## Lec. 5

## Dental plaque biofilm

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Bacteria are the primary etiologic agents in periodontal disease. It has been found more than 500 distinct microbial phenotypes present in dental plaque. These kinds of bacteria have evolved to survive in the environment of tooth surface, gingival epithelium and oral cavity.

Dental plaque is defined as a soft yellow-greyish deposits that form the biofilm adhering to the tooth surface or other hard surfaces in the oral cavity such as removable and fixed restorations.

-Dental plaque consists primarily from microorganisms and intercellular matrix along with scattering epithelial cells, leukocytes and macrophages.
- The presence of tough extracellular matrix makes it impossible to remove by rinsing or using spays

**Biofilm** is defined as the relatively undefinable microbial community associated with a tooth surface or any other hard non shedding material.



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## Dental plaque as a biofilm

Structurally dental plaque is now considered to be as a biofilm of complex and dynamic microbial community areas.

- It contains areas of high and low bacterial biomass interlaced with aqueous channels of different sizes comprise the nutrient channels for bacterial colonization.
- The intercellular matrix forms a hydrated gel in which bacteria can survive and proliferate.
- Biofilm adheres firmly to the tooth surface and resists to mechanical removal as well as antibiotics
- Biofilm is a fascinated structure, which functions look like multicellular organisms ,characterised by shedding of bacterial surface components (antigens, which can activate a host immune response) and release of various toxins (endotoxin, which can activate a host inflammatory response) which cause host tissue damage
- The biofilm plays a major role in protecting the colonisation species from host defence mechanisms

### **Biofilm structure**

- Biofilm is composed of microcolonies of bacterial cells (15-20% by volume), which are distributed in matrix or glycocalyx (70-80% by volume)
- Thick biofilms have demonstrated presence of water channels between the microcolonies.
- These water channels permit the passage of nutrient and other agents through out the biofilm acting as a circulating system
- Some of the functions of the biofilm depend on the ability of bacteria and microcolonies within the biofilm to communicate with each other
- This activity is called 'quorum sensing' in which bacteria secrete a signalling molecule that accumulates in the local environment and triggers a response such as a change in the expression of specific genes once they reach a critical threshold concentration
- The threshold concentration reached at a high-cell density and therefore bacteria sense that the population has reached a critical mass or quorum
- Some evidence showed that the intercellular communication can occur after cell-cell contact and herein may not involve secreted signaling molecules



Plaque is different from other deposits that may be found on the tooth surface such as Materia alba and calculus

Materia alba refers to soft accumulations of bacteria, food matter and tissue cells that lack of the organised structure of dental plaque and can easily displaced with a water spray

Calculus is a hard deposit that forms by mineralisation of dental plaque and is generally covered by a layer of unmineralized plaque

Classification of dental plaque

- 1. Supragingival plaque
- 2. Subgingival plaque

Supragingival plaque is found on or above gingival margin, and where it located in a direct contact with the gingival margin is referred to a marginal plaque

Subgingival plaque is found below the gingival margin between the tooth and the gingival sulcular epithelium



Microbiological studies indicate that there is a difference between tooth-associated and tissue associated regions of subgingival plaque

- Different regions of plaque have significant different processes associated with disease of periodontium
- For example, marginal plaque is of importance in the development of gingivitis
- While supragingival and tooth-associated bacteria are critical **in calculus** formation and **root caries**
- Tissue-associated subgingival plaque is important in the soft tissue destruction (different forms of periodontitis)

**Dental plaque** is composed of microorganisms, where one gram of plaque in a wet condition contains 2x10<sup>11</sup> bacteria - The number of bacteria in supragingival plaque on a single tooth surface can range from 10<sup>3</sup> on a healthy crevice compared to >10<sup>8</sup> bacteria in a deep pocket

In addition to bacteria dental plaque contains non bacterial microorganisms such as yeasts, protozoa and viruses - It contains cells such as epithelial cells, macrophages and leukocytes

The inter cellular matrix , estimated to comprise 20-30% of plaque mass

- It consists of **organic** and **inorganic** materials derived from saliva, gingival crevicular fluid
- Organic constituents include polysaccharides, proteins glycoproteins and lipid material
- Inorganic constituents is mainly calcium and phosphorus with trace amount of other minerals such as sodium, potassium and fluoride
- The source of inorganic component of supragingival plaque is from saliva
- Whereas in subgingival it is derived from crevicular fluid

## Formation of dental plaque

Dental plaque may be visualised on teeth after 1-2 days with no oral hygiene measures

- Movement of tissues and food materials over teeth results in mechanical removal of plaque
- Such removal of plaque is effective on the coronal two thirds of the tooth surface
- Therefore, plaque is typically observed on the gingival third of the tooth surface

