Dental plaque

Oral biofilms are functionally and structurally organized polymicrobial communities that are embedded in an extracellular matrix of exopolymers on mucosal and dental surfaces. **Dental plaque** is defined clinically as a structured, hard, yellow-grayish substance that adheres to the intraoral hard surfaces, including removable and fixed restorations. The tough extracellular matrix makes it impossible to remove plaque by rinsing or with the use of sprays. Plaque can be differentiated 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 the organized structure of dental plaque and that are easily displaced with a water spray. **Calculus** is a hard deposit that forms via the mineralization of dental plaque and that is generally covered by a layer of unmineralized plaque **(Table 1).**

Table 1:- Differences Between Tooth Deposits :-

Materia Alba	Dental Plaque	Calculus
1-White, cheese like accumulation	1-Resilient, clear to yellow grayish substance	1-Hard deposit that forms via the mineralization of dental plaque
2-Soft accumulation of salivary proteins, some bacteria, many desquamated epithelial cells, and food debris	2-Primarily composed of bacteria in a matrix of salivary glycoproteins and extracellular polysaccharides	
3-Lacks an organized structure and is therefore not as complex as dental plaque	3-Considered to be a biofilm	2-Generally covered by a layer of unmineralized dental plaque

4-Easily displaced with a water spray	4-Impossible to remove by rinsing or with the use of sprays	Removed by scaling

Dental plaque is composed primarily of microorganisms. **One gram** of plaque (wet weight) contains approximately $\mathbf{10^{11}}$ bacteria. The number of bacteria in **supragingival plaque** on a single tooth surface can exceed $\mathbf{10^{9}}$ cells. In a **periodontal pocket**, counts can range from $\mathbf{10^{3}}$ bacteria in a healthy crevice to more than $\mathbf{10^{8}}$ bacteria in a deep pocket.

According to its position the dental plaque can be classified into supragingival or subgingival.

• **Supragingival plaque** is found at or above the gingival margin. When the plaque in direct contact with the gingival margin, it is referred to as **marginal plaque**.

• **Subgingival plaque** is found below the gingival margin, between the tooth and the gingival epithelium.

Supragingival plaque typically demonstrates the stratified organization of a multilayered accumulation of bacteria. **Gram-positive cocci and short rods** predominate at the tooth surface, whereas **gram-negative rods, filaments, and spirochetes** predominate in the outer surface of the mature plaque mass.

The subgingival plaque differs in composition from the supragingival plaque, **because of** the local availability of blood products and a low reduction–oxidation (redox) potential, which characterizes the anaerobic environment.

The environmental parameters of the subgingival region differ from the supragingival region. The gingival crevice or pocket is bathed by the flow of crevicular fluid, which contains many substances that bacteria may use as

nutrients. Host inflammatory cells and mediators are likely to have considerable influence on the establishment and growth of bacteria in the subgingival region.

Both morphologic and microbiologic studies of subgingival plaque reveal distinctions between the **tooth-**associated regions and the **soft tissue**–associated regions of subgingival plaque.

The tooth-associated cervical plaque that adheres to the root cementum does not differ from that observed in gingivitis. At this location, filamentous microorganisms dominate, but cocci and rods also occur. This plaque is dominated by gram positive rods and cocci, including S. mitis, S. sanguinis, Actinomyces oris. However, in the deeper parts of the pocket, the filamentous organisms become fewer in number. **In the apical portion**, it seem to be absent. Instead, the microbiota is dominated by smaller organisms without a particular orientation. The apical border of the plaque mass is separated from the junctional epithelium by a layer of host leukocytes, and the bacterial population of this apical-tooth–associated region shows an increased concentration of gram-negative rods.

The layers of microorganisms that face the soft tissue lack a definite inter microbial matrix and contain primarily gram-negative rods and cocci, as well as large numbers of filaments, flagellated rods, and spirochetes.

The composition of the subgingival plaque depends on the pocket depth. The apical part is more dominated by spirochetes, cocci, and rods, whereas in the coronal part more filaments are observed.

The site specificity of plaque is significantly associated with diseases of the periodontium. Marginal plaque, for example, is important in the initiation and development of gingivitis. Supragingival plaque and tooth-associated subgingival plaque are critical in calculus formation and root caries, whereas tissue-associated

subgingival plaque is important in the tissue destruction that characterizes different forms of periodontitis. Biofilms also establish on artificial surfaces exposed to the oral environment, such as prostheses and implants.

