**Lecture 1&2 dr. Ayser najah**

**Dental Plaque Biofilm**

Dental plaque is defined clinically as a structured resilient yellow-grayish substance that adheres tenaciously to the intraoral hard surfaces, including removable and fixed restorations. The tough extracellular matrix makes it impossible to remove plaque by rinsing or the use of sprays.

Plaque can thus 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 are easily displaced with a water spray.

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

Dental plaque is composed primarily of microorganisms. One gram of plaque (wet weight) contains approximately 1011 bacteria.

The number of bacteria in supragingival plaque on a single tooth surface can exceed 109.

In a periodontal pocket, counts can range from 103 bacteria in a healthy crevice to > 108 bacteria in a deep pocket.

Using highly sensitive molecular techniques for microbial identification, it has been estimated that more than 500 distinct microbial phenotypes can be present as natural inhabitants of dental plaque. In fact, this number may be much greater...

Any individual may harbor 150 or more different species. Non bacterial microorganisms that are found in plaque include archaea , yeasts, protozoa, and viruses

**Differences between bacterial deposits**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Materia alba | Dental plaque | Claculus |
| 1 | White cheese-like accumulations | Resilient clear to yellow grayish | Hard deposits formed by mineralization of dental plaque |
| 2 | A soft accumulation of salivary proteins | Primarily composed of bacteria in a matrix of salivary proteins |  |
| 3 | Lack of organized structure (not complex) as dental plaque) | Considered as a biofilm | Generally covered by a layer of un-mineralized dental plaque |
| 4 | Easily displaced by a water spray | Removed only by mechanical rinsing (tooth brushing) |  |

Dental plaque is broadly classified as supragingival or subgingival based on its position on the tooth surface toward the gingival margin

**Supragingival plaque is** found at or above the latter; when 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 pocket epithelium

Supragingival plaque typically demonstrates a stratified organization of a multilayered accumulation of bacterial morphotypes.

Gram-positive cocci and short rods predominat at the tooth surface, whereas gram-negative rods and filaments, as well as spirochetes, predominate in the outer surface of the mature plaque mass

In general, the subgingival microbiota differs in composition from the supragingival plaque, primarily 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 those of the supragingival region. The gingival crevice or pocket is bathed by the flow of crevicular fluid, which contains many substances which 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 and soft tissue-associated regions of subgingival plaque. .

The tooth-associated cervical plaque, adhering to the root cementum , does not markedly 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 numbers, and in the apical portion they seem to be virtually 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 popultion of this apical tooth-associated region shows an increased concentration of gram-negative rods.

The layers of microorganisms facing the soft tissue lack a definite intermicrobial matrix and contain primarily gram-negative rods and cocci as well as large numbers of filaments, flagellated rods, and spirochets.

Host tissue cells (e.g., white blood cells and epithelial cells) may also be found in this region Bacteria are also found within the host tissues, such as in the soft tissues and within epithelial cells, as well as in the dentinal tubules.

The composition of the subgingival plaque depends on 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 of prime importance 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 form on artificial surfaces exposed to the oral environment such as prostheses and implants. A large series of papers compared the microbiota in pockets around teeth with those around implants of partially edentulous patients. The similarities were striking.

**Accumulation of a Dental Plaque Biofilm**

The process of plaque formation can be divided into several phases

1-the formation of the pellicle on the tooth surface,

2-initial adhesion/attachment of bacteria

3- colonization/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 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 2 hours, the pellicle is essentially in equilibrium between adsorption and detachment, although further pellicle maturation can be observed for several hours. Consequently, bacteria that adhere to tooth surfaces do not contact the enamel directly but interact with the acquired enamel pellicle; however, the pellicle is not merely a passive adhesion matrix.

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 once the bacteria are in loose contact with the acquired enamel pellicle. It is the specific interactions between microbial cell surface “adhesin” molecules and receptors in.

The salivary pellicle that determines whether a bacterial cell will remai n 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 shortly after cleaning. Over the first 4 to 8 hours, 60% to 80% of bacteria present are members of the genus Streptococcus. Other bacteria 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 provide new binding sites for adhesion by other oral bacteria. 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.

**Colonization and Plaque Maturation** The primary colonizing bacteria adhered to the tooth surface provide a new receptors for attachment by other bacteria in a process known as “coadhesion.” Together with growth of adherent microorganisms, coadhesion leads to the development of micro colonies and eventually to a mature biofilm. 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.

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-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 Treponema denticola.

**Factors Affecting Supragingival Dental dental plaque formation**

Clinically, early undisturbed plaque formation on teeth follows an exponential growth curve when measured planimetrically. 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.

Several reports have shown that the microbial composition of the dental plaque will change with a shift toward a more anaerobic and a more gram-negative flora including an influx of fusobacteria, filaments, spiral forms, and spirochetes.

This was in this beautifully illustrated in experimental gingivitis studies ecologic shift within the biofilm, there is a transition from the early aerobic environment characterized by gram-positive facultative species to a highly oxygen-deprived environment in which gram negative anaerobic microorganisms predominate. Bacterial growth in older plaque is much slower than in newly formed dental plaque presumably because nutrients become limiting for much of the plaque biomass.

**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.

**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. Ample 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. There seems to be threshold for surface roughness (Ra 0.2 micrometers) above which bacterial adhesion is facilitated.

**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. , 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 of, 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 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 de novo 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 is paralleled by a physiologic transition in the developing plaque .The growth of P. gingivalis is enhanced by metabolic byproducts produced by other microorganisms, such as succinate from C. ochre cea and protoheme from Campylobacter rectus . Overall, the total plaque population is more efficient than any one constituent organism at releasing energy from the available substrates.

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 interdependencies are probably critical to the growth and survival of microorganisms in dental plaque and may partly explain the evolution of highly specific structural inter actions observed among bacteria in plaque.

**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.

**Microbiologic Specificity of Periodontal Diseases**

**Nonspecific Plaque Hypothesis**

In the mid1900s, periodontal diseases were believed to result from an accumulation of plaque over time, eventually in conjunction with a diminished host response and increased host susceptibility with age.

The nonspecific plaque hypothesis maintains that periodontal noxious products by the entire plaque flora. According to this thinking, when only small amounts of plaque are present, the noxious products are neutralized by the host. Similarly, large amounts of plaque would produce large amounts of noxious products, which would essentially overwhelm the host’s defenses. Several observations contradicted these conclusions. First, some individuals with considerable amounts of plaque and calculus, as well as gingivitis, never developed destructive periodontitis. Furthermore, individuals who did present with periodontitis demonstrated considerable site specificity in the pattern of disease. Some sites were unaffected, whereas advanced disease was found in adjacent sites. In the presence of a uniform host response, these findings were inconsistent with the concept that all plaque was equally pathogenic. Recognition of the differences in plaque at sites of different clinical status (i.e., disease versus health) led to a renewed search for specific pathogens in periodontal diseases and a conceptual transition from the nonspecific to the specific plaque hypothesis. Although the nonspecific plaque hypothesis has been discarded in favor of the specific plaque hypothesis or the ecologic plaque

Hypothesis, much clinical treatment is still based on the nonspecific Plaque hypothesis

**Specific Plaque Hypothesis**

The specific plaque hypothesis states that only certain plaque is pathogenic, and its pathogenicity depends on the presence of or increase in specific microorganisms. This concept predicts that plaque harboring specific bacterial pathogens results in a periodontal disease because these organisms produce substances that mediate the destruction of host tissues. Acceptance of the specific plaque hypothesis was spurred by the recognition of A. actinomycetemcomitans as a pathogen in localized aggressive periodontitis.

**Ecologic Plaque Hypothesis**

Both the total amount of dental plaque and the specific microbial composition of plaque may contribute to the transition from health to disease. The health associated dental microfloral is considered to be relatively stable over time and in a state of dynamic equilibrium or ‘microbial homeostasis. 1- The excessive accumulation of nonspecific plaque.

2- plaque-independent host factors (e.g., the onset of an immune disorder, changes in hormonal balance such as in pregnancy), or environmental factors (e.g smoking , diet ),Changes in the host status, such as inflammation.

3-tissue degradation, and/or high GCF flow, may lead to a shift in the microbial population in plaque, culminating in periodontal disease.

**Bacterial adherence**

Characteristics of bacterial surfaces most bacteria in nature are surrounded by

highly hydrated matrices called “glycocalyces”; these are often made up of “heterolpolysaccharides”, which bacteria can produce from any carbohydrate source, many bacteria bear long appendages at their surfaces which may extend beyond the surface of the glycol calyx, these appendages are called pilli or fimbriae. .

**Characteristics of bacteria adherence**

An important characteristic of living cells is that they carry negative electric charge and thus tend to repel each other electrostatically, the tooth surface is also negatively charged and repels the cells, the cells are also influenced by electrodynamic forces (van der waal’s force) which are attractive forces, the attractive and repulsive forces will create a gap between the bacteria and the tooth surface, this gap is influenced by the presence of ions, hydrogen ions and cations (+ve) charge will narrow the gap, the importance of the glycocalyx has extension beyond the highly charged surface of the bacterial cell and can bridge the gap between bacteria and tooth surface .

The bacterial pili or fimbriae are long enough to protrude beyond the glycocalyx and assist in bridging the gap and establishing the contact between the bacteria and the tooth surface, the adhesion of bacteria to tooth surface is highly specific mechanism, there are molecules called “adhesins” on the bacteria recognize specific receptor molecules on the tooth surface, these adhesins located on the pili.

The carbohydrate groups of glycoproteins of the pellicle may serve as receptors for such bacterial adhesins, so the pellicle will facilitate adherence and serve as solid ground for long lasting microbial life.

On the other hand some of the salivary mucins (agglutinins and secretory immunoglobulin A) may react with the bacterial surface structure and block adhesins so in that way it will prevent the bacteria from adhering to the oral surfaces.

**Adhesive Surface Proteins and Fibrils**

To colonize the periodontal pocket, bacteria must adhere to specific receptors on cells or tissues in the region, such as teeth, the existing microbial  biofilm, or the pocket epithelium.

Adhesion is mediated by hair-like filaments extend from the cell surface called fimbriae or pili. Pili are composed of repeating Subunits of protein pilin.

pili were once thought to be unique to gram -Negative bacteria, but they have now been identified in several gram positive organisms including streptococci

and actinomyces.

Teeth and implants provide hard, non-shedding surfaces that allow for development of extensive plaque deposits. teeth are considered as the portal of entry of periodontal pathogens.

Strains of P. Gingivalis produce two types of fimbriae that are known as

The**major fimbriae** and the **minor fimbriae.**

Major and minor fimbriae interact  with oral streptococci .

Major fimbriae have also been shown to bind host extracellular matrix

proteins fibronectin and type I collagen, salivary proline rich proteins  and statherin, and epithelial cells.  Major sheath protein (msp) of***T. denticola*** *is* a peri-plasmic protein

 o n the cell surface.

Msp interacts with the host extracellular matrix proteins fibrinogen Fibronectin

and laminin.  In addition ,msp recognizes receptors on **P. Gingivalis** and

**F.Nucleatum**  and mediates coaggregation.

Therefore, msp is a multifunctional adhesin that promotes binding to host and bacterial receptors.

**Some anaerobic sub-gingival bacteria:**

1. Prophyromonas gingivalis.
2. Prevotella intermedia.
3. Aggregatibacter actinomycetem comitans (A.a), formally called actinobacillus actinomycetem comitans.
4. Capnocytophaga species.
5. Actinomyces naeslundii.
6. Fusobacterium nucleatum.
7. Streptococcus sanguis
8. Tannerella forsythia.

**Microbial flora are associated with:**

1. **Clinically healthy gingiva**

If the teeth are kept clean with proper oral hygiene measures, the gingiva remains healthy and few bacteria are found along the gingival margin. If the person with such gingiva stop cleaning his teeth, bacteria will be accumulated on his teeth within few hours. The most predominant bacteria are streptococcus (G+ve cocci) and actinomyces (G+ve rods) also G-ve rods and facultative anaerobic rods are found in small proportions.

1. **Gingivitis**
   1. In mild to moderate gingivitis: for at least 2-3 months, streptococci account for around 25% of the microbial flora of subgingival plaque (streptococcus mitis & streptococcus sanguis) are predominant species. Another 25% of subgingival bacteria is composed of Actinomyces species. Another 25% are G-ve anaerobic rods as fusobacterium, bacteroids and campylobacter. Other 25% are miscellaneous bacteria. The spirochetes are very difficult to be cultured so it can be identified by dark field microscopy. Their percentage is very small forming about 2%.
   2. Pregnancy gingivitis: there is increase in the proportion of prevotella intermedia (or black pigmented bacteroids species) and capnocytophaga. The increase in these organisms is related to increased levels of estrogen and progesterone hormones in gingival fluid. These hormones are used by these organisms as growth factors.
   3. Acute necrotizing ulcerative gingivitis: the microflora is composed primarily of fusiform bacteria & spirochetes to form **fusospirochetal complex**. These organisms are capable of invading the epithelium and the connective tissue of the gingiva.
2. **Chronic periodontitis**

The microflora is dominated by anaerobic microorganisms. G-ve rods form about 75% like bacteroids and fusobacterium nucleatum. Spirochetes form about 50% of the flora.

1. **Aggressive periodontitis**

Dominated by anaerobic G-ve rods which form about 60%, and 7% spirochetes. From the G-ve rods attention has been paid for aggregatibacter actinomycetem comitans (A.a) which is almost always present in aggressive periodontitis and less prevalent in chronic periodontitis

**Experimental Gingivitis**

In an investigation called “experimental gingivitis in man” (Loe et al 1965), the cause and effect relationship between dental plaque and gingival inflammation was demonstrated. In that study, the oral hygiene of a group of healthy individuals (12 patients who were 9 dental students, 1 instructor and 2 laboratory technicians) was improved during several weeks of intensive instruction in the use of tooth brush and tooth picks.

This resulted in excellent gingival condition. Then, all oral hygiene measures were withdrawn allowing plaque to re accumulate along the gingival margin. All subjects developed gingivitis within 10-21 days. The mean gingival index score increased from 0.27 at base line to 1.05 at the end of the no brushing period. Gingival inflammation resolved in all subjects within 1 week of resuming hygiene measures. During the experimental period, plaque samples were obtained at regular intervals and subjects to bacteriological examination of gram stained smear. The bacteria present in the samples were classified according to their gram reaction and morphology.

With healthy gingiva, very few bacteria were present on the cervical surfaces of teeth. The removable deposit was dominated by desquamated epithelial cells between which few bacteria could be seen about 90% of these bacteria were G+ve cocci and rods. The remainder 10% were G-ve bacteria.

When all oral hygiene measures stopped, the following phases of plaque development occurred:

**1st phase**:

Initial 2 days of the experiment, not only all types of bacteria increase but their proportional distribution change as well. G+ve cocci and rods forming a greater proportion of the flora.

**2nd phase:**

Days 3 and 4 are characterized by proliferation of fusobacteria and filamentous bacteria.

**3rd phase:**

Days 5-9 are characterized by the appearance of spirilla and spirochetes.

After about 7 days, G+ve cocci and rods which initially predominated now only form about 50% of the complex flora and up to 3 weeks, no further major changes in the bacterial distribution occur.

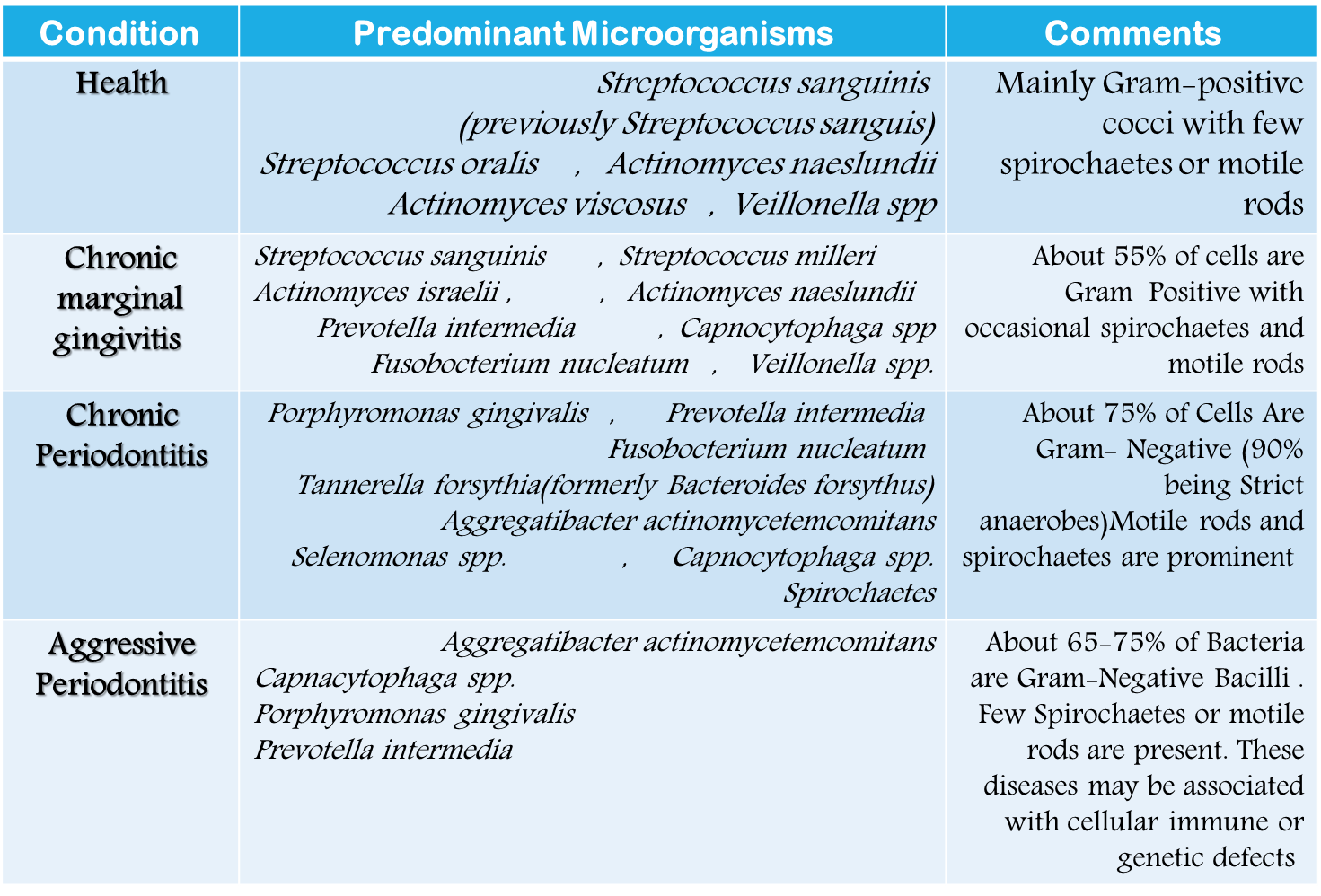
The experiment only gives information about G+ve and G-ve bacteria but not the type of species of bacteria and it’s an important and dependable experiment which proved clinically that dental plaque is the main etiological factor in the development of periodontal disease.

**Why the bacterial composition of sub-gingival plaque is different from supra-gingival plaque?**

1. The access to the oral cavity is limited, which favors anaerobic bacteria (growing only In the absence of oxygen)
2. Nutrients are readily available from gingival exudates, the volume of which is increased as a result of inflammation in the gingiva.
3. Detachment of already established micro-organisms is limited due to the protecting gingival tissue making it possible for the organisms without special adhesion mechanisms to survive.
4. The chance of arrival of additional bacteria from saliva is limited.

The nutrients of sub-gingival bacteria are provided by the following:

1. Gingival exudates or fluid which contains proteins, carbohydrates, minerals and vitamins and they form a good nourishment to the bacteria.
2. Dead cells of the periodontal tissue when periodontal lesions are initiated.
3. The metabolic products produced by one group of bacteria may serve as energy source for other bacteria.



**Microoranisms associated With Various types of periodontal diseases**

**Criteria for the Identification of** **Periodontopathogens**

During the 1870s, robert koch developed the classic criteria  By which a microorganism can be judged to be a causative agent  in human infections.

These criteria, known as*Koch's postulates,*

1. Be routinely isolated from diseased individuals

2. Be grown in pure culture in the laboratory

3. Produce a similar disease when inoculated into susceptible laboratory animals

4. Be recovered from lesions in a diseased laboration the case of periodontitis and if the ecologic plaque hypothesis   proves correct, it impossible to fulfill Koch’s postulates, because no single organism or group of organisms is responsible for all cases of disease. The periodontal pathogen that fulfilled *Koch's postulates* is

Aggregatibacter actinomycetemcomitans in the form of localized aggressive periodontitis

**There are three primary problems:**

(1) The inability to culture all of the organisms that have been  associated  with disease (e.g., many of the oral spirochetes);

(2) The difficulties inherent in defining and culturing sites of active disease ;and

 (3) The lack of a good animal model system for the study of periodontitis.

 It must be inherently impossible to fulfill Koch’s postulates, because

no single organism or group of organisms is responsible for all cases of disease.

Criteria of periodontal pathogens proposed by Sigmund Socransky

1. Be associated with disease, as evidenced by increases in the number of

organisms at diseased sites.

* 2. Be eliminated or decreased in sites that demonstrate the clinical resolution of disease with treatment.
* 3. Humoral immune response.
* 4. Be capable of causing disease in experimental animal models.
* 5. Produce demonstrable virulence factors that are responsible for enabling the microorganism to cause the destruction of the periodontal tissues

The two periodontal pathogens that have most thoroughly fulfilled Socransky s criteria are Aggregatibacter actinomycetemcomitans in the form of localized aggressive periodontitis and Perphynomonas gingivalis in chronic periodontitis.