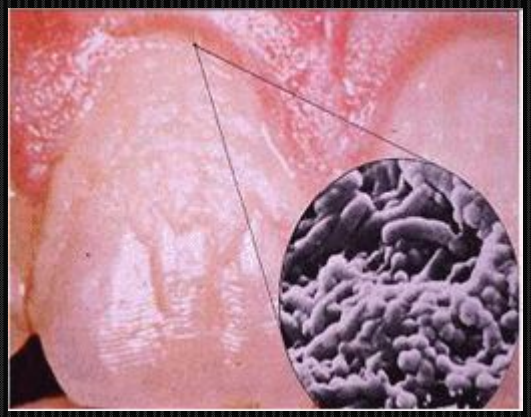

PERIODONTAL
MICROBIOLOGY
Dr. Nisha K.J

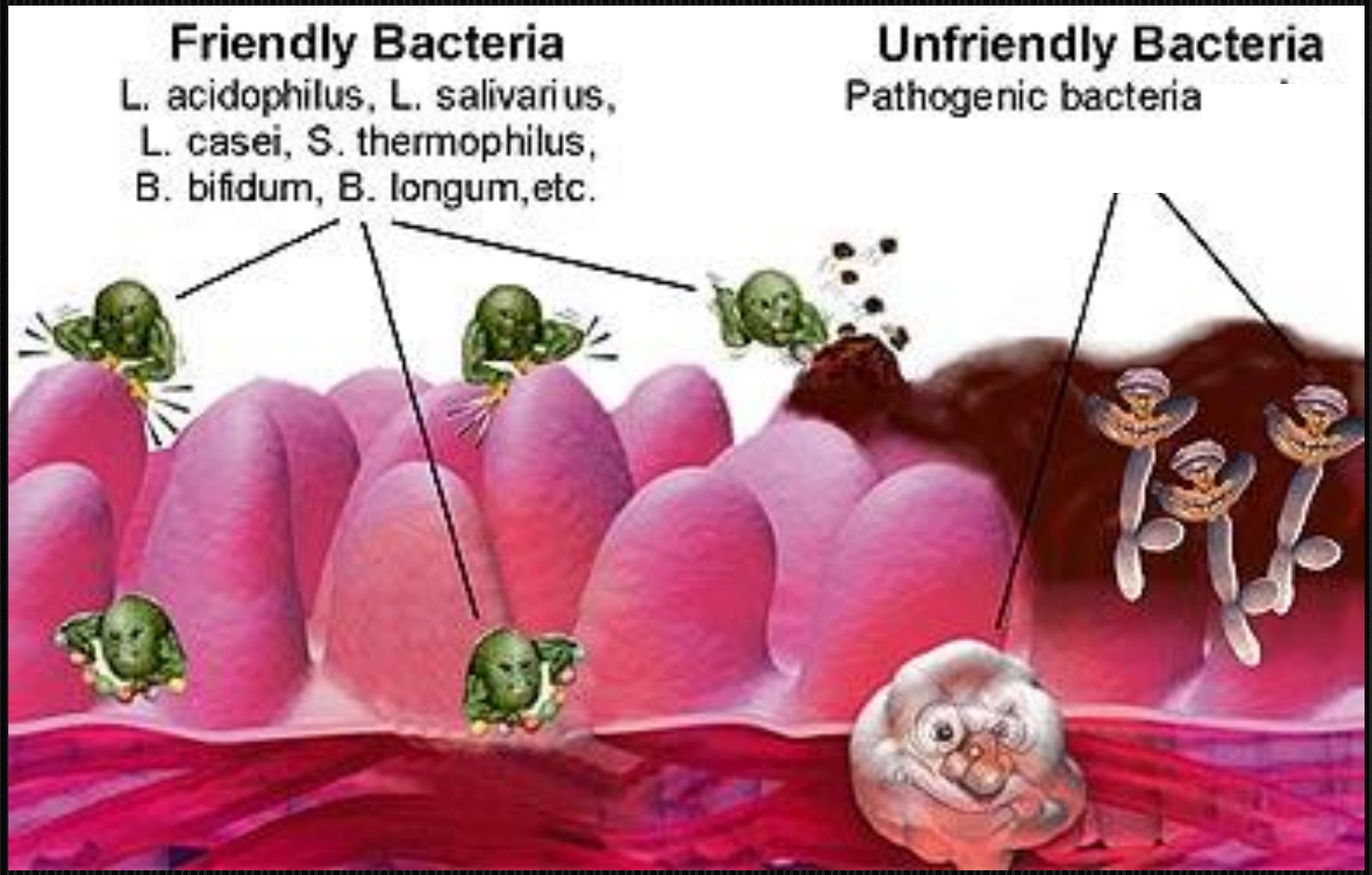
CONTENTS

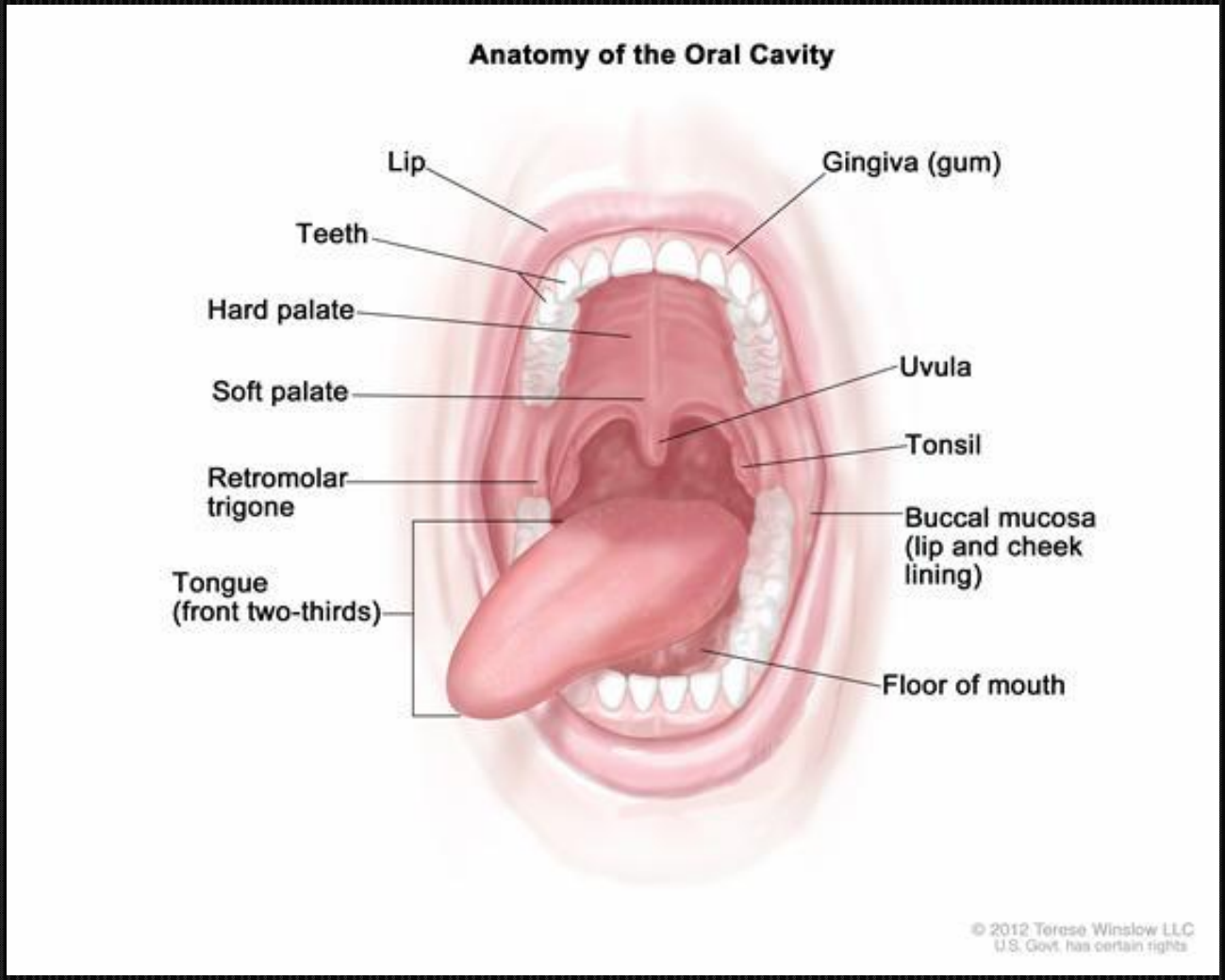
- Intrauterine – fetus sterile

  But after passing through the b i r t h canal, the fetus acquires vaginal and fecal microorganisms
- Colonization of oral cavity by microorganisms – at birth
- Within hours – mainly facultative and aerobic bacteria
- By 2nd day – anaerobic bacteria
- Within 2 weeks – nearly mature microbiota
- After weaning – complex collection of more than 500 different species



Commensal and pathogenic bacteria

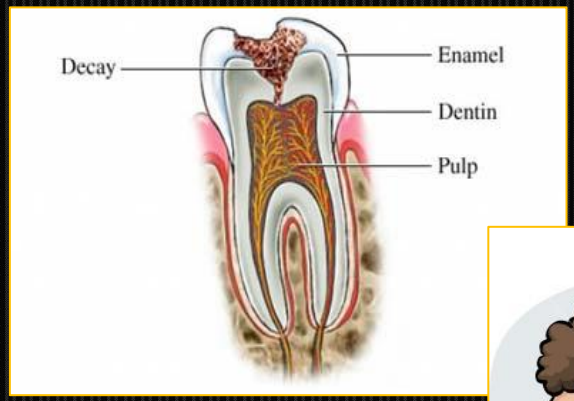


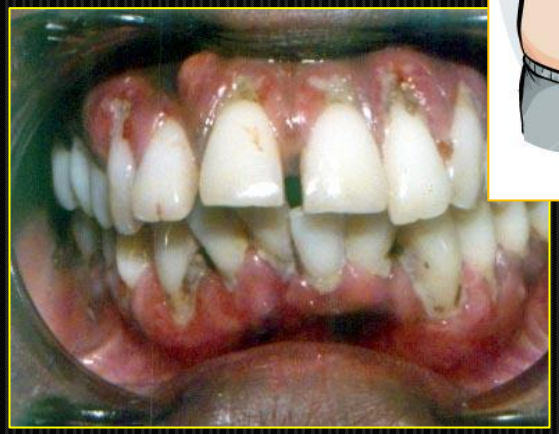

Anatomy of the Oral Cavity
Lip
Gingiva (gum)
Teeth
Hard palate
Uvula
Soft palate
Tonsil
Retromolar trigone
Buccal mucosa (lip and cheek lining)
Tongue (front two-thirds)
Floor of mouth
© 2012 Terese Winslow LLC
U.S. Govt. has certain rights

Teeth

- Unique
- Hard, non-shedding surface – allows the development of extensive structured bacterial deposits

Accumulation of bacteria





Soft deposits on teeth

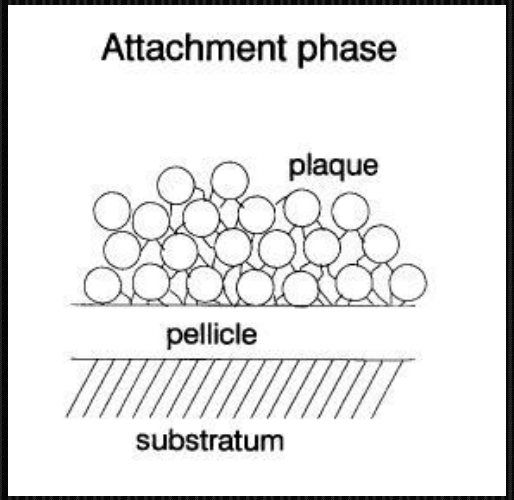
Acquired pellicle

Dental Plaque

Materia alba

Acquired pellicle

- Within nanoseconds after vigorously polishing teeth, the acquired pellicle covers the tooth surface!
- Saliva- derived layer
- Composed of glycoproteins from the saliva, other proteins, receptors for bacteria
- It's that "slick"feeling on your teeth
- Initial phase of plaque development

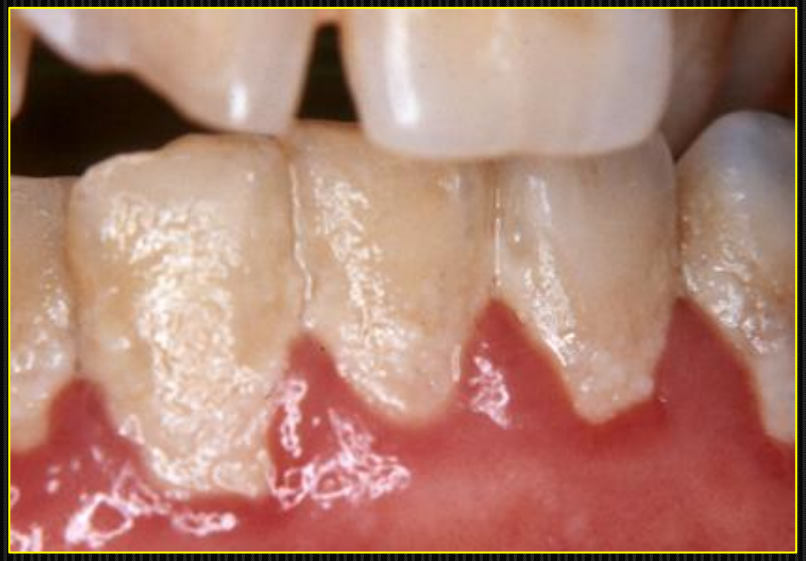


PLAQUE

W.H.O DEFINITION

'A specific but highly variable structural entity resulting from the growth and colonization of microorganisms on the surface of teeth and restorations, consisting of numerous microbial species and strains embedded in an extracellular matrix'

WHO [1978]



- Structured, resilient, Yellowish-grayish
- Adheres tenaciously to intra-oral hard surfaces
- Impossible to remove by rinsing or water spray

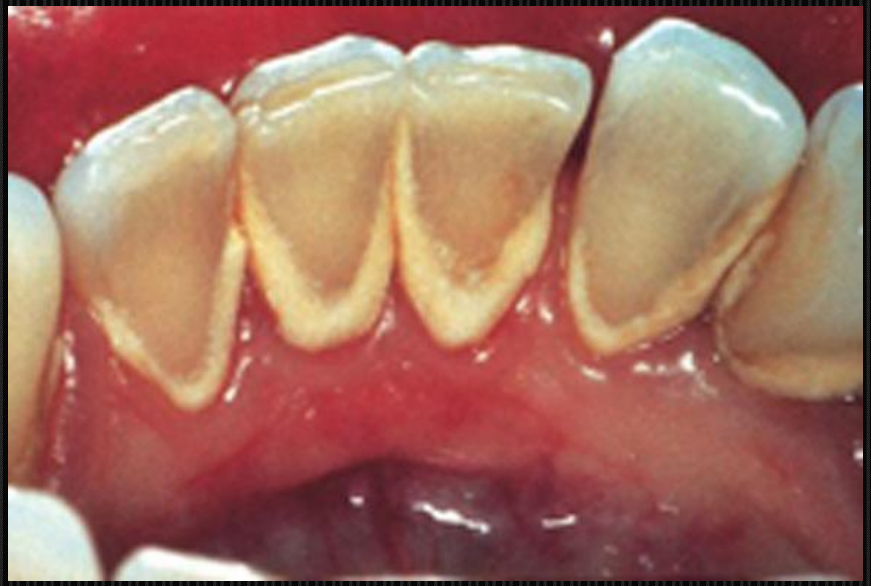
- Complex microbial community, with greater than 10^{11} bacteria per gram
- More than500 distinct bacterial species
- Contains bacterial cells, a small number of epithelial cells, leukocytes, and macrophages.
- The cells are contained within an extracellular matrix, which is formed from bacterial products and saliva.
- The extracellular matrix contains protein, polysaccharide, lipids and glycoproteins

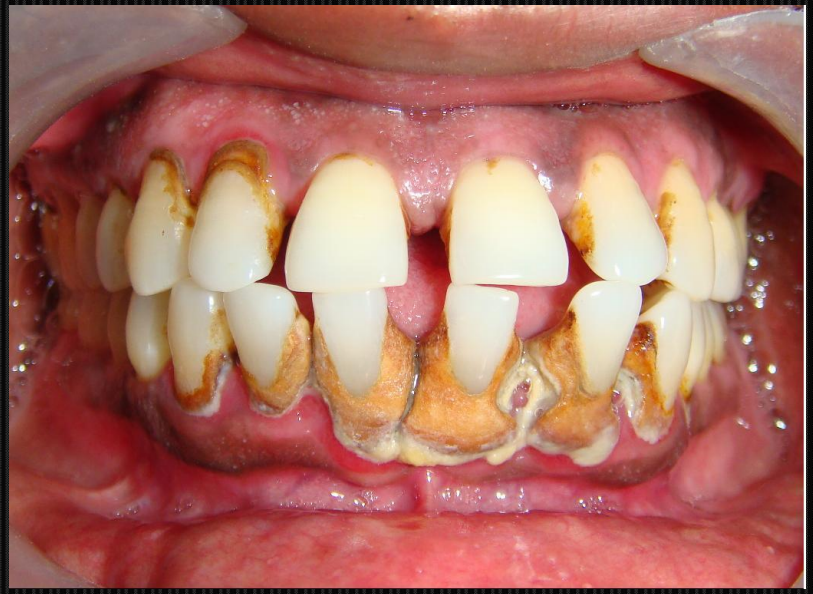
Materia alba

- Soft accumulations of food debris, dead cells, bacteria
- loosely adherent, grayish-white mass of oral debris and bacteria
- lack the organized structure of dental plaque
- Easily displaced with a water spray

Calculus

- Calculus is hard deposits that form by mineralization of dental plaque and is generally covered by a layer of unmineralised plaque.

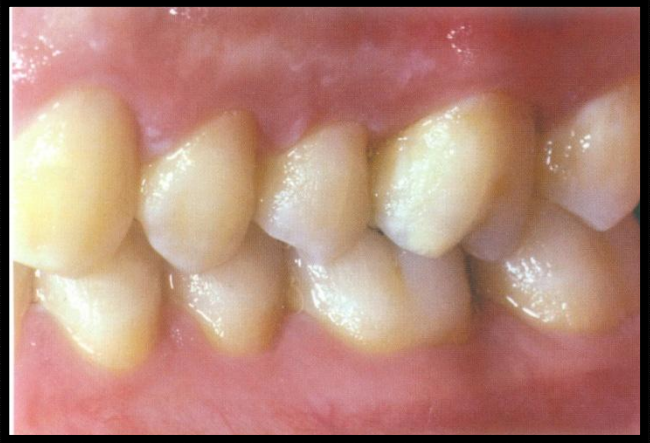


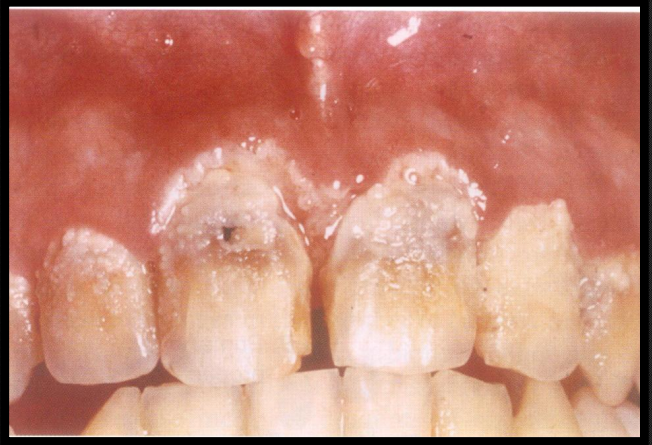


Detection of plaque

A] DIRECT VISION

- *Thin plaque translucent [not visible]*
- *May have extrinsic stains (yellow, green, tobacco)*
- *Thick plaque – dull, hazy colour [visible] - materia alba and food debris may collect over biofilm*

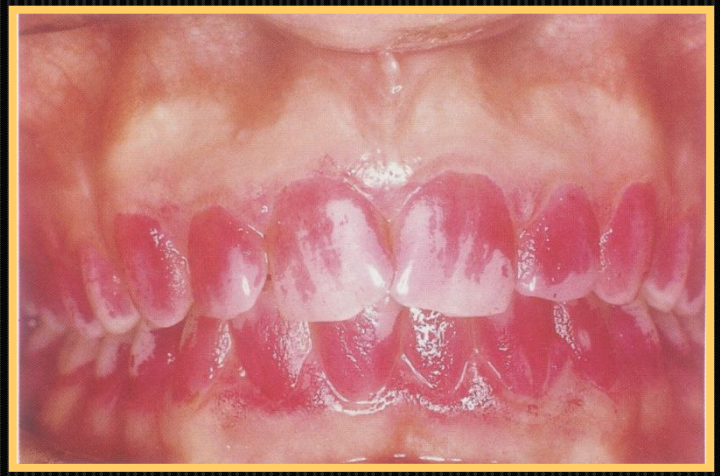




B] USE OF EXPLORER OR PROBE

- when not visible, it may be detected by passing the side of the tip of probe and if plaque present it will adhere to instrument

C] USE OF DISCLOSING AGENT



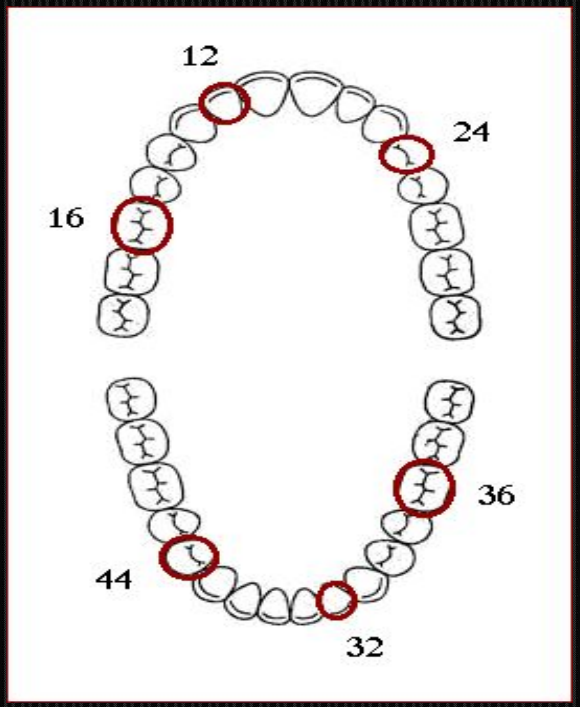
INDICES FOR RECORDING AND COMPARISON

- Plaque Index (PI) Silness and Loe
- Simplified Oral Hygiene Index (OHI-S) (Greene and Vermillion)
- Turesky Modification of Quigley-Hein Index

Plaque Index (PlI) - Silness and Loe in 1964

This index measures the thickness of plaque on the gingival one third.

The six index teeth are: 16, 12, 24, 36, 32, 44

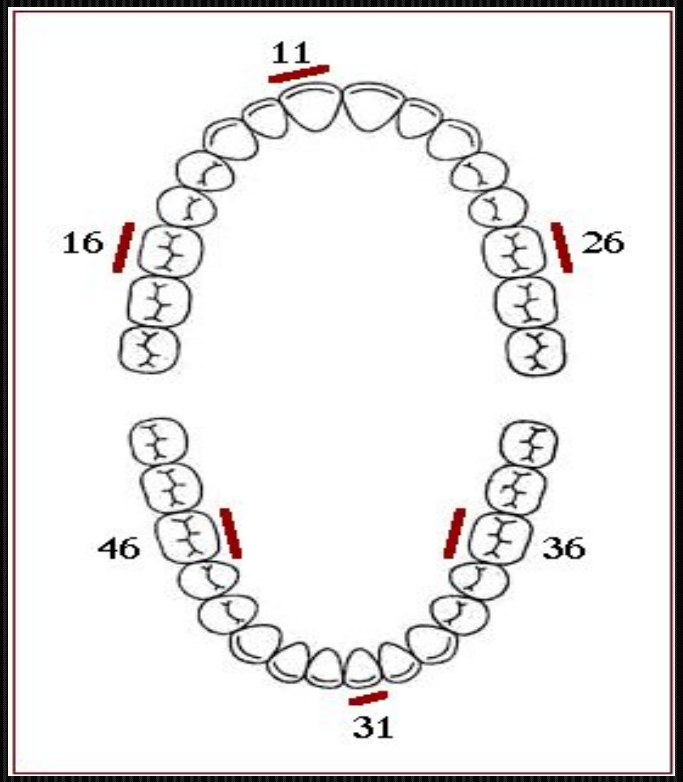


Score	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth, which cannot be seen with the naked eye. But only by using disclosing solution or by using probe.
2	Moderate accumulation of deposits within the gingival pocket, on the gingival margin and/ or adjacent tooth surface, which can be seen with the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

Calculation: PII = Total scores / No. of surfaces examined

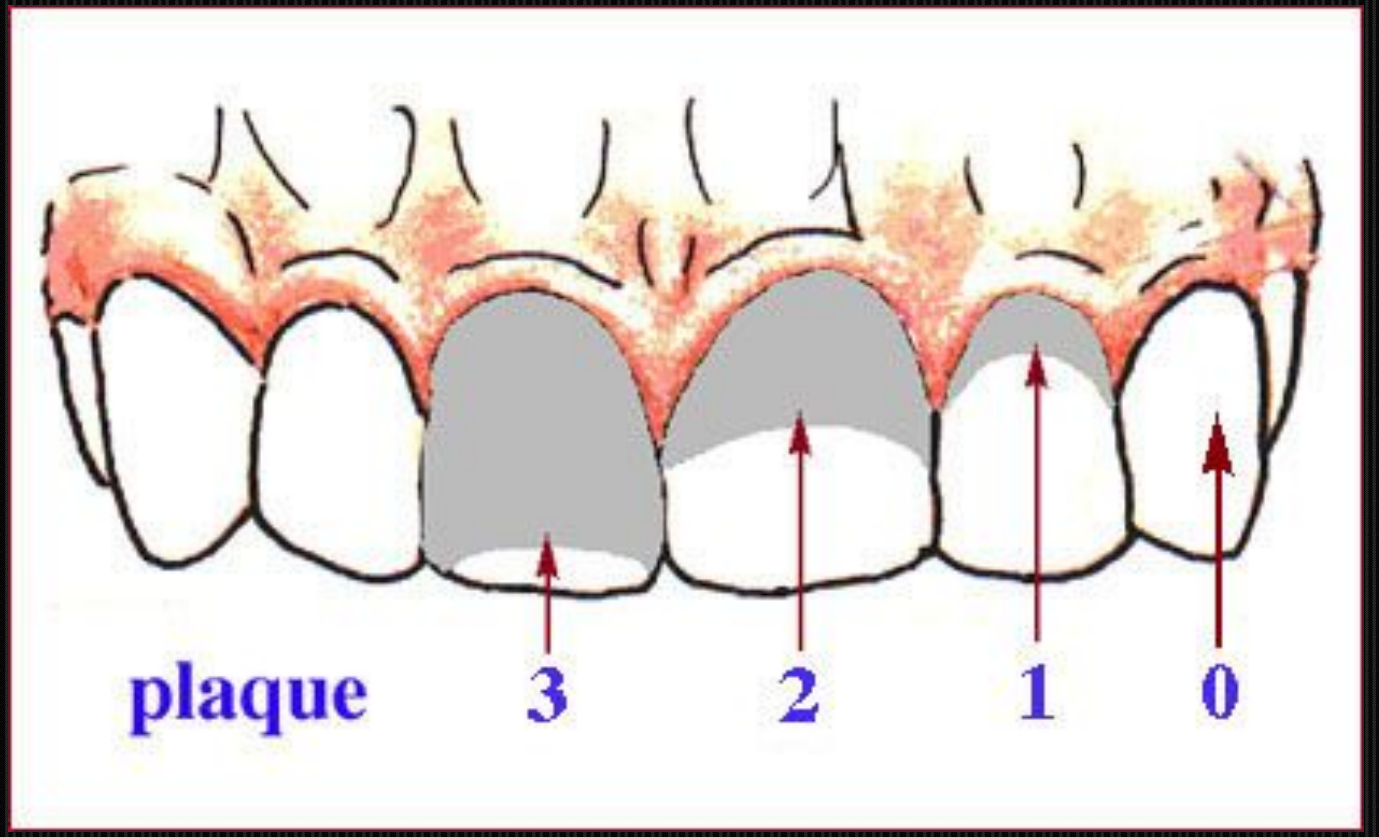
Simplified Oral Hygiene Index (OHI-S) (Greene and Vermillion, 1964)

- Two components - the Debris Index and the Calculus Index.



Criteria for classifying debris

Scores	Criteria
0	No debris or stain present
1	Soft debris covering not more than one third of the tooth surface, or presence of extrinsic stains without other debris regardless of surface area covered
2	Soft debris covering more than one third, but not more than two thirds, of the exposed tooth surface.
3	Soft debris covering more than two thirds of the exposed tooth surface.


plaque
3
2
1
0

Dental plaque

- *Composition*
- *Classification*
- *Structure*
- *Formation*

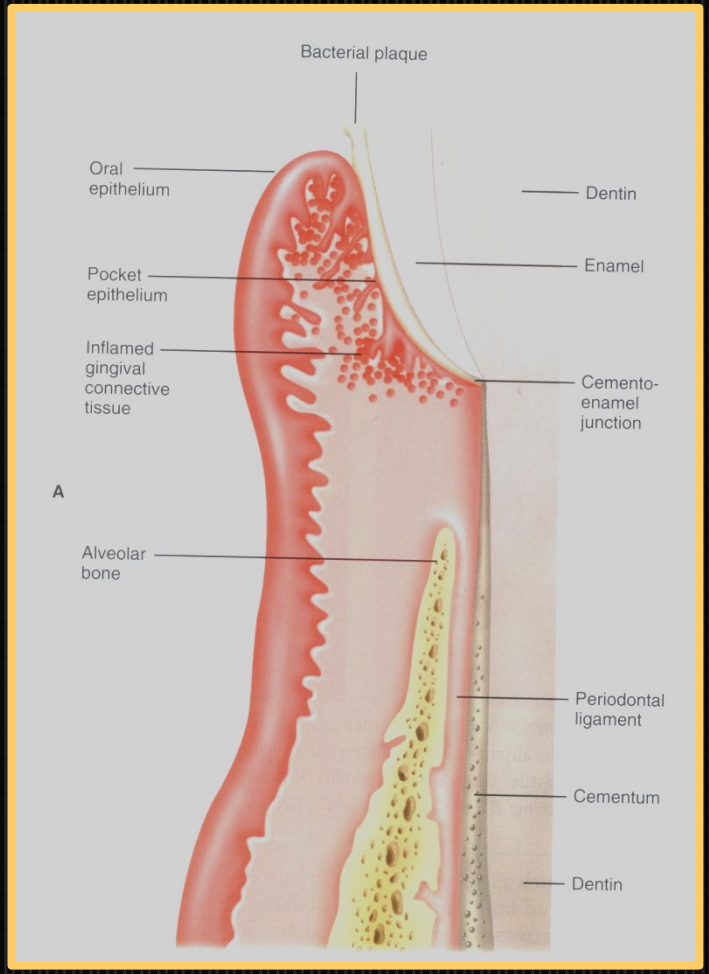
Plaque is primarily composed of bacteria in a matrix of salivary glycoproteins and extracellular polysaccharides.

COMPOSITION

SOLID 20 % **WATER 80 %**

70 -80 % - bacteria

20 -30 % - intercellular matrix



- ***Intercellular matrix***
  - *Organic*
    - *Carbohydrates – 30%*
    - *Proteins – 30%*
    - *Lipids – 15%*
  - *Inorganic*
    - *Major – Ca, PO_4*
    - *Minor - Mg, K,Na*

- *Bacteria*

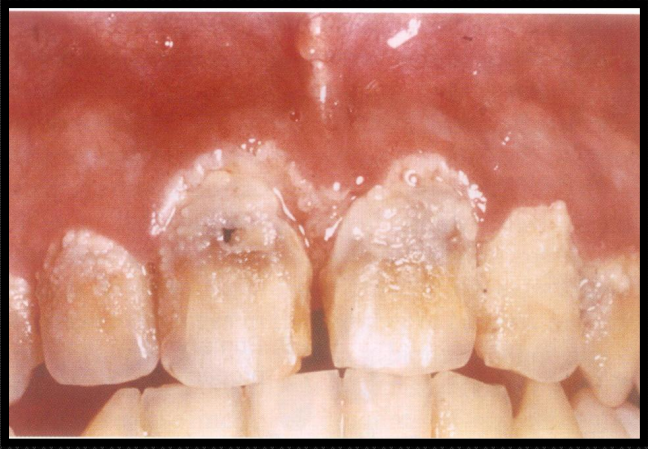
- *Organic substances*
- *-protein*
- *-polysaccharide*
- *-glycoprotein*
- *-lipids*

Classification

Based on relationship to gingival margin

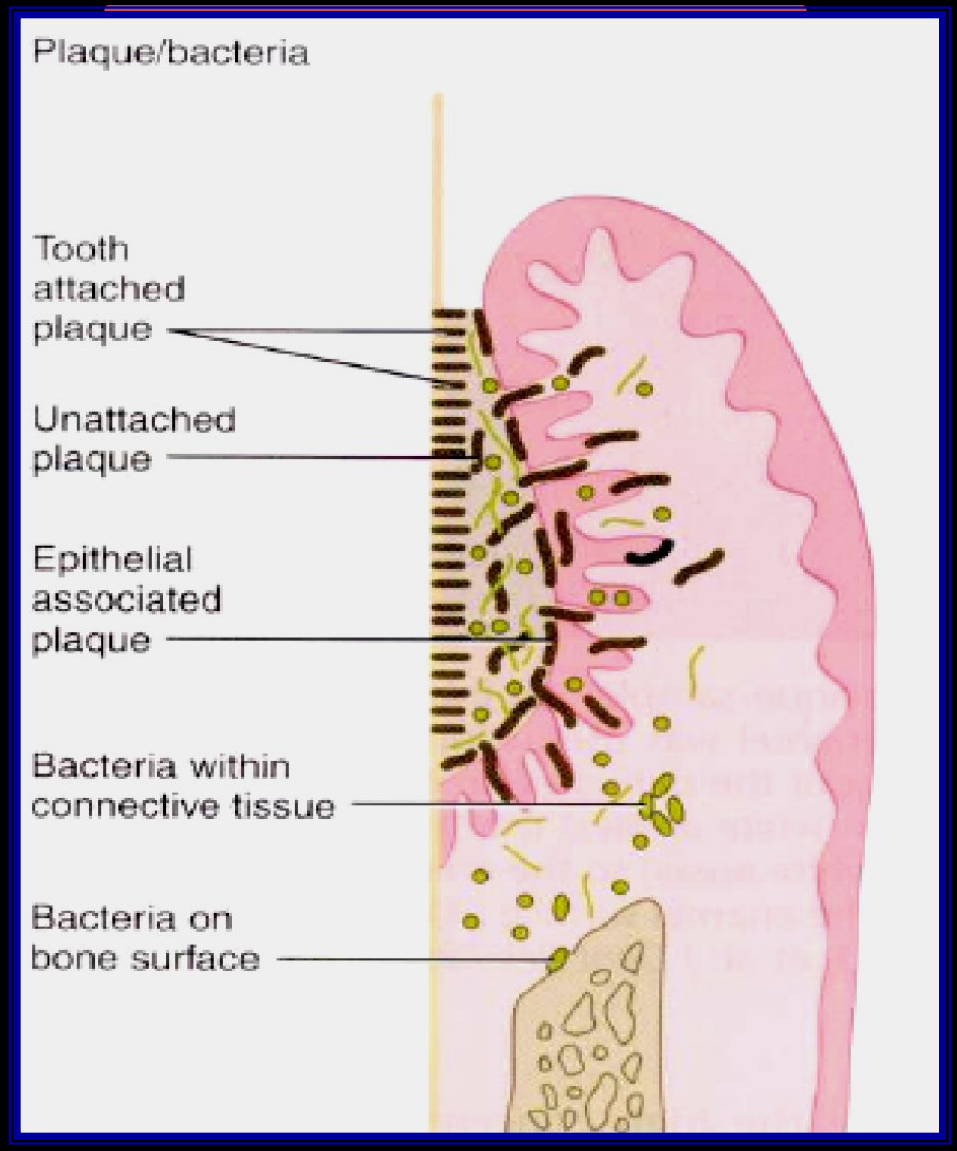
Supragingval plaque

Subgingival plaque

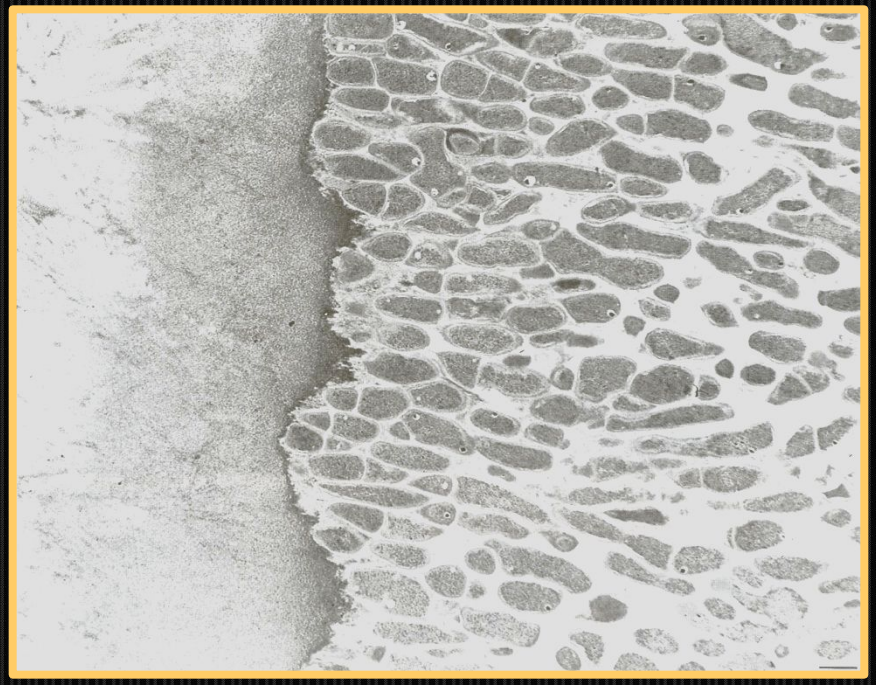


- ***Supragingival***
  - ***Coronal***
  - ***Marginal***

- ***Subgingival***
  - ***Tooth associated***
  - ***Unattached***
  - ***Epithelial associated***

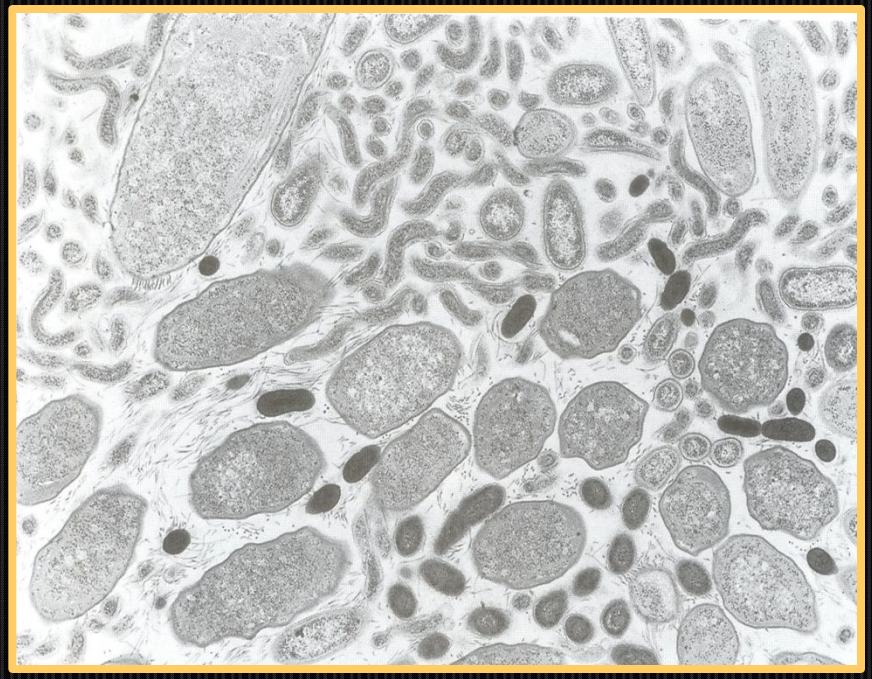

Plaque/bacteria
Tooth attached plaque
Unattached plaque
Epithelial associated plaque
Bacteria within connective tissue
Bacteria on bone surface

SUPRAGINGIVAL PLAQUE



Tooth surface: Stratified organisation of multilayered accumulation of Gm +ve cocci and short rods.
Outer surface: Gm –ve rods, filaments and spirochetes.

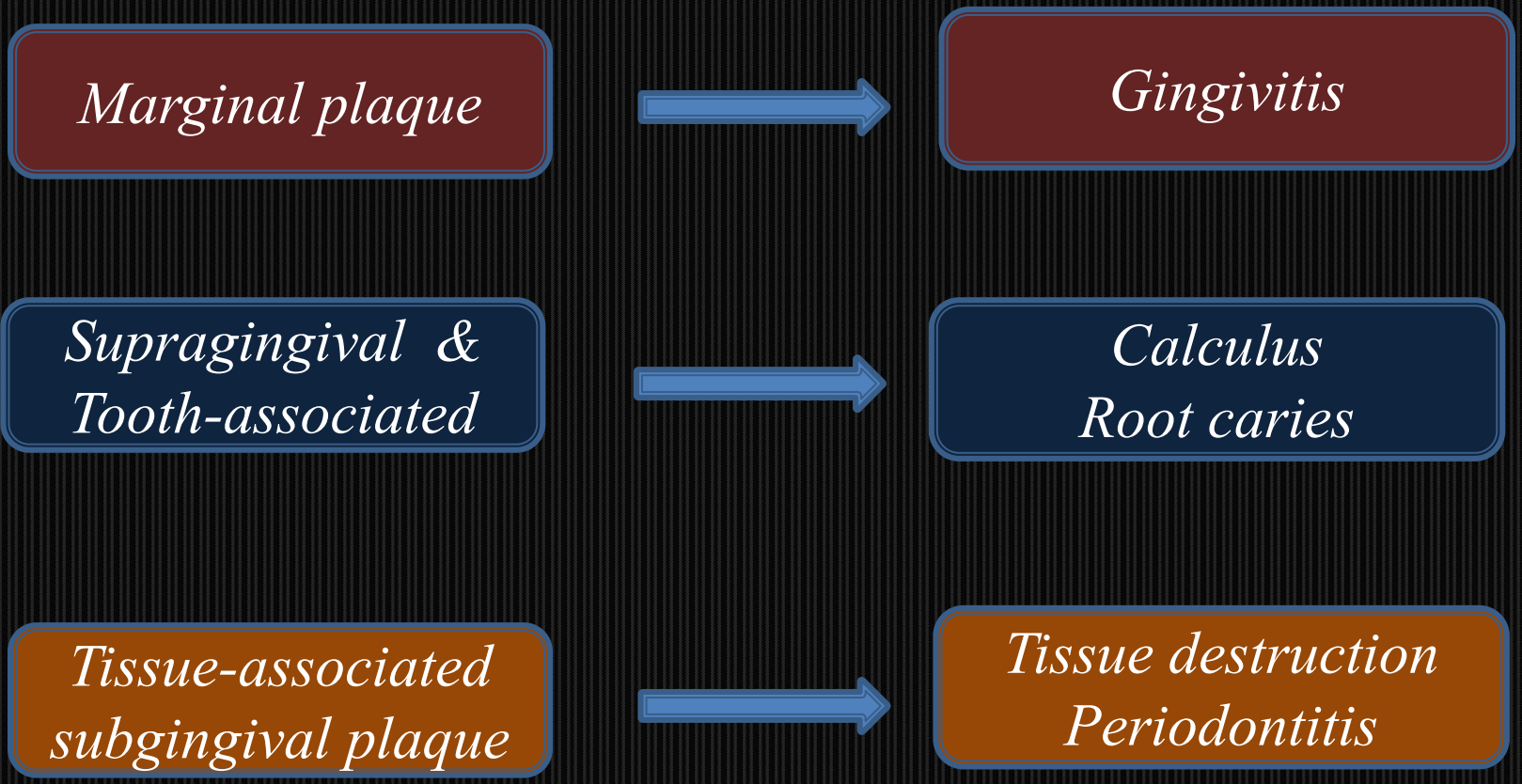
SUBGINGIVAL PLAQUE



Fastidious , strict anaerobes.
Local availability of blood products and a low redox potential, characteristic of anaerobic environment.
GCF – nutrients, contain inflammatory cells and mediators

Subgingival plaque

Tooth-associated cervical plaque	*•Filaments* *•Gram +ve rods and cocci*
Apical tooth-associated plaque	*•Gram –ve rods* *•Lack a definite intermicrobial matrix*
Unattached plaque	*•Gram -ve rods and cocci, filaments, flagellated rods, spirochetes*
Tissue-associated plaque	Gram –ve anaerobic rods like P.gingivalis, P. intermedia, T. forsythia


Marginal plaque
Gingivitis
Supragingival &
Tooth-associated
Calculus
Root caries
Tissue-associated
subgingival plaque
Tissue destruction
Periodontitis

DIFFERENCE BETWEEN SUPRAGINGIVAL AND SUBGINGIVAL PLAQUE

	SUPRAGINGIVAL PLAQUE	SUBGINGIVAL PLAQUE
1. LOCATION	Coronal to marginal gingiva	Apical to marginal gingiva
2. ORIGIN	Salivary glycoproteins, acquired pellicle	Downgrowth of bacteria from supragingival plaque and GCF

	SUPRAGINGIVAL PLAQUE	SUBGINGIVAL PLAQUE
3. **DISTRIBUTION**	Proximal surfaces, cracks, pits, fissures, overhanging margins of restoration, artificial crowns, orthodontic bands etc.	Shallow & deep pockets,.
4. **ADHESION**	Acq.Pellicle, other bacteria, tooth surface	Tooth surface, subgingival pellicle, calculus

	SUPRAGINGIVAL PLAQUE	SUBGINGIVAL PLAQUE
5. **STRUCTURE**	Adherent, densely packed microbial layer over pellicle on tooth surface.	Tooth attached, unattached, epithelial attached.
6. **M.O**	Gram + ve cocci, filaments	Gram – ve spirochetes

	SUPRAGINGIVAL PLAQUE	SUBGINGIVAL PLAQUE
7. SOURCE OF NUTRIENTS	Saliva and ingested food	GCF, exudate and leucocytes
8. SIGNIFICANCE	Supragingival calculus	Gingivitis, periodontitis and subgingival calculus.

FORMATION OF PLAQUE

STAGES

- *Formation of acquired pellicle.*
- *Initial Adhesion and Attachment.*
- *Colonization and Plaque maturation.*

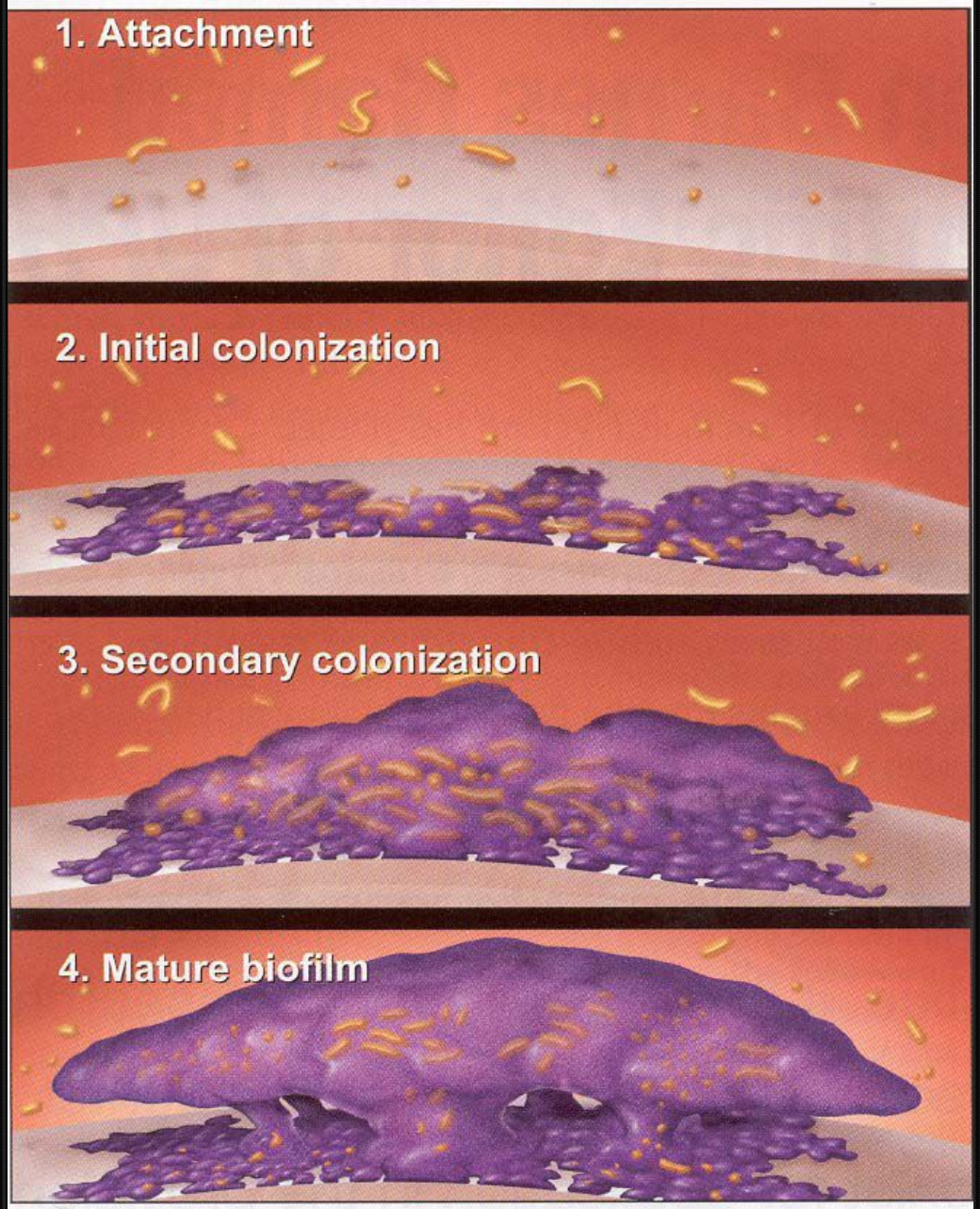


Figure 7. The Pattern of Biofilm Development. The stages of biofilm maturation are: attachment, initial colonization, secondary colonization, and mature biofilm.

FORMATION OF ACQUIRED PELLICLE

Is an amorphous organic tenacious membranous layer that forms over exposed tooth surfaces as well as restorations and dental calculus.

Within nanoseconds after polishing the teeth, a thin saliva derived layer forms on the tooth.

Pellicle forms by ***selective adsorption of environmental macromolecules.***

This pellicle consists of numerous components, including

glycoproteins (mucins),

proline-rich proteins,

phosphoproteins (e.g., statherin),

histidine-rich proteins,

enzymes (e.g., a-amylase),

molecules that can function as adhesion sites for bacteria (receptors).

INITIAL ADHESION AND ATTACHMENT OF BACTERIA

Phase 1

1.Transport to the surface:

Initial transport of bacteria to tooth surface.

Random contact can occur, through-

- Brownian motion
- Sedimentation of micro-organisms
- Liquid flow
- Active bacterial movement.

Phase 2

Initial adhesion:

- Reversible adhesion:

Interaction between the bacteria and the surface from a certain distance(50 nm) through long range and short range forces, including van der Waal and electrostatic forces .

Its a week reversible adhesion

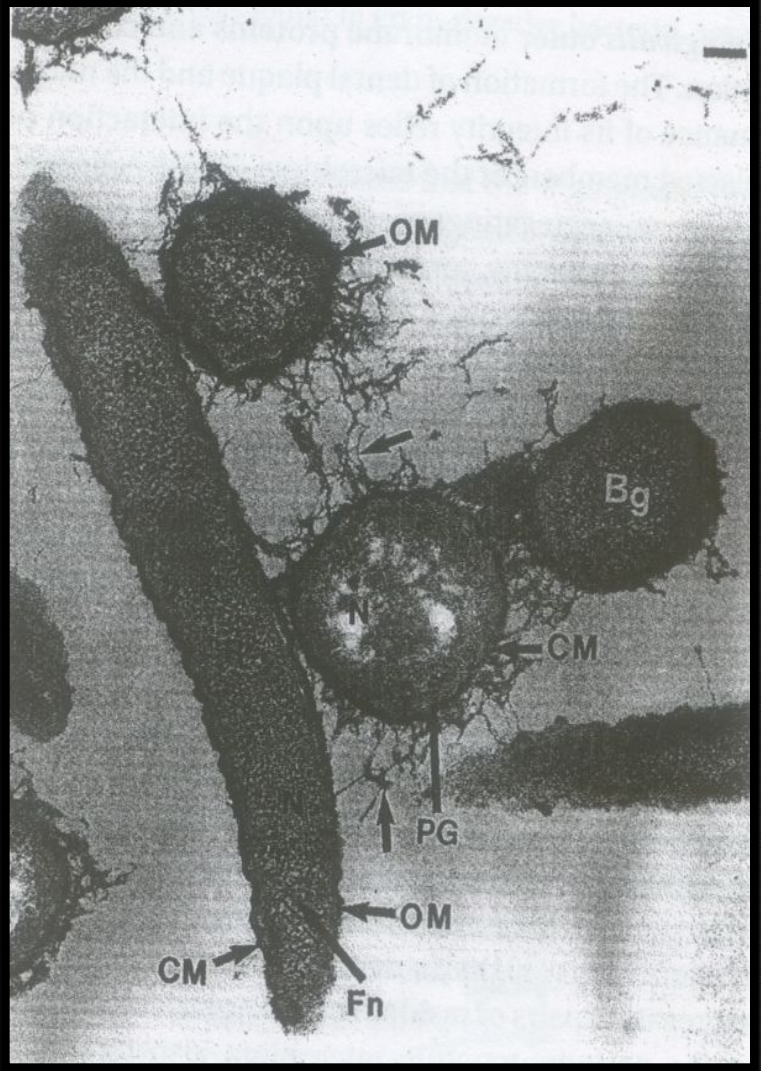
Phase 3

- Attachment: firm anchorage between bacteria & surface.
- Bonding b/w bacteria & pellicle – *adhesions* of microorganisms and complementary ***receptors*** in pellicle.
- Eg- Streptococci bind to acidic proline rich protein and other receptors in the pellicle like α-amylase and sialic acid.
- Actinomyces viscosus posess fimbriae that contains adhesins that specifically bind to proline rich proteins of the dental pellicle.

Phase 4: COLONIZATION AND PLAQUE FORMATION

Coaggregation

Adherence between different bacterial strains



- Process occurs primarily through highly specific stereochemical interaction of protein and carbohydrate molecules on bacterial surfaces.
- Interaction of secondary colonizers with early colonizers
- Eg: Fusobacterium nucleatum with Sreptococcus sanguis, Prevotella loescheii with Actinomyces viscosus, Capnocytophaga ochracea with A.viscosus

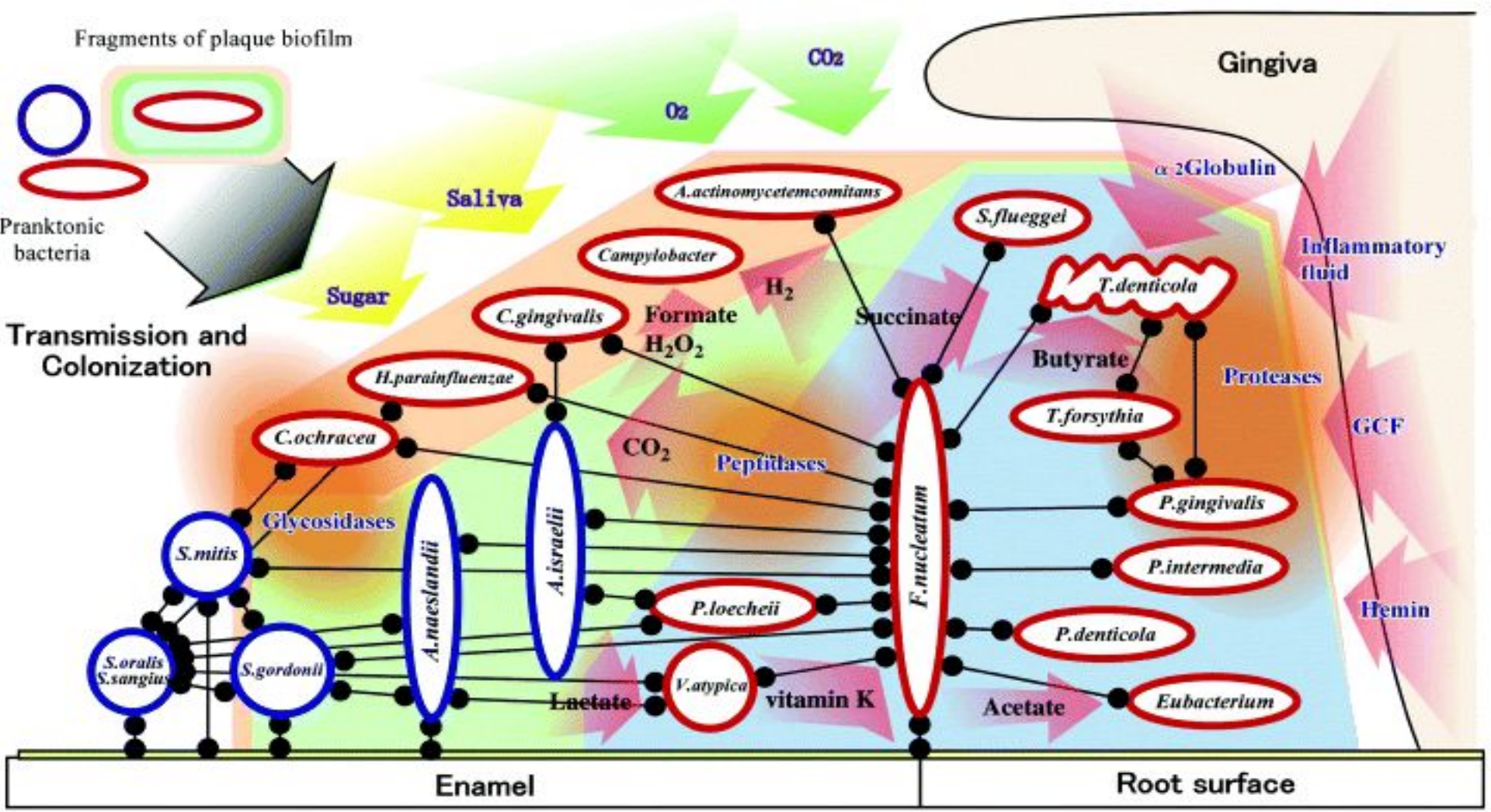
- Interactions among different Gm +ve species.
- Between Gm +ve and Gm –ve species.
- Both the primary colonisers- streptococci and actinomycetes are facultative anaerobes.
- They prepare favorable environment for secondary colonizers which have more fastidious growth requirements.

- They do not initially colonize the clean tooth surface but adhere to bacteria already in plaque mass.
- In later stages of plaque formation, coaggregation b/n different Gm –ve species is likely to predominate.
- Eg- Fusobacterium nucleatum with Porphyromonas gingivalis or Treponema denticola

Corncob structure

Steptococci adhere to filaments of Bacterionema matruchotii or Actinomyces species.

‘Test tube brush’ composed of filamentous bacteria to which Gm –ve rods adhere.



Early colonizer ⇨ Secondary colonizer ⇨ Late colonizer : Microbial succession

(Gram ⊕ cocci) (Gram ⊕ ⊖ rod) (Gram ⊖ rod/ motile) (Dominant microorganisms)

Aerobic ⇨ Capnophilic / Facultative anaerobic ⇨ Anaerobic : Environment modification

Saliva (saccharolytic) ⇨ GCF (proteolytic) : Nutrition supply