Dental plaque biofilm formation

The process of plaque formation can be divided into several phases:

(1) The formation of the pellicle on the tooth surface,

(2) The initial adhesion/attachment of bacteria,

(3) Colonization/plaque maturation.

☒ Formation of the acquired Pellicle

All surfaces in the oral cavity including hard and soft tissues, are coated with a layer of organic material known as the **acquired pellicle**. The pellicle on tooth surface **consists of** more than 180 peptides, proteins, and glycoproteins including keratins, mucins, proline-rich proteins, phosphoproteins e.g., statherin, histidine-rich proteins, and other molecules that can **function as** adhesion sites (receptors) for bacteria. Salivary pellicle can be detected on clean enamel surfaces within **1 minute** after introduction into the mouths of volunteers. By two hours, the pellicle is essentially in equilibrium between adsorption and detachment, although further pellicle maturation can be observed for several hours. Therefore, bacteria that adhere to tooth surfaces do not contact the enamel directly but interact with the acquired enamel pellicle. Many proteins retain enzymatic activity when incorporated into the pellicle, and some of these, such as peroxidases, lysozyme and α-amylase, may affect the physiology and metabolism of adhering bacterial cells.

☒ Initial Adhesion/Attachment of Bacteria

Tooth brushing removes most but not all bacteria from the exposed surfaces of teeth. However, recolonization begins immediately and bacteria can be detected within 3 minutes of introducing sterile enamel into the mouth.

The initial steps of transport and interaction with the surface are essentially **nonspecific** (i.e., they are the same for all bacteria). The proteins and carbohydrates that are exposed on the bacterial cell surface become important when the bacteria are in loose contact with the acquired enamel pellicle. The **specific interactions** between microbial cell surface **"adhesin"** molecules and **receptors** in the salivary pellicle determine whether a bacterial cell will remain associated with the surface. Only a relatively small proportion of oral bacteria possess adhesins that interact with receptors in the host pellicle, and these organisms are generally the most abundant bacteria in biofilms on tooth enamel. Over the **first 4 to 8 hours**, the **genus Streptococcus** tends to dominate, usually accounting for >20% of bacteria present. Other bacteria that commonly present at 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 **Actinomyces spp. and Veillonella spp**. These species are considered the "**primary colonizers"** of tooth surfaces. The **primary colonizers 1**-provide new binding sites for adhesion by other oral bacteria. **2-**The metabolic activity of the primary colonizers modifies the local microenvironment in ways that can influence the ability of other bacteria to survive in the dental plaque biofilm. **For example**, by removing oxygen, the primary colonizers provide conditions of low oxygen tension that permit the survival and growth of obligate anaerobes.

The initial steps in bacteria attachment include:-

Step 1 Transport to the surface,

Step 2 Initial reversible adhesion,

Step 3 Strong irreversible attachment.

Phase 1: Transport to the Surface

The first stage involves the initial transport of the bacterium to the tooth surface. Random contacts may occur, **for example**, through **Brownian motion**, through **sedimentation of microorganisms**, through **liquid flow**, or through **active bacterial movement** (chemotactic activity). However, saliva flow or mechanical contact between oral soft tissues and teeth are more important for bringing the primary colonizing bacteria into contact with teeth.

Phase 2: Initial Adhesion

The second stage results in an initial reversible adhesion of the bacterium. This is initiated when the bacterial cell comes into close proximity to the surface. **Long- and short-range forces, including van der Waals attractive forces and electrostatic repulsive forces**, work at this stage.

Phase 3: Strong irreversible Attachment

After initial adhesion, a firm anchorage between the bacterium and the tooth surface. The binding between the bacteria and the pellicle is mediated by **specific adhesins** on the bacterial cell surface (usually proteins) and **complementary receptors** (proteins, glycoproteins, or polysaccharides) in the acquired pellicle. Many proteins in the acquired pellicle can act as receptors for streptococci, including α-amylase, acid proline-rich proteins, statherin. Molecules (adhesins) on these early bacterial colonizers (mainly streptococci, e.g. Streptococcus mitis,

Streptococcus oralis) can bind to complementary receptors in the acquired pellicle to make the attachment stronger. Individual species can organize several adhesins, in **Gram-positive bacteria**, several families of surface proteins can act as adhesins, including serine-rich proteins and antigen I/II. In **Gram-negative bacteria**, auto-transporters, extracellular matrix-binding proteins, and pili function as adhesins