## The **process of plaque formation** can be divided into three phases

- 1- Formation of the pellicle coating on the tooth surface
- 2- Initial colonisation by bacteria
- **3** Secondary colonisation and plaque maturation

## Formation of the pellicle

All surfaces in the oral cavity including hard and soft tissues are coated with a layer of organic material known as pellicle

- Its derived from saliva and crevicular fluid components, bacterial and host tissue cells products and debris
- The pellicle on the tooth surface consists of more than 180 peptides, proteins and glycoproteins including keratins, mucins, histidine-rich proteins, proline rich proteins and phosphoproteins
- The mechanisms involved in enamel pellicle formation include electrostatic van der Waals and hydrophobic forces
- Salivary pellicle can be detected on clean surfaces within 1 min
- By 2 hours, the pellicle is essentially in equilibrium between adsorption and detachment, although further pellicle maturation can be observed for several hours



## **Pellicle functions**

1- Protective barrier, providing lubrication for surfaces and preventing tissue desiccations

2- It provides a substrate to which bacteria can attach, as bacteria do not contact the enamel directly but interact with the enamel pellicle ( the pellicle is not merely a passive adhesion matrix

3- Many proteins retain enzymatic activity when incorporated into the pellicle and some of these proxidases and amylase may affect the physiology and metabolism of adhering bacterial cells

#### Initial colonisation of the tooth surface

Tooth brushing removes most but not all bacteria from the exposed surfaces of the teeth

- However, recolonization begins immediately and bacteria can be detected within 3mins of introducing sterile enamel into he mouth
- Ther is a specific molecules on the bacterial surface called **adhesin**, which interact with receptors present in the dental pellicle
- This can determine whether or not a bacterial cell will remain associated with the surface
- Only a small proportion of oral bacteria possess adhesin that interact with receptors in the host pellicle and these microorganisms are generally the most abundant bacteria in the biofilms on tooth enamel shortly after cleaning
- Over the first 4-8 hours, 60-80% of bacteria present are members of the genus streptococcus

- Other bacteria commonly present at the this time include species that cannot survive without oxygen (
  obligate aerobes) such as Haemophilus spp and Neisseria spp, as well as organisms that can grow in the
  presence or absence of oxygen (facultative anaerobes) including Actiomyces spp and Veillonella spp
- These species are considered the primary colonisers on the tooth surface, which provide new binding sites for adhesion by other oral bacteria
- The metabolic activity of the primary colonisers modifies the local microenvironment in a way that can influence the ability of other bacteria to survive in the dental plaque biofilm
- For example, by removing oxygen, the primary colonisers provide conditions of low oxygen tension that permit the survival and growth of obligate anaerobes

## Secondary colonisation and plaque maturation

- The primary colonising bacteria adhered to the tooth surface provide new receptors for attachment with other bacteria in a process known as **co-adhesion**
- Co-adhesion leads to the development of microcolonies and eventually to a mature biofilm
- Different species or even different strains of a single species have distinct sets of coaggregation partners
- Secondary colonisers microorganisms include *Prevotella intermedia, Capnocytophaga spp., Fusobacterium nucleatum* and *Porphyromonas gingivalis*
- Fuso bacteria coaggregate with all other human oral bacteria while Veilloella spp, Capnocytophaga spp and Prevotella spp bind to stpretococci and or actinomyces

- Each newly accreted cell becomes itself a new surface and therefore may act as a coaggregation bridge to the next potentially accreting cell type that passes by well-charachterised interactions of secondary colonisers with early colonisers include the coaggregation of *F. nucleatum* with S. *sanguinis*
- The transition from early supragingival dental plaque to mature plaque growing below the gingival margin involves a shift in the microbial population from primarily gram-positive organisms to high numbers of gram-negative bacteria
- Examples of these types are coaggregation of *F. nucleatum* with *P. gingivalis* or *Treponema denticola*



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# Dental biofilms: difficult therapeutic targets

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Periodontal diseases are infections caused by microorganisms that colonize the tooth surface at or below the gingival margin. While these infections have many properties in common with other infectious diseases, they exhibit unique properties conferred by their site of colonization and the nature of the environment in which they reside. Table 1 presents an overly simplified summary of four crude categories body and the infection is usually rapidly resolved by a "cure", by removal of some body part or by demise of the patient. Treatment of these infections is usually supportive, although antibiotics are often used in more severe cases. Examples of such infections include local abscesses caused by organisms such as *Staphylococcus aureus*, upper respiratory infections caused by organisms such as *Streptococcus*