Research Paper Medical Science

Interspecies communication in Oral bio-films

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ABSTRACT

Until recently, bacteria were considered to live rather asocial, reclusive lives. New research shows that, infactmature dental biofilms consist of towering microcolonies in which the resident bacterial cells interact with one another and exchange messages in the form of signalling molecules and metabolites. These structures have been compared with the apartment buildings of busy cities. There is mounting evidence that mutually beneficial interactions between microbial cells are essential to the development of biofilms in the oral cavity. This review discusses the mutualistic partnerships that form between oral bacteria, and the contribution of interspecies communication to the formation of mixed microbial communities.

Keywords : Oral Biofilm, micro colonies, interspecies communication

INTRODUCTION

The micro-flora of the oral cavity is diverse, and more than 700 bacterial species have been detected. These bacterial species are thought to play important roles in the maintenance of oral health and in the aetiology of oral diseases in humans. Antony van Leeuwenhoek (1632–1723) made the first observations using his primitive microscopes. "I didn't clean my teeth for three days and then took the material that had lodged in small amounts on the gums above my front teeth. I found a few living animalcules". In recent years, with the advent and application of new molecular and imaging technologies, a more complete understanding of the biology of dental plaque as a biofilm and microbial community has been possible.Interactions among human oral bacteria are integral to the development of plaque. From the early stages of colonization to the formation of mature supragingival and subgingival plaque, diverse arrays of bacterial species colonize into densely populated communities. Interactions among different bacterial cell types are proposed to drive the maturation of plaque. These interactions occur at several levels, including physical contact, metabolic exchange, small-signal-molecule-mediated communication and exchange of genetic material.

Interactions which include:

• Competition between bacteria
• Synergistic interactions
• Production of an antagonist by one resident
• Neutralization of a virulence factor produced by one organism by another resident
• Interference in the growth-dependent signalling mechanisms of one organism by another

ORAL BIOFILMS

DEFINITIONS

- Matrix-enclosed bacterial population's adherent to each otherand/or to surfaces or interfaces, includingmicrobial aggregates within pore spaces of porous media. (Costerton et al,1995)[1]
- Microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription. (Donlan and Costerton ,2002)[2]
- Orientated aggregations of microorganisms attached to each other or to a surface and enclosed in extracellular polymeric substance (EPS) produced by themselves. (Marsh Pd,2005)[3]

STRUCTURE OF A BIOFILM

Fig 1: Illustration of Biofilm Structure

- Biofilms are composed of micro colonies of bacterial cells (15–20% by volume) that are non-randomly distributed in a shaped matrix or glycocalyx. Individual microcolonies can consist of a single species but morefrequently are composed of several different species

- The water channels permit the passage of nutrients and other agents throughout the biofilm acting as a primitive ''circulatory'' system".
- Nutrients make contact with the sessile (attached) microcolonies by diffusion from the water channel to the microcolony rather than from the matrix.
- At low shear force, the colonies are shaped like mushrooms, while at high shear force; the colonies are elongated & capable of rapid oscillation.

EXOPOLYSACCHARIDES (the back bone of the biofilm)

Exopolysaccharides (EPS), which are produced by the bacteria in the biofilm, are major components of the biofilm making up 50-95% of the dry weight. (Sutherland IW, 1999)4

i. They play a major role in maintaining the integrity of the biofilm as well as preventing desiccation & attack by harmful agents.
ii. They may also bind essential nutrients such as cations to create a local nutritionally rich environment favouring specific microorganisms.
iii. The EPS matrix could also act as a buffer & assist in the retention of extracellular enzymes (& their substrates) enhancing substrate utilization by bacterial cells.
iv. One distinguishing feature of oral biofilms is that many of the microorganisms can both synthesize & degrade the EPS.