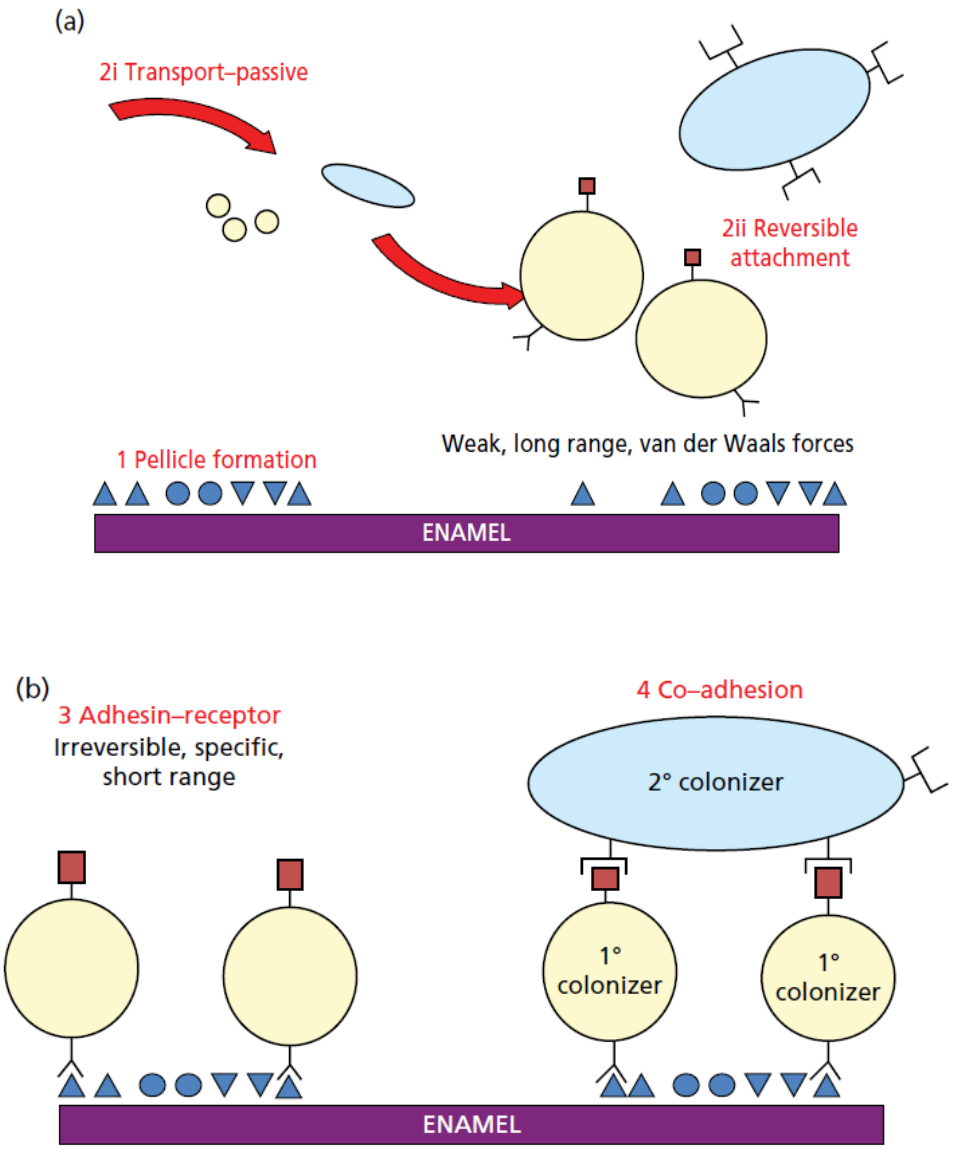

Fig.1:-Schematic representation of the different stages in the formation of dental biofilms.

☒ Colonization/plaque maturation

Once attached, the primary colonizers start to multiply. The metabolism of these bacteria that attach early will modifies the local environment, for example by making it more anaerobic condition by consumption of oxygen and the production of reduced end products of metabolism.

As the biofilm develops, adhesins on the cell surface of secondary colonizers, such as obligate anaerobes, bind to receptors on bacteria that are already attached by a process termed **co-adhesion or co-aggregation**, and the composition of the biofilm becomes more diverse (a process termed **microbial succession**).

