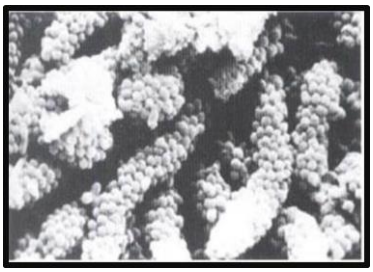

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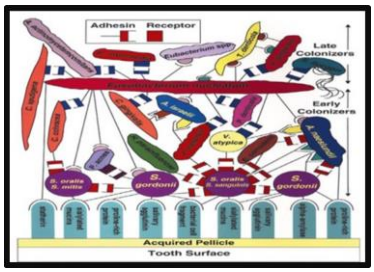

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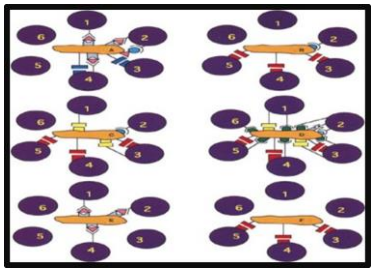

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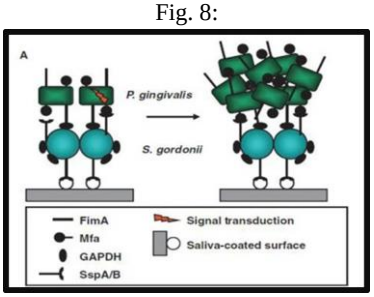

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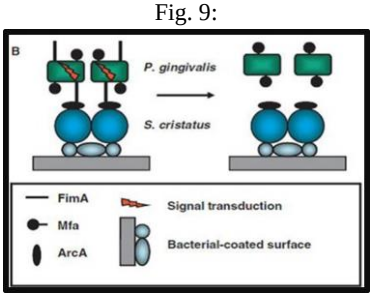

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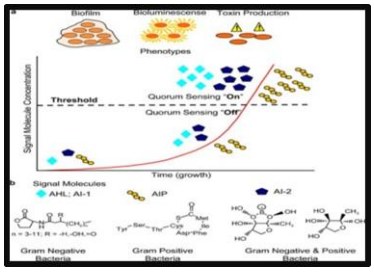

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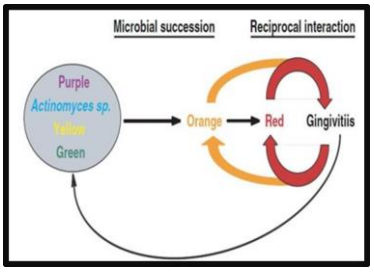

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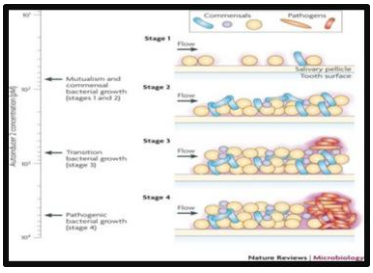
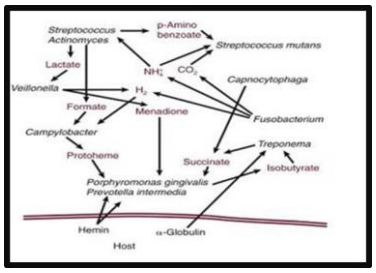


Fig. 15:

- with the formation of the pellicle, an acellular material on the tooth surface that is mostly composed of glycoproteins. (formation of acquired pellicle)
- Step 2: pioneer micro-organisms settle in the pellicle and form colonies.(initial adherence and colonization)
- Step 3: plaque maturation, (Secondary colonization)

Phase 1: Molecular adsorption Phase 2: Bacterial adhesion by single organisms. Phase 3: Growth of extracellular matrix production and multiplication of the adhering bacteria. Phase 4: Sequential adsorption of further bacteria to form a more complex and mature biofilm (Fig. 2, Fig. 3 & Fig. 4).