PROPERTIES OF BIOFILM

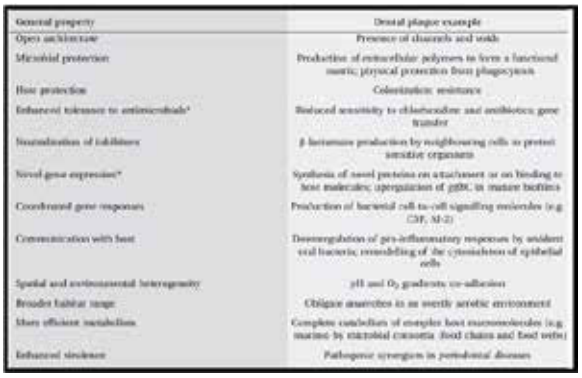

Fig 2: Properties of Biofilm

FORMATION OF BIOFILM

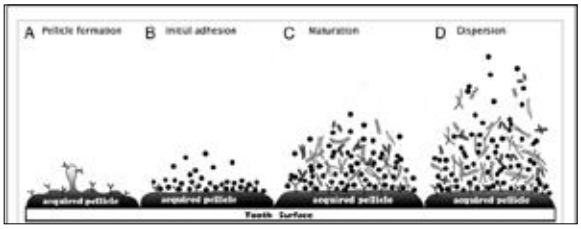

Fig 3: Formation of Biofilm

Process by which genetically distinct bacteria become attached to one another via specific molecule (Rickard et al, 2003)[5]

- Gibbons and Nygaard,1970 discovered coaggregation inter-bacterial aggregation.
- Coadhesion-Recognition between a suspended cell type and one already attached to a substratum. (Bos R et al,1994)
- Highly specific
- Protein 'adhesin' and saccharide 'receptor'
- Coaggregation partnerships are central to the development of biodiversity in supragingival and subgingival plaque
- 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.

Organisms of the same or different genera

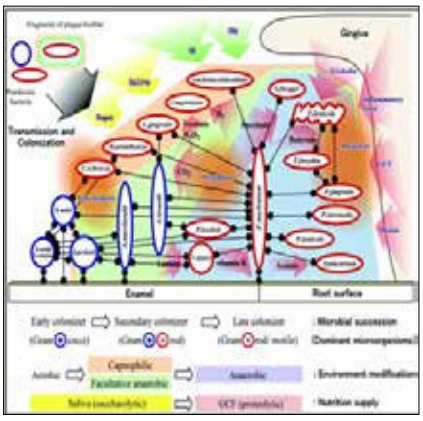

Fig 4: Coaggregation

- First bacteria to colonize -Streptococci and Gram-positive rods
- Within the first 4 hours: Streptococcus mitis, Streptococcus sanguinis and Streptococcus oralis represent 60–90% of the cultivable streptococci
- After 24 hours: Morphological types of bacteria, which coaggregate, to form intricate structures such as 'corn cobs' and 'bristle-brush formations'.
- Other early colonizers include Actinomyces spp., Capnocytophagaspp., Eikenella spp., Haemophilus spp., Prevotella spp, Propioni bacterium spp, and Veillonellaspp

The first organisms to attach are the primary (early) colonizers and primary colonization is mediated through specific or non-specific physico-chemical interactions with components of an adsorbed, organic conditioning film. If conditions are suitable, the primary colonizers can then multiply on the substratum to form micro colonies. As environmental conditions change within the young biofilm and the substratum becomes covered by bacteria, secondary (late) colonizers are then able to attach to the primary colonizers and the biofilm begins to develop into a multispecies community. Coaggregation interactions are believed to contribute to the development of biofilms by two routes . The first route is by single cells in suspension specifically recognizing and adhering togenetically distinct cells in the developing biofilm. The second route is by the prior coaggregation in suspension of secondary colonizers followed by the subsequent adhesion of this coaggregate to the developing biofilm. In both cases, bacterial cells in suspension (planktonic cells) specifically adhere to cells in the biofilm in a process known as coadhesion.

Coadhesion: Fusobacterium nucleatum can co-aggregate with many oral bacteria, including streptococci and obligate anaerobes.Therefore, this species is a key component of dental biofilms and serves as a coordinator that bridges the late and early colonizers. The bacteria representing early colonizers coaggregate with only a specific set of other early colonizers but not with all of them and generally not with any of the late colonizers. Members of red complex coaggregate strongly in vitro (Grenier 1992) 6, (Onagawa et al, 1994. Yao et al. 1996) and one species of the complex may produce growth factors required by another in that complex.

Many coaggregation adhesins have been identified on the cell surfaces of dental plaque bacteria and theexternal appendages thus enabling cells to make more effective contact with prospective partners.

Dental plaque organisms can express more than one coaggregation adhesin simultaneously on the cell surface; this will also optimize the chances of a cell finding a suitable partner in the competition for survival in the high-shear oral environment All these factors are consistent with the suggestion that theseadhesins contribute to the buildup of a multi-species plaque community.