Different species or even different strains of a single species have distinct sets of coaggregation partners. **Fusobacteria** coaggregate with **all** other human oral bacteria while **Veillonella spp., Capnocytophaga spp. and Prevotella spp.** bind to **streptococci and/or actinomyces**.

A key organism in plaque biofilm development is Fusobacterium nucleatum. This species can co-adhere to most oral bacteria, and acts as an important bridging organism between early and late colonizing species.

Well-characterized interactions of secondary colonizers with early colonizers include the coaggregation of F. nucleatum with S. sanguinis, Prevotella loescheii with A. oris, and Capnocytophaga ochracea with A. oris. Streptococci show intrageneric coaggregation, allowing them to bind to the nascent monolayer of already bound streptococci.

Secondary colonizers ,such as Prevotella intermedia, Prevotella loescheii, Capnocytophaga spp., F. nucleatum, and P. gingivalis **do not** initially colonize clean tooth surfaces but adhere to bacteria already in the plaque mass.

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. Therefore, in the later stages of plaque formation coaggregation between different gram-negative species is likely to predominate. **Examples** of these types of interactions are the co aggregation of **F. nucleatum with P. gingivalis or T. denticola.**

☒ Plaque maturation

Some of the attached bacteria synthesize extracellular polymers (the plaque matrix) that can consolidate attachment of the biofilm. The matrix not only scaffold for the biofilm but also it can bind and retain molecules, including enzymes, and also delay the penetration of charged molecules into the biofilm. Biofilms are functionally-organized, and the diverse conditions within the biofilm induce novel patterns of bacterial gene expression, while the close proximity of different species provides the opportunity for interactions. Examples of these interactions include:

- ✓ **Development of food chains** (in which the end product of metabolism of one organism is used as a primary nutrient by secondary colonizers).
- ✓ **Cell–cell signaling**. Plaque bacteria have been shown to communicate with one another in a cell density-dependent manner via small diffusible molecules, using strategies, for example by the secretion of **small peptides** by **Gram-positive bacteria** to coordinate gene expression among cells of a similar species and **autoinducer-2 (AI-2)** other communication systems may function between different oral species (**Gram-positive and Gram-negative bacteria**).