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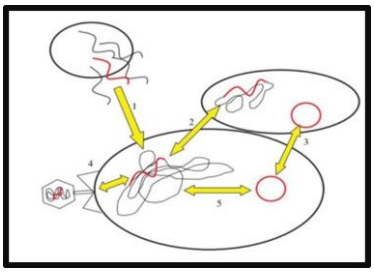

Fig. 17:

The predominant early colonizers of the subgingival plaque biofilms are the *Actinomyces* species and streptococci. A complex microbial community then develops within the space of only a few days , and the secondary colonizers tend to be the more pathogenic species such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium nucleatum* and *Aggregatibacter* acnumerous adhesions that enable attachment to the earlier bacterial inhabitants of the region, often choosing partners that are metabolically compatible (Fig. 5).[5]

Mechanisms of inter-species binding/interaction

1. Co-aggregation.
2. Quorum sensing and autoinducer-2
3. Metabolic communication.
4. Genetic exchange within communities

Coaggregation

Coaggregation is defined as the specific cell-to-cell recognition that occurs between genetically distinct cell types.[6] Each cell type bears on its surface one or more types of coaggregation mediator, which are called adhesins and receptors. Gibbons and Nygaard (1970)[7] discovered coaggregation among plaque bacteria and called it interbacterial aggregation. The term coaggregation was coined to describe a clumping phenomenon that occurred when sucrose-grown streptococci were paired with actinomyces and was used to distinguish this intergeneric type of clumping from the dextran-mediated intraspecies aggregation of actinomyces. The sequential arrangement of coaggregating cells and the formation of multiple bridges in the accretion of cells as multigeneric coaggregates.

Purple cells- *Streptococcus oralis*; green cells- *Actinomyces naeslundii*; orange cells- *Capnocytophaga ochracea*; blue cell-, A. *israelii*; red cells- C. *gingivalis* (Fig. 6).

Coaggregation Bridges

A coaggregation bridge (Fig. 7) is formed when the common partner bears two or more types of coaggregation mediators. These mediators can be various types of receptor polysaccharides, or various types of adhesins, or a mixture of the two (Fig. 8). The corncob configuration results from the growth of cocci on the surface of a filamentous microorganism. Corn cob formations were occasionally seen as a feature of plaque present on teeth associated with gingivitis (Fig. 9), while bristle-brush formations, composed of a central axis of a filamentous bacterium with perpendicularly associated short filaments, were commonly seen in the subgingival plaque of teeth associated with periodontitis.[5]

The *Streptococcus* - *Actinomyces* coaggregation groups

Streptococcus spp. And *Actinomyces* spp., are two of the initial colonizing genera on enamel surfaces. Thousands of types of interactions could result if *streptococcus-actinomyces* coaggregations are random, but instead six coaggregation groups of streptococci (*Streptococcus* coaggregation groups 1–6) and six coaggregation groups of *Actinomyces* (*Actinomyces* coaggregation groups A–F) are found (Fig. 10).[8]

Streptococcus coaggregation groups	Actinomyces coaggregation groups
Group 6	Group D
All streptococcal groups(1-6)	Group D (Most reactive)
Group 2, 3, 4, 5	Group B
Group 3, 4, 5	Group F
Group 2, 3, 4, 5 plus Lactose inhibitable co-aggregation with streptococcal group 1 and 3	Group C
Group 3, 4, 5 bear receptors for lactose inhibitable co-aggregations as well as few adhesions, whereas streptococcal coaggregation groups 1, 2, 6 bear only adhesins	

Interactions between *P. gingivalis* and *Streptococcus gordonii*

Co-adhesion between *P. gingivalis* and *S. gordonii* is mediated by two sets of adhesion–receptor pairs: the long (major- FimA) and short (minor - Mfa) fimbrial subunit proteins of *P. gingivalis* that interact with *Streptococcal* glyceraldehyde-3-

phosphate dehydrogenase and Ssp surface proteins, respectively (Fig. 11).

Interactions between *P. gingivalis* and *S. cristatus*

Down regulation of fimA in *P. gingivalis* due to contact with arginine deaminase on the surface of *S. cristatus*, results in no community formation between *P. gingivalis* and *S. cristatus* (Fig. 12).[3]

In addition to adhesins, a number of streptococcal processes contribute to community development with *P. gingivalis*. These can be grouped into broad categories, as follows:[3]

i. Intercellular or intracellular signaling (chorismate-binding enzyme, pyruvate oxidase, MarR family transcriptional regulator);
ii. Cell wall integrity and maintenance of adhesive proteins [methionine sulfoxide reductase, UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelateligase (MurE)];
iii. Extracellular capsule biosynthesis (cell wall polysaccharide biosynthesis protein); and
iv. Physiology (glutamate dehydrogenase, ABC transporter ATP-binding protein, V-type ATP synthase).

Quorum Sensing

The capacity of bacteria to communicate with each other is called quorum sensing. This involves the regulation of expression of specific genes through the accumulation of signalling compounds that mediate intercellular communications. When these signalling compounds reach a threshold level (quorum cell density), gene expression can be activated. Such quorum sensing seems to play a role in expressing genes for antibiotic resistance and in encouraging the growth of beneficial species to the biofilm and discouraging the growth of competitors (Fig. 13).[9]

Autoinducer-2 as a universal signal mediating mutualism among oral bacteria

In 2001, Schauder *et al.*, proposed that a small molecule called autoinducer-2 was a universal signal mediating messages among the species in mixed species communities.[8] Autoinducer-2 is an umbrella designation that covers a collection of molecules formed from the spontaneous rearrangement of 4,5-dihydroxy-2,3pentanedione (DPD), which is the product of LuxS. Frias *et al.*, 2001 support a hypothesis that commensal oral bacteria respond to low levels of autoinducer-2, whereas periodontopathogenic bacteria respond to higher levels of autoinducer-2. Kolenbrander et al hypothesised that the orange and red complex bacteria send and receive autoinducer-2 signals at much higher concentrations and grow rapidly (Fig. 14 & Fig. 15).[10]

Metabolic Communication

Subgingival bacteria often have complex nutritional requirements that can be met, in part, through the release of a metabolite by another organism in the community. These interactions can be considered signaling, in the broad sense, in that they represent sensing and responses to environmental conditions by the organisms (Fig. 16).[5]

Genetic Exchange within Communities

Horizontal gene transfer by transformation, conjugation or transduction is a principal driver of bacterial evolution. The closely packed environment in biofilm communities facilitates genetic exchange among constituent cells (Fig. 17 [Transformation (1), conjugation of a conjugative transposon (2) and a conjugative plasmid (3), and transduction (4) are shown. Also shown is the integration of a plasmid into the chromosome (5). The mobile DNA is shown in red, and chromosomal).[11]

Transformation

Transformation is defined as the uptake and maintenance of DNA. Competence is the physiological state in which the cell can take up DNA. Some oral bacteria, including members of the genus Neisseria, Streptococcus and Actinobacillus are naturally competent and have specialized systems for DNA uptake. One of the rate-limiting steps for transformation in the oral cavity is the longevity of DNA molecules in this environment. Therefore, it is likely that both interspecies and intergeneric gene transfer can occur by this route in this environment.[12,13]

Transduction

Transduction is a process where bacterial DNA is packaged into bacteriophage heads. When the phage infects a suitable host it injects this bacterial DNA, instead of phage DNA, into the new host.[11] One of the main barriers to the activity of bacteriophage in oral biofilms is the access to the cells within the extracellular polymeric substances secreted by the cells themselves when growing as a biofilm. Periodontal bacteria, such as *A. actinomycetemcomitans*, *Fusobacteria* and *T. denticola* have been shown to possess bacteriophage.[14]

Conjugation

Conjugation is the polar transfer of genetic material through direct cell-to-cell contact and is mediated by a variety of specialized genetic elements, such as conjugative transposons and conjugative plasmids.

It requires intimate cell-to-cell contact between the donor and recipient cells.[11] Tetracycline-resistant *A. actinomycetemcomitans* have been shown to possess the efflux gene tet(B), which was transferable between strains of *A. actinomycetemcomitans* and between *A. actinomycetemcomitans* and *Haemophilus influenzae*. Both chromosomal and plasmid-borne antibiotic-resistance markers have been transferred between oral streptococci and oral black pigmented *Bacteroides* spp.[15]

CONCLUSION

The subgingival biofilm is more than a random assemblage of organisms seeking shelter from the hostile environment of the oral cavity. Rather, there exists 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. 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. The success of these strategies is evidenced by the fact that in the absence of host intervention, the subgingival area is colonized by biofilm communities from shortly after birth until death.

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