QUORUM SENSING

Cell-density linked, coordinated gene expression in popula-

tions that experience threshold signal concentrations to induce a synchronized population response. (Fuqua W. C et al, 1994)[7]

• Found both in bacteria and in fungi (Miller &Bassler 2001) [8], (Keller &Surette 2006) 9 , (Diggleet al . 2007)

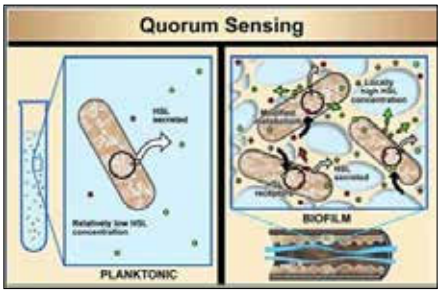

Fig 5: Quorum Sensing

- The term "Quorum Sensing" : Dr. Steven Winans in 1994
- Within biofilms, a sophisticated system of cell–cell communication are used by some bacteria to co-ordinate gene expression and involves a wide variety of secreted compounds known as autoinducers, including some packaged in vesicles.
- Quorum sensing may give biofilms their distinct properties.
- Quorum sensing is dependent on cell density. Once the signalling compounds reach a threshold level (quorum cell density), gene expression is activated. E.g. expression of genes for antibiotic resistance at high cell densities may provide protection.
- Quorum sensing also has the potential to influence community structure by encouragingthe growth of beneficial species (to the biofilm) and discouraging the growth of competitors.

❖ Therapeutic enzymatic degradation of the signalling molecules will prevent the formation of biofilms and possibly weaken established biofilms. This is called as "Quorum Quenching".

ANTIBIOTIC RESISTANCE

- The antibiotic resistance of bacterial cells in biofilm was reported to be 1,000 to 1,500 times greater than planktonic cells.(unattached cells) (Levy SB ,1998)
- Structural organization of biofilms and the subsequent altered pattern of gene expression results intheir reduced susceptibility of cells to antimicrobial agent (Gilbert et al.1997)
- It has been shown in many studies that the resistance of bacteria to antibiotics, biocides or preservatives is affected by their nutritional status, growth rate, temperature, pH and prior exposure to sub effective concentrations of antimicrobial agents
- Variations in any of these parameters can lead to a varied response to antibiotics within a biofilm.

METABOLIC COMMUNICATION

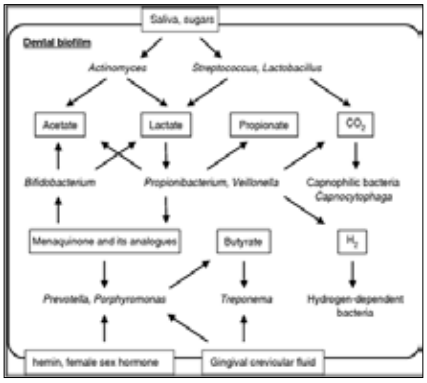

Fig 6: Metabolic Communication

- For oral bacteria, nutrients are available from saliva, gingival crevicular fluid, food containing sugars, food debris, and metabolicproducts of other bacteria.
- Metabolic cooperation among bacteria is central to the establishment of stable multispecies biofilm communities.
- 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 createsbiologically available substrates for different organisms.

BACTERIOCINS AND THEIR ROLE

- Proteinaceous toxins which may exert either specific or nonspecific effects on other bacteria.
- Narrow killing spectrum. (Chatterjee, et al,2005)10
- S.mutans strains bacteriocins, termed mutacins. (Qi, F. et al,2001): greatest capacity for bacteriocinproduction,mutacins I to V. (Nes, I. F et al,2007)
- Bacteriocins may also affect interspecies interactions by acting as analogues of signalling molecules

GENETIC EXCHANGE:

The transfer of genetic information can occur by:

- Conjugation
- Transduction
- Transformation
- Transposition

METHODS TO STUDY BACTERIAL INTERACTIONS IN ORAL BIOFILM

- Confocal Laser Scanning Microscopy
- Combination of vital fluorescence staining and confocal laser scanning microscopy:
  - o Structure of intact human dental biofilms
  - o Spatial distribution of living and dead bacteria in the different biofilm layers in situ. (Netuschil et al., 1998; Zaura-Arite et al., 2001; Auschill et al., 2001)
- Proteomics and transcriptomics
- DNA microarray - an assay that can be used to measure the level of expression in a collection of cells for thousands of genes. (Simon et al., 2002)
- To characterize genetic differences among isolates and closely related species. (Gibson, 2002)

CONCLUSION

- Bacterial interactions can affect the growth of individual organisms or groups of related organisms. In addition, such interactions may have specific effects in terms of the virulence properties of biofilm residents which could influence the overall pathogenicity of such structures.
- A better understanding of the mechanisms involved in periodontitis for investigators, the development of novel diagnostic, preventive, and treatment strategies against polymicrobial infection.

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