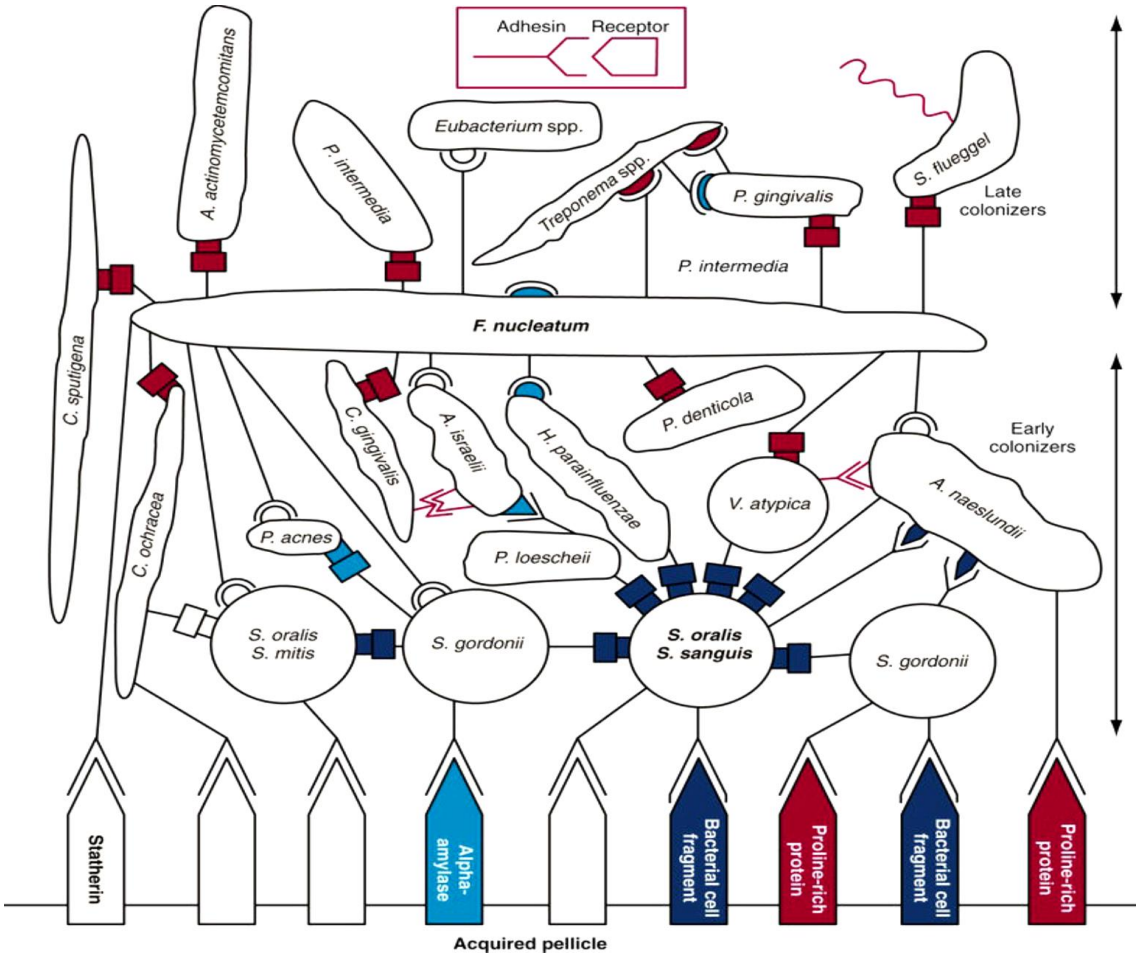

Fig. :- Diagrammatic representation of initial plaque formation. Early colonizers bind to receptors in the pellicle. Each adherent cell becomes in turn the nascent surface and bridge for additional species (secondary colonizers).

Factors Affecting Supragingival Dental dental plaque formation

During the first 24 hours starting from a clean tooth surface, plaque growth is negligible from a clinical viewpoint (**<3%** coverage of the vestibular tooth surface, which is an amount nearly undetectable clinically). This “lag time” is due to the fact that the microbial population must reach a certain size before it can be easily detected by the clinician. During the following 3 days, coverage progresses rapidly to the point where, after 4 days, on average **30%** of the total coronal tooth area will be covered with plaque.

Topography of Supragingival Plaque

Early plaque formation on teeth follows a typical topographic pattern with initial growth along the gingival margin and from the interdental space (areas protected against shear forces). Later, a further extension in the coronal direction can be observed. This pattern may fundamentally change when the tooth surface contains irregularities that offer a favorable growth path. Plaque formation can also start from grooves, cracks, or pits. By multiplication, the bacteria subsequently spread out from these starting up areas as a relatively even monolayer. Surface irregularities are also responsible for the so-called “individualized plaque growth pattern, which is reproduced in the absence of optimal oral hygiene. This phenomenon illustrates the importance of surface roughness in plaque growth, which should lead to proper clinical treatment options.

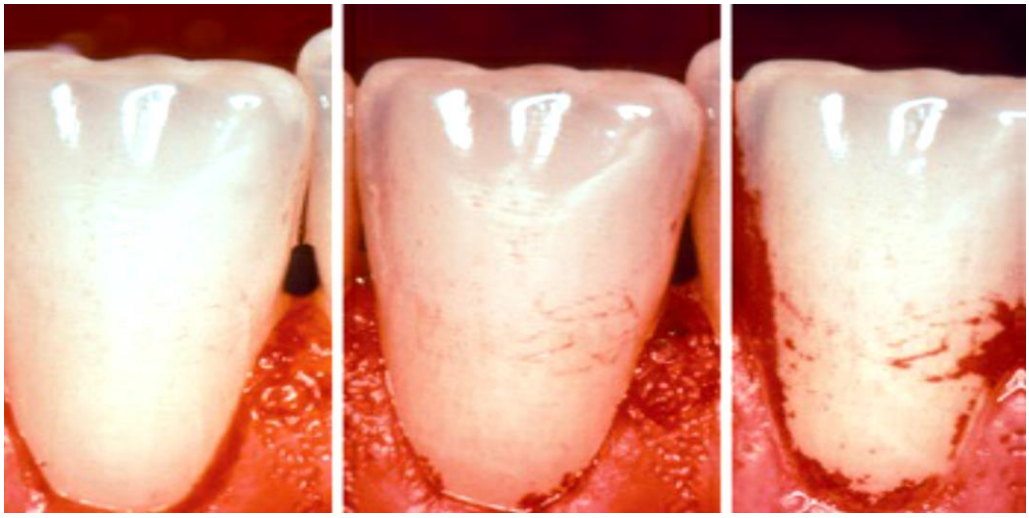

Fig. Typical topography of plaque growth. Initial growth starts along the gingival margins and from the interdental spaces (i.e., areas protected from shear forces) to extend farther in a coronal direction.

