Biofilm—Plaque Formation on Tooth and Root Surfaces

The oropharynx is an open ecosystem wherein bacteria are always present; bacteria attempt to colonize in all favorable locations. Most bacteria, however, can only persist after the formation of a biofilm upon desquamation-free surfaces, i.e., hard substances (tooth and root surfaces, restorative materials, implants, prostheses etc.). In the presence of healthy dental and gingival relationships, there is a balance between the additive and retentive mechanisms of biofilms vis-à -vis the abrasive forces that tend to reduce biofilm formation, e.g., self-cleansing by the cheeks and tongue, diet and mechanical oral hygiene measures.

The existence of a biofilm results within a matter of hours or days, in the phases described below (Darveau et al. 1997, Descouts & Aronsson 1999, Costerton et al. 1999).

The establishment and stabilization of bacteria within a biofilm are important not only for the etiology of periodontitis, but also for adjunctive systemic and topical medicinal treatment for periodontitis (p. 287): Biofilm bacteria imbedded within a matrix of extracellular polysaccharides are more than 1,000 times less sensitive to antimicrobials (e.g., antibiotics) than free-floating ("planktonic") bacteria.

44 Dental Plaque—Development

Within minutes after completely cleansing the tooth surface, a *pellicle* forms from proteins and glycoproteins in saliva.

A *Association*: Through purely physical forces, bacteria associate loosely with the pellicle.

B *Adhesion*: Because they possess special surface molecules (adhesins) that bind to pellicle receptors, some bacteria become the "primary colonizers," particularly streptococci and actinomyces. Subsequently, other microorganisms adhere to the primary colonizers.

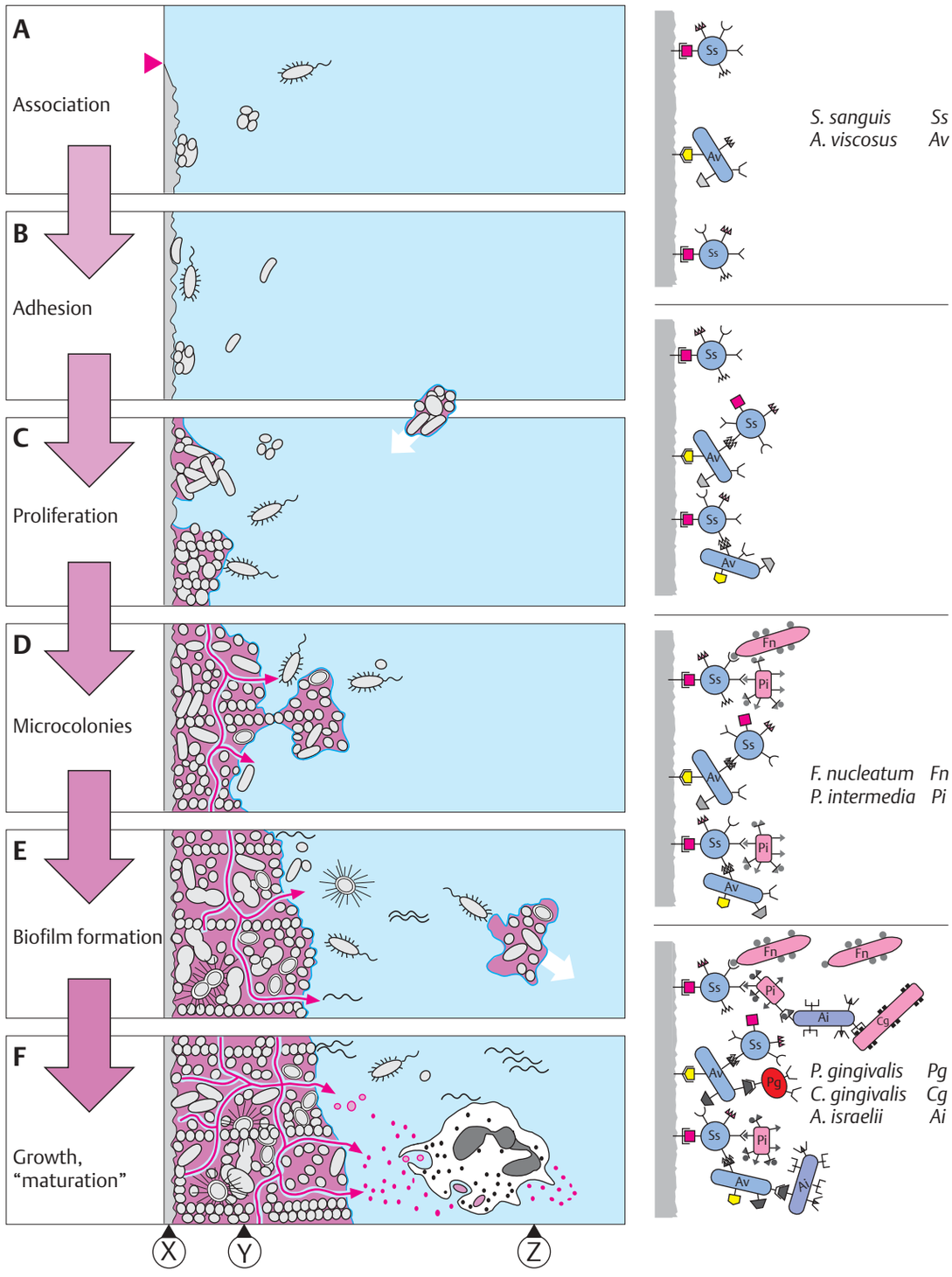
C Bacterial *proliferation* ensues.

D *Microcolonies* are formed. Many streptococci secrete protective extracellular polysaccharides (e.g., dextrans, levans).

E *Biofilm ("attached plaque")*: Microcolonies form complex groups with metabolic advantages for the constituents.

F *Plaque growth—maturation*: The biofilm is characterized by a primitive "circulatory system." The plaque begins to "behave" as a *complex organism*! Anaerobic organisms increase. Metabolic products and evulsed cell wall constituents (e.g., lipopolysaccharides, vesicles) serve to activate the host immune response (p. 38). Bacteria within the biofilm are protected from phagocytic cells (PMN) and against exogenous bacteriocidal agents.

X Pellicle
Y Biofilm—"Attached Plaque"
Z Planktonic Phase

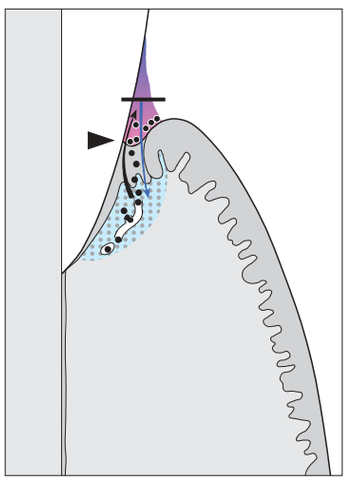


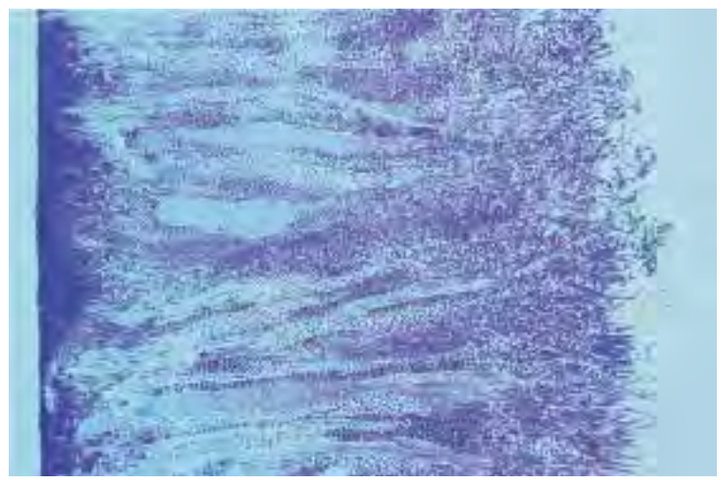
Supragingival Plague

... and its Initial Subgingival Expansion

The first bacteria that accumulate *supragingivally* on the tooth surface are mostly gram-positive (*Streptococcus* sp, *Actinomyces* sp.). In the course of the following days, gram-negative cocci as well as gram-positive and gram-negative rods and the first filamentous forms begin to colonize (Listgarten et al. 1975, Listgarten 1976). By means of a variety of *metabolic products*, the bacterial flora provoke the tissue to increased exudation and migration of PMN leukocytes into the sulcus ("leukocyte walls" against the bacteria).

The increase in PMN diapedesis and the flow of sulcus fluid lead to initial disintegration of the junctional epithelium. This makes it possible for bacteria to more easily invade between the tooth and the junctional epithelium, and invade the subgingival area (gingivitis, gingival pocket formation). In the total absence of oral hygiene, plaque formation and an initial host defensive response within gingival tissue occur. With optimum—including interdental—oral hygiene, the formation of biofilm is repeatedly disrupted and gingival health is maintained.

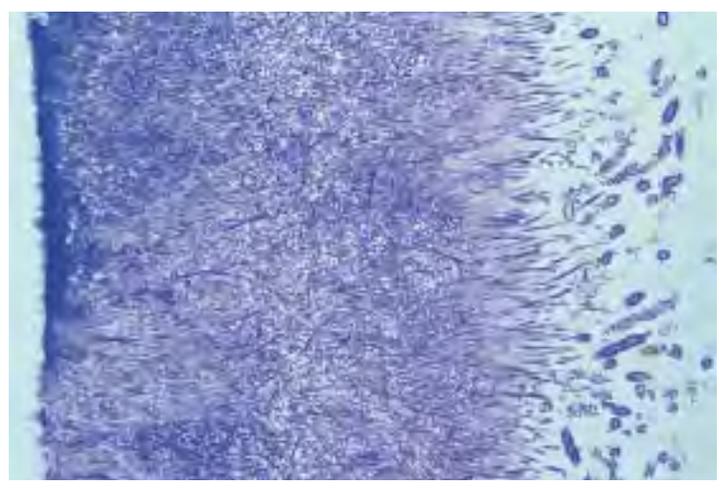




45 One-week-old Plaque—Interactions

Thick zone of early colonizers on the enamel surface and the column-like structures that result from rapid proliferation of streptococci. On the plaque surface, one observes rods and filaments.

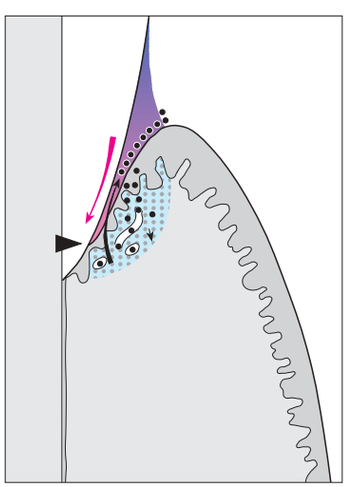
Left: Interaction between host and plaque. Chemotactically regulated immigration of polymorphonuclear granulocytes (PMN, arrow). The black horizontal line indicates the level from which this sample of plaque was taken.

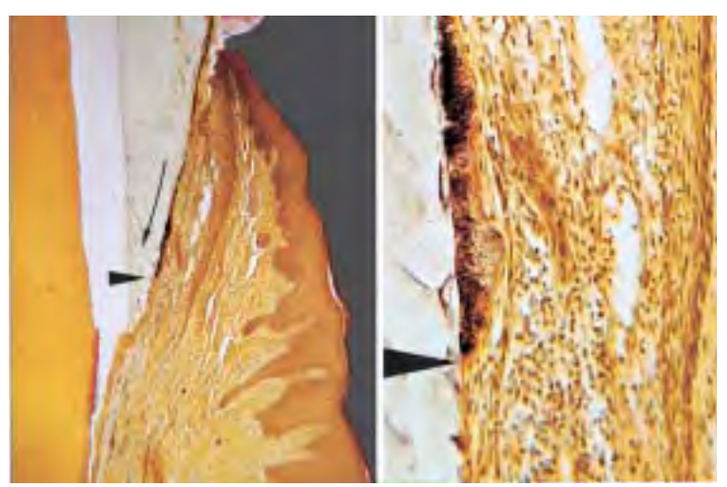


46 Three-week-old Plaque

The composition of the supragingival plaque has changed markedly. Filamentous organisms now predominate. Conspicuous forms resembling "corn cobs" are observed at the plaque surface.
Left: In this transmission electron photomicrograph, the structure of such a "corn cob" is revealed. At the center is a gram-negative filamentous organism (**F**), surrounded by gram-positive cocci (**C**).

Histology and TEM courtesy *M. Listgarten*





47 Expansion of Supragingival Plaque—Gingival Pocket

Middle and Right: Weakening of the epithelial attachment to the tooth permits apical migration of gram-positive plaque bacteria in a thin layer between the tooth and the junctional epithelium (thin arrow). Gram-negative bacteria colonize subsequently, and a *gingival* pocket forms (Fig. 150).
Histology courtesy *G. Cimasoni*

Left: Schematic representation of the interaction between plaque and host tissue.

Natural Factors Favoring Plaque Retention

The formation of a plaque biofilm can be enhanced by natural retention factors, which can also render biofilm removal by means of oral hygiene more difficult. These retention factors include:

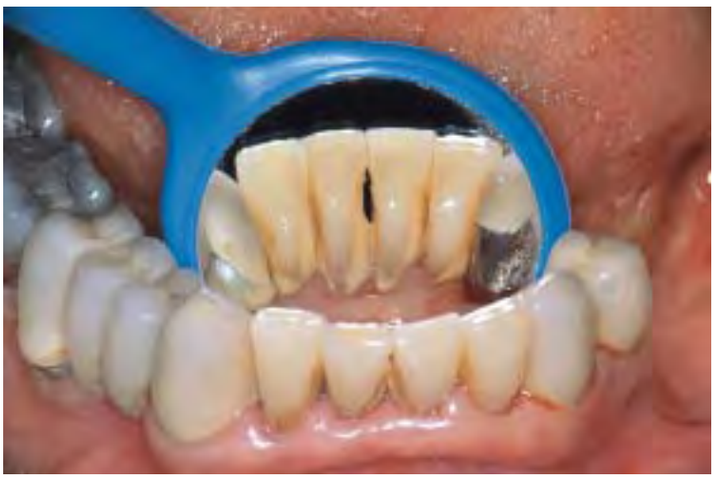
- Supra- and subgingival calculus
- Cementoenamel junctions and enamel projections
- Furcation entrances and irregularities
- Tooth fissures and grooves
- Cervical and root surface caries
- Crowding of teeth in the arch.

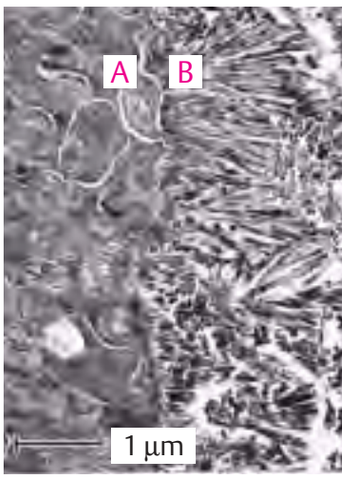
By itself, *calculus* is not pathogenic. However, its rough surface presents a retention area for vital, pathogenic bacteria. At the microscopic level, the *cementoenamel junction* is very irregular, and offers retentive roughness. Enamel projections and "pearls" also inhibit soft tissue attachment. *Furcation entrances, fissures,* etc. are retentive niches for plaque. *Carious lesions* represent a huge bacterial reservoir. *Crowding of teeth* reduces self-cleansing and renders oral hygiene more difficult.

48 Supragingival Calculus
Lingual surfaces of mandibular incisors and buccal surfaces of maxillary molars near the orifices of salivary ducts often exhibit massive accumulations of supragingival calculus.

Right: TEM of old supragingival calculus. Calcified plaque (**A**) close to the tooth surface. Note the accumulation of cell-free hexagonal monocrystals (**B**) upon the calcified plaque.

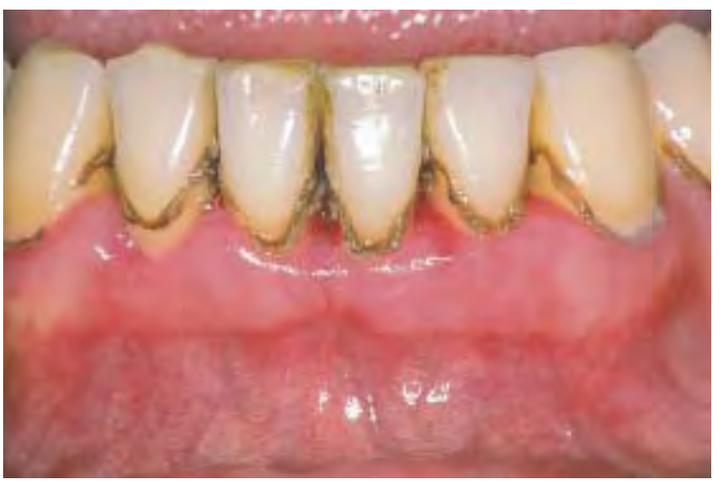
Courtesy *H. Schroeder*

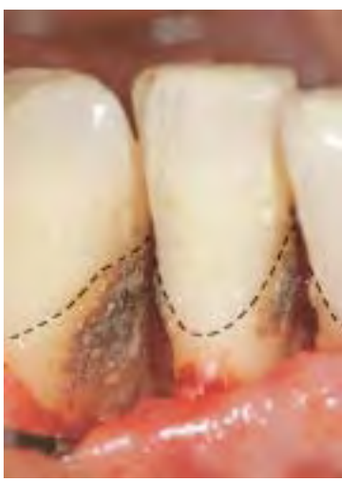




49 Subgingival Calculus
In this patient with long-standing periodontitis, the gingiva has receded. Calculus that was formerly subgingival is now supragingival.

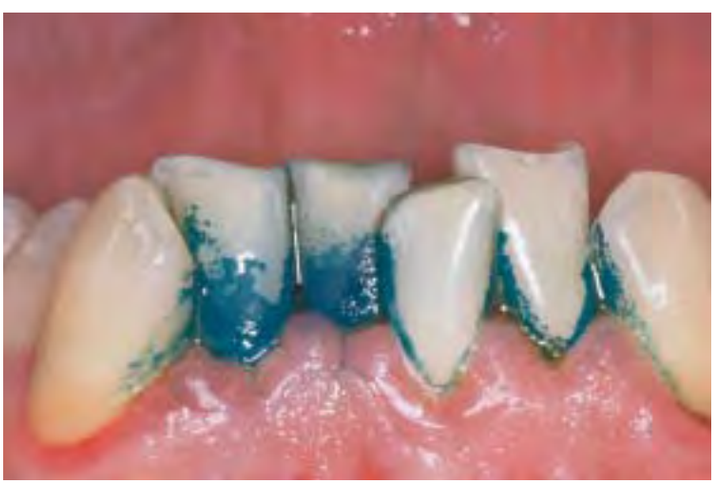
Right: Subgingival calculus is observed clinically after reflecting the gingival margin. Subgingival calculus is usually dark in color (Fe minerals) and harder than the more loosely structured supragingival calculus (calcium phosphates). The cementoenamel junction is indicated by the dashed line.

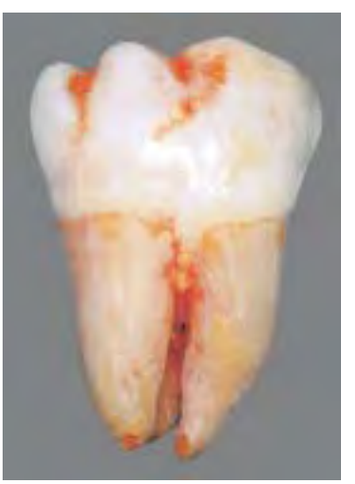




50 Crowding, Enamel Projection (Enamel "Pearl")
The lingually displaced mandibular incisors do not benefit from the natural self-cleansing action of the lower lip. Oral hygiene is also rendered more difficult.

Right: The furcation on this molar is filled by a projection of enamel that ends in a bulbous pearl, extending into the interradicular area. When a pocket forms in such an area, plaque control is particularly difficult.





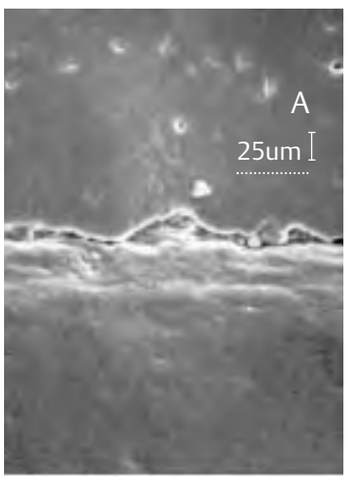
Iatrogenic Factors Favoring Plaque Retention

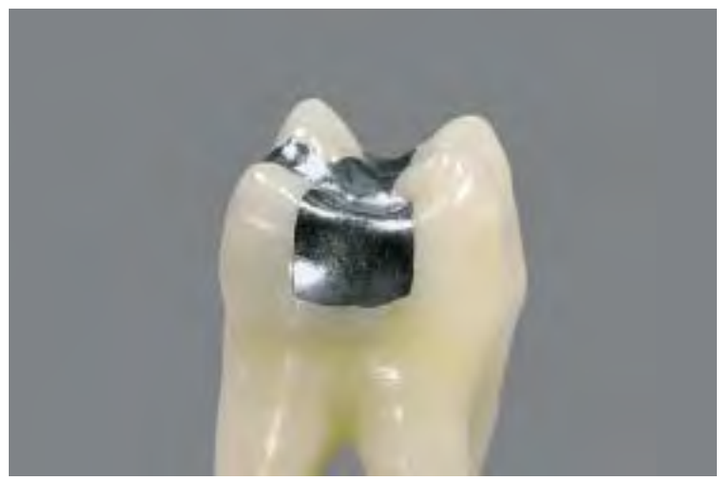
Restorative dentistry—from a simple restoration to a full-mouth reconstruction—can do more harm than good to the patient's oral health if performed improperly! Placing only optimum restorations is synonymous with preventive periodontics (tertiary prevention, p. 198).

Fillings and crowns that appear to be perfect clinically and macroscopically almost always exhibit deficiencies at the margins when viewed microscopically. When margins are located subgingivally, they always present an irritation for the marginal periodontal tissues.

Overhanging margins of restorations and crowns accumulate additional plaque. Gingivitis ensues. The composition of the plaque changes. The number of gram-negative anaerobes (e. g., *Porphyromonas gingivalis*), the organisms responsible for initiation and progression of periodontitis, increases rapidly (Lang et al. 1983).

Gross iatrogenic irritants such as poorly designed *clasps* and *prosthesis saddles* may exert a direct traumatic influence upon periodontal tissues.

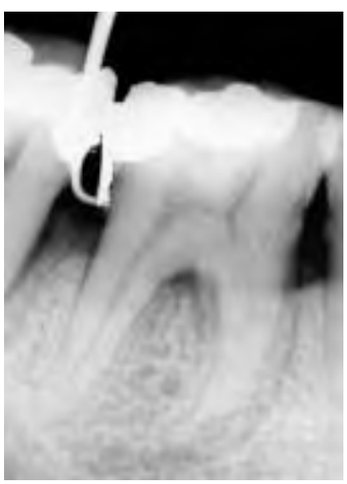


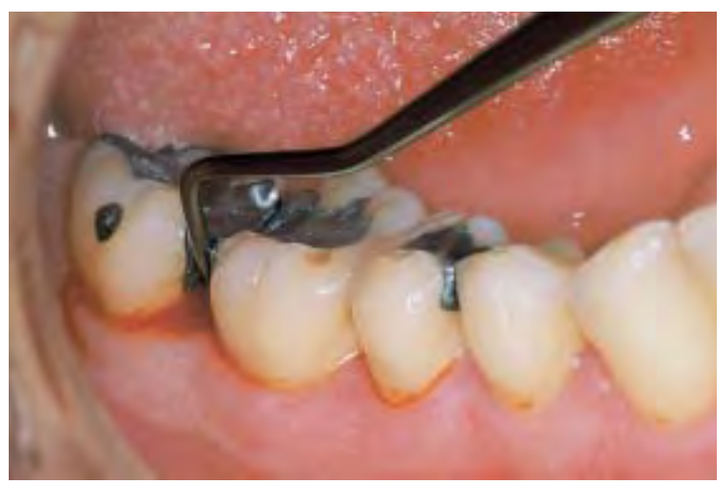


51 Amalgam Restoration—Clinical View and SEM

Left: Viewed in the scanning electron microscope, a clearly visible margin defect is observed. Such a defect is a perfect niche for the accumulation of plaque. The amalgam restoration (**A**) is at the top of this figure, the adjacent enamel below. The white dot under the 25 µm legend are representative of the size of coccoid microorganisms (ca. 1 µm).

Courtesy *F. Lutz*

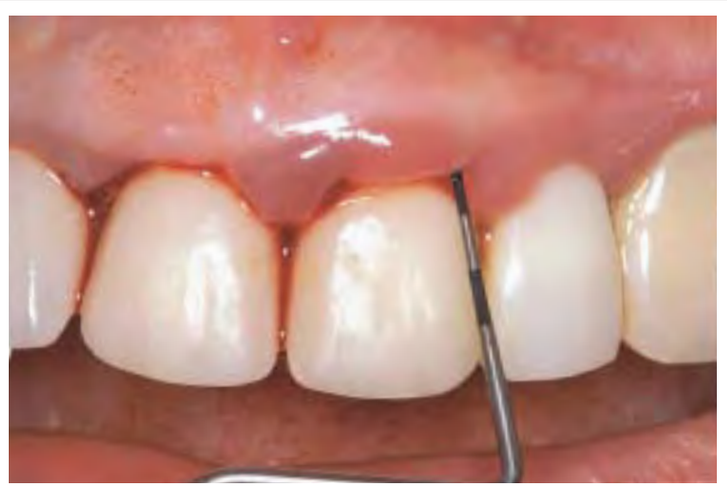




52 Amalgam—Proximal Overhang

Gross overhangs such as this, located subgingivally, invariably lead to plaque accumulation and to gingivitis (note hemorrhage). Pathogenic, gram-negative anaerobes are frequently observed. In contrast to amalgam and especially gold restorations, composite resin restorations are particularly retentive of bacteria.

Left: Radiograph of the same case.



53 Crown Margin Overhang and Open Margins

The cement that was used to cement this crown has begun to extrude from the open margin. The massive retention of plaque between the crown and the prepared tooth leads to severe gingivitis and establishment of a pathogenic bacterial flora.

Left: Section through a porcelain-fused-to-metal crown with a margin that is both overhanging (arrows) and open. Calculus (**C**) and plaque have accumulated apically.

Subgingival Plaque

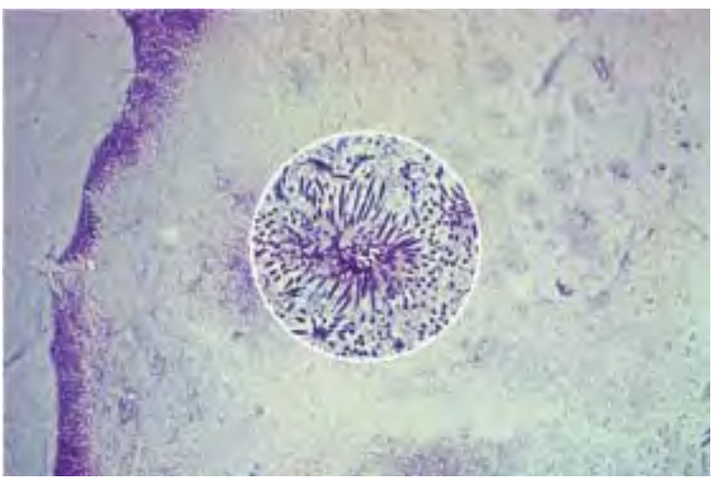
Extending apically from the supragingival region, a subgingival plaque biofilm will often form within the existing gingival sulcus/pocket; this was previously called the "adherent" plaque. In addition to gram-positive bacteria such as streptococci, actinomyces, etc., as the probing depth increases so does the number of anaerobic *gram-negative* bacteria (p. 36).

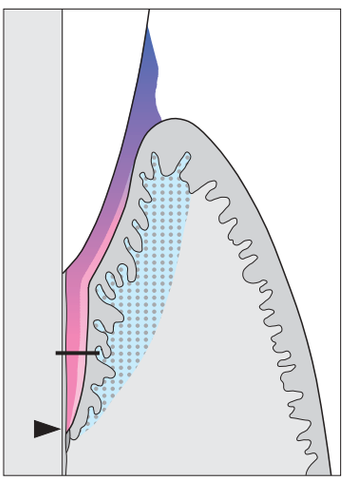
This subgingival biofilm can also calcify. A dark, hard and difficult to remove calculus ("serum calculus") accumulates. In addition, the gingival pocket also contains loose agglomerates of non-adherent, often mobile bacteria (with a high concentration of gram-negative anaerobes and spirochetes). In acute phases, *periodontopathic* bacteria often increase dramatically. These include *Actinobacillus actinomycetemcomitans, P. gingivalis, T. forsythia,* spirochetes etc. (pp. 30, 33, 38). Despite these alterations in the subgingival plaque, periodontitis, even in the acute stage, cannot be characterized as a "highly specific" infection because large differences have been reported in the bacterial composition between patients and even within different pocket locations in the same patient (Dzink et al. 1988, Slots & Taubmann 1992, Lindhe 1997).

54 Subgingival Pocket Flora
This is a relatively thin adherent biofilm (blue-violet). One observes loose accumulations of gram-negative, anaerobic, and also motile bacteria. Formations resembling test tube brushes, consisting of filamentous bacteria are also observed (inset).

Histology courtesy *M. Listgarten*

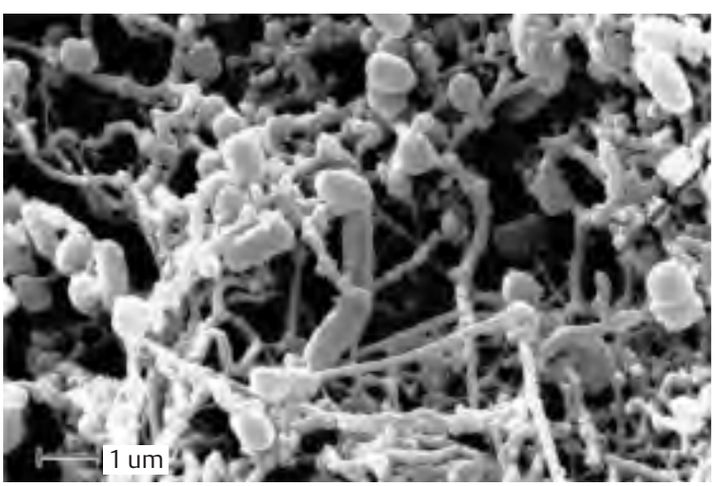
Right: As pocket depth increases (arrow), the resident flora becomes increasingly gram-negative and anaerobic.





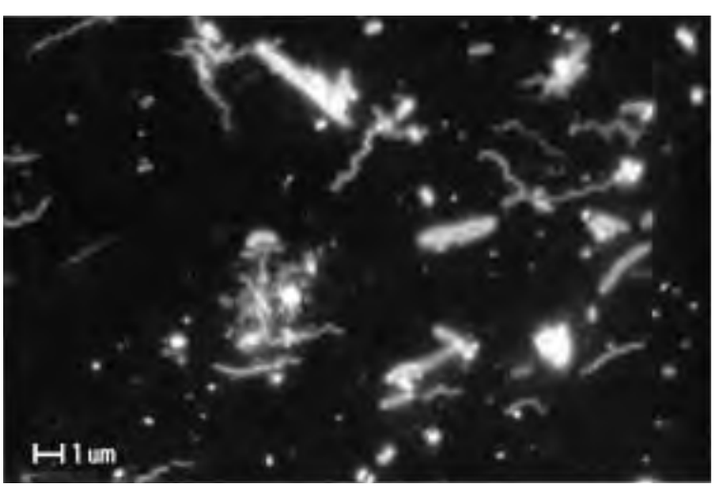
55 Surface of the Biofilm on the Root
Within a pocket, the root surface of a tooth manifesting periodontitis is covered with a densely intertwined bacterial colonization composed of many different bacterial morphotypes (scanning electron photomicrograph).

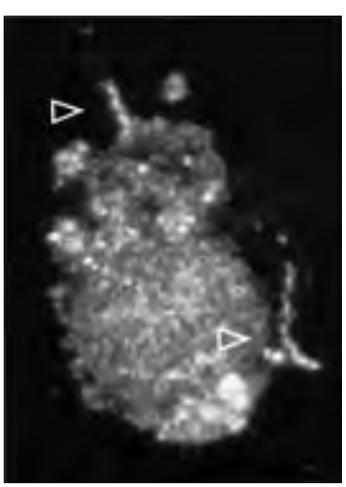
The *morphology* of the bacteria permits neither a determination of the species nor any clues concerning pathogenicity.



56 Microorganisms of the Non-adherent Plaque—"Planktonic" Phase
In a dark-field preparation, motile rods and small to larger spirochetes predominate, while cocci and filaments are rare: Typical signs of an active pocket (exacerbation; cf. p. 63).
Right: Intact phagocytes (PMN) in the pocket exudate do not lose their capacity for phagocytosis. The arrow depicts a spirochete being engulfed.

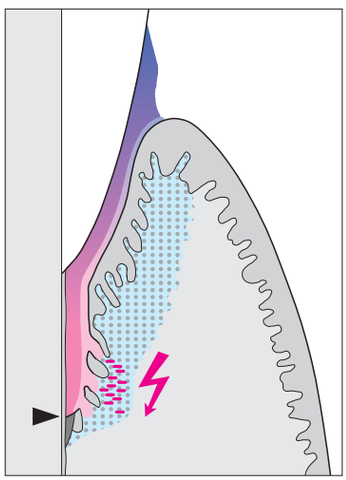
Courtesy *B. Guggenheim*

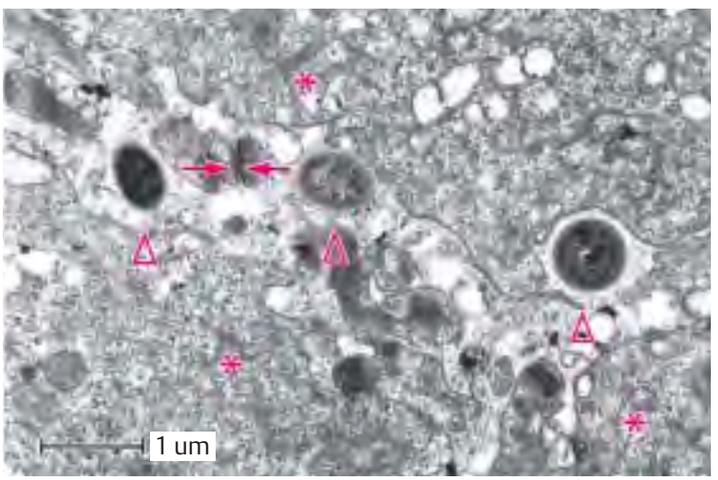




Bacterial Invasion Into Tissue?

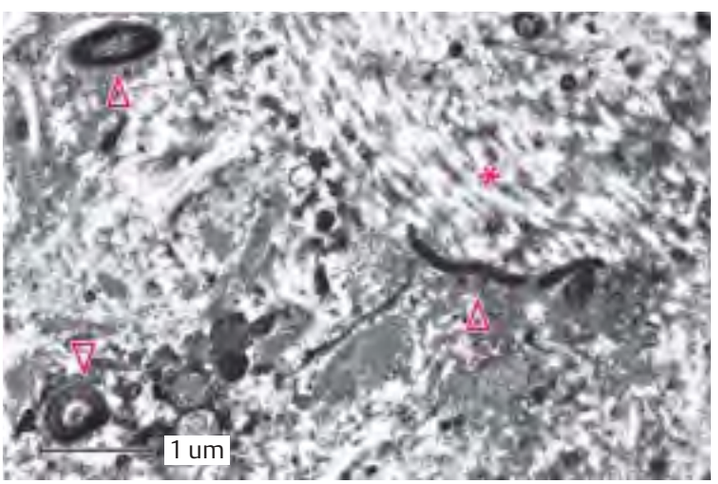
In patients with a compromised immune response, for example in cases of early onset, aggressive periodontitis (p. 96), bacteria with pathogenic potential have the ability to invade the cells of the pocket epithelium and the subepithelial connective tissue, and to remain viable there for varying periods of time. This usually occurs only in the depth of the pockets where the bacteria can avoid the infiltrate (defense), which is usually located close to the gingival margin. The periodontopathic bacteria (p. 33) produce virulence factors (leukotoxins from *Actinobacillus actinomycetemcomitans,* lipopolysaccharides and enzymes), which can initially shut down the chemotactic guidance of defense cells (e. g., PMN) or even kill PMNs. With time, the invading microorganisms are recognized by the activated immune system and killed. If tissue invasion is mild, small areas of necrotic tissue resorption may result, but if the bacterial invasion is massive, acute suppurative abscesses can result (Allenspach-Petrzilka & Guggenheim 1983, Christensson et al. 1987, Frank 1988, Saglie et al. 1988, Slots 1999, Van Winkelhoff & Slots 1999). It is unclear whether oral microorganisms that invade tissue cells actually *colonize*, or whether the individual microorganisms simply invade.





57 Bacteria within the Pocket Epithelium
Bacteria (red triangles) in the widened intercellular spaces of the pocket epithelium. Three epithelial cells (✻) and one desmosome (double arrow) are observed.
The exudate and PMNs have significantly widened the intercellular spaces between the JE cells.

Left: In the depth of the active pocket, one observes ulcerated epithelium, through which bacteria may invade the connective tissue (red bars).



58 Bacterial Invasion—Infection
Bacteria of various species are observed within the connective tissue (arrows) adjacent to a deep periodontal pocket. Tissue damage (✻ = degraded collagen) may result, or tissue may remain completely healthy in appearance.

Left: A gram-negative bacterium (**G**B) is observed in the midst of otherwise essentially intact collagen fibrils

59 Necrosis—Suppuration
Almost the entire photomicrograph is filled by a dead phagocytic cell (PMN, ✻). The cell contains phagolysosomes, some of which exhibit digested material (arrow).
The dead phagocyte is surrounded by dead bacteria and bacterial cell walls. This pus must either be resorbed by the host tissue, or expelled (abscess, fistula).

TEMs courtesy *B. Guggenheim*

Classification of Oral Microorganisms

Thanks to new laboratory techniques (e. g., 16S rRNA analyses), over 500 species and subspecies have been isolated and classified from subgingival and supragingival bacterial samples (Slots & Taubman 1992, Moore & Moore 1994, Socransky et al. 1999). Some of the cultivable species are listed below.

Today, only about a dozen microorganisms are classified as *periodontal pathogens.* Foremost among these are gram-negative organisms, including *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia* (formerly *Bacteroides forsythus)* and *Prevotella intermedia* (p. 33).

Some of these bacteria possess significant biochemical capacities for the pathogenesis of inflammatory periodontal diseases. For example, they are capable of colonizing root surfaces and cell surfaces, and therefore maintain a secure position in the micro-ecological cosmos of the pocket flora. The microorganisms are usually capable of co-aggregation, i. e., they aggregate with one or more other types of bacteria to form a so-called complex or "cluster" (Socransky et al. 1998, 1999). Such complexes have been characterized as highly pathogenic or only slightly pathogenic.

60 Microorganisms in the Plaque Biofilm and in the Non-adherent Planktonic Phase

Prokaryotes	Gram ⊕ positive		Gram ⊖ negative	
	Facultative anaerobes	**Obligate anaerobes**	**Facultative anaerobes**	**Obligate anaerobes**
Cocci ●	**Streptococcus** – *S. anginosus (S. milleri)* – *S. mutans* – *S. sanguis* • **Ss** – *S. oralis* – *S. mitis* – *S. intermedius*	**Peptostreptococcus** – *P. micros* • **Pm** **Peptococcus**	**Neisseria** **Branhamella**	**Veillonella** – *V. parvula*
Rods ▬	**Actinomyces** – *A.naeslundii* • **An** – *A. viscosus* • **Av** – *A. odontolyticus* – *A. israelii* **Propionibacterium** **Rothia** – *R. dentocariosa* **Lactobacillus** – *L. oris* – *L. acidophilus* – *L. salivarius* – *L. buccalis*	**Eubacterium** – *E. nodatum* • **En** – *E. saburreum* – *E. timidum* – *E. brachy* – *E. alactolyticum* **Bifidobacterium** – *B. dentium*	**Actinobacillus** – *A. actinomycetemcomitans* • **Aa** **Capnocytophaga** – *C. ochracea* – *C. gingivalis* – *C. sputigena* **Campylobacter** – *C. rectus* • **Cr** – *C. curvus* – *C. showae* **Eikenella** – *E. corrodens* • **Ec** **Haemophilus** – *H. aphrophilus* – *H. segnis*	Porphyromonas – *P. gingivalis* • **Pg** – *P. endodontalis* **Prevotella** – *P. intermedia* • **Pi** – *P. nigrescens* – *P. melaninogenica* – *P. denticola* – *P. loescheii* – *P. oris* – *P. oralis* **Bacteroides** – *T. forsythia* • **Tf** – *B. gracilis* **Fusobacterium** – *F. nucleatum* • **Fn** – *F. periodonticum* **Selenomonas** – *S. sputigena* – *S. noxia*
Spirochetes and mycoplasms ≈	**Mycoplasm** – *M. orale* – *M. salivarium* – *M. hominis*		**Spirochetes of ANUG** **Treponema sp.** – *T. denticola* • **Td** – *T. socranskii* – *T. pectinovorum* – *T. vincentii*	
Eukaryotes	**Candida** – *C. albicans*	**Entamoeba**		**Trichomonas**