Surface Micro roughness.

Rough intraoral surfaces (e.g. crown margins, implant abutments, and denture bases) accumulate and retain more plaque and calculus in terms of thickness, area, and colony-forming units. Sufficient plaque also reveals an increased maturity/pathogenicity of its bacterial components, characterized by an increased proportion of motile organisms and spirochetes and/or a denser packing of them. Smoothing an intraoral surface decreases the rate of plaque formation.

Individual Variables Influencing Plaque Formation.

The rate of plaque formation differs significantly between subjects, differences that might overrule surface characteristics. A distinction is often made between "heavy" (fast) and "light" (slow) plaque formers. It has shown that the clinical wettability of the tooth surfaces, the saliva-induced aggregation of oral bacteria, and the relative salivary flow conditions around the sampled teeth explained 90% of the variation. Moreover, the saliva from light plaque formers reduced the colloidal stability of bacterial suspensions, for example, S. sanguinis.

Variation within the Dentition.

Within dental arch large differences in plaque growth rate can be detected. In general early plaque formation occurs faster: in the **lower jaw** (when compared to the upper jaw); in **molar areas**; on the buccal tooth surfaces when compared to palatal sites (especially in the upper jaw); and in the **interdental regions** when compared to the buccal or lingual surfaces.

Impact of Gingival Inflammation and Saliva.

Several studies clearly indicate that early in vivo plaque formation is more rapid on tooth surfaces facing inflamed gingival margins than on those adjacent to healthy

gingival. These studies suggest that the increase in crevicular fluid production enhances plaque formation. Probably, some substance(s) from this exudate (e.g. minerals, proteins, or carbohydrates) favor both the initial adhesion and/or the growth of the early colonizing bacteria. Additionally, it is known that during the night, plaque growth rate is reduced by some 50%. This seems surprising, since one would expect that reduced plaque removal and the decreased salivary flow at night would enhance plaque growth. The fact that the supragingival plaque obtains its nutrients mainly from the saliva appears to be of greater significance than the antibacterial activity of saliva.

The Impact of Patient's Age.

Although older studies were contradictory, more recent papers clearly indicate that a subject's age **does not influence** in plaque formation. The developed plaque in the older patient group resulted however, in a more severe gingival inflammation, which seems to increased susceptibility to gingivitis with aging.

Spontaneous Tooth Cleaning.

Many clinicians still believe that plaque is removed spontaneously from the teeth such as during eating. However, based on the firm attachment between bacteria and surface, this seems unlikely. Even in the occlusal surfaces of the molars, plaque remains, even after chewing fibrous food carrots, apples, or chips.

Metabolism of Dental Plaque Bacteria

The majority of nutrients for dental plaque bacteria originate from saliva or GCF, although the host diet provides an occasional but nevertheless important food supply. The transition from gram positive to gram negative microorganisms observed in the structural development of dental plaque.

The growth of **P. gingivalis** is enhanced by metabolic byproducts produced by other microorganisms, such as **succinate** from Capnocytophaga ochrecea and **protoheme** from Campylobacter rectus.

Metabolic interactions occur also between the host and plaque microorganisms. Increases in steroid hormones are associated with significant increases in the proportions of P. intermedia found in subgingival plaque. These nutritional inter-dependencies are probably critical to the growth and survival of microorganisms in dental plaque and may partly explain the evolution of highly specific structural interactions observed among bacteria in plaque.

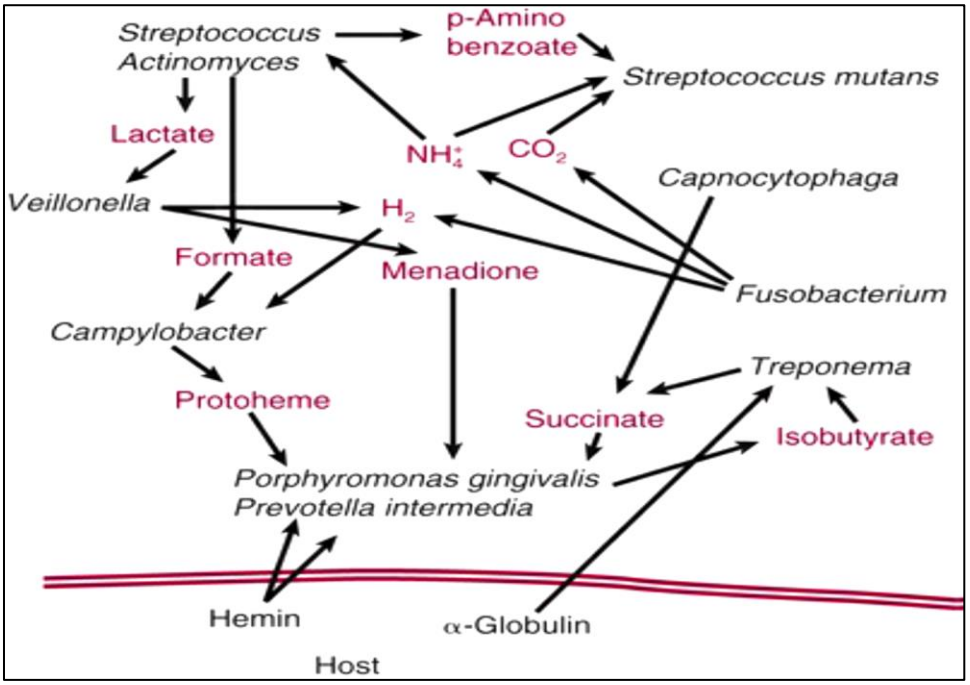

Fig.:-Schematic illustration of metabolic interactions among different bacterial species found in plaque and between the host and the plaque bacteria. These interactions are likely to be important to the survival of bacteria in the periodontal environment.

Communication between Biofilm Bacteria

Bacterial cells do not exist in isolation. In a biofilm, bacteria have the capacity to communicate with each other. One example of this is quorum sensing, in which bacteria secrete a signaling 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 is reached only at a high-cell density, and therefore bacteria sense that the population has reached a critical mass, or quorum. There is some evidence that intercellular communication can occur after cell-cell contact and in this case, may not involve secreted signaling molecules. Two types of signaling molecules have been detected from dental plaque bacteria: **peptides** released by **gram-positive** organisms during growth and a **"universal"** signal molecule **autoinducer 2(AI-2)**. Peptide signals are produced by oral streptococci and are recognized by cells of the same strain that produced them. Responses are induced only when a threshold concentration of the peptide is attained, and thus the peptides act as cell density, or quorum sensors.

Biofilms and Antimicrobial Resistance

Bacteria growing in microbial communities adherent to a surface do not "behave" the same way as bacteria growing suspended in a liquid environment (in a planktonic or unattached state). For example, the resistance of bacteria to antimicrobial agents is dramatically increased in the biofilm. Almost without exception, organisms in a biofilm are 1000 to 1500 times more resistant to antibiotics than in their planktonic state. The mechanisms of this increased resistance differ from species to species, from antibiotic to antibiotic, and for biofilm growing in different habitats.

It is generally accepted that the resistance of bacteria to antibiotics 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 will thus lead to a varied response to antibiotics within a biofilm. An important mechanism of resistance appears to be the slower rate of growth of bacterial species in a biofilm, which makes them less susceptible to many but not all antibiotics. The biofilm matrix, although not a significant physical barrier to the diffusion of antibiotics, does have certain properties that can retard antibiotic penetration.

In addition, extracellular enzymes such as β-lactamases, formaldehyde lyase, and formaldehyde dehydrogenase may become trapped and concentrated in the extracellular matrix, thus inactivating some antibiotics (especially positively charged hydrophilic antibiotics).

Recently, “super-resistant” bacteria were identified within a biofilm. These cells have multidrug resistance pumps that can extrude antimicrobial agents from the cell. Since these pumps place the antibiotics outside the outer membrane, the process offers protection against antibiotics that target, for example, cell wall synthesis. The penetration and efficacy of antimicrobials against biofilm bacteria are critical issues for the treatment of periodontal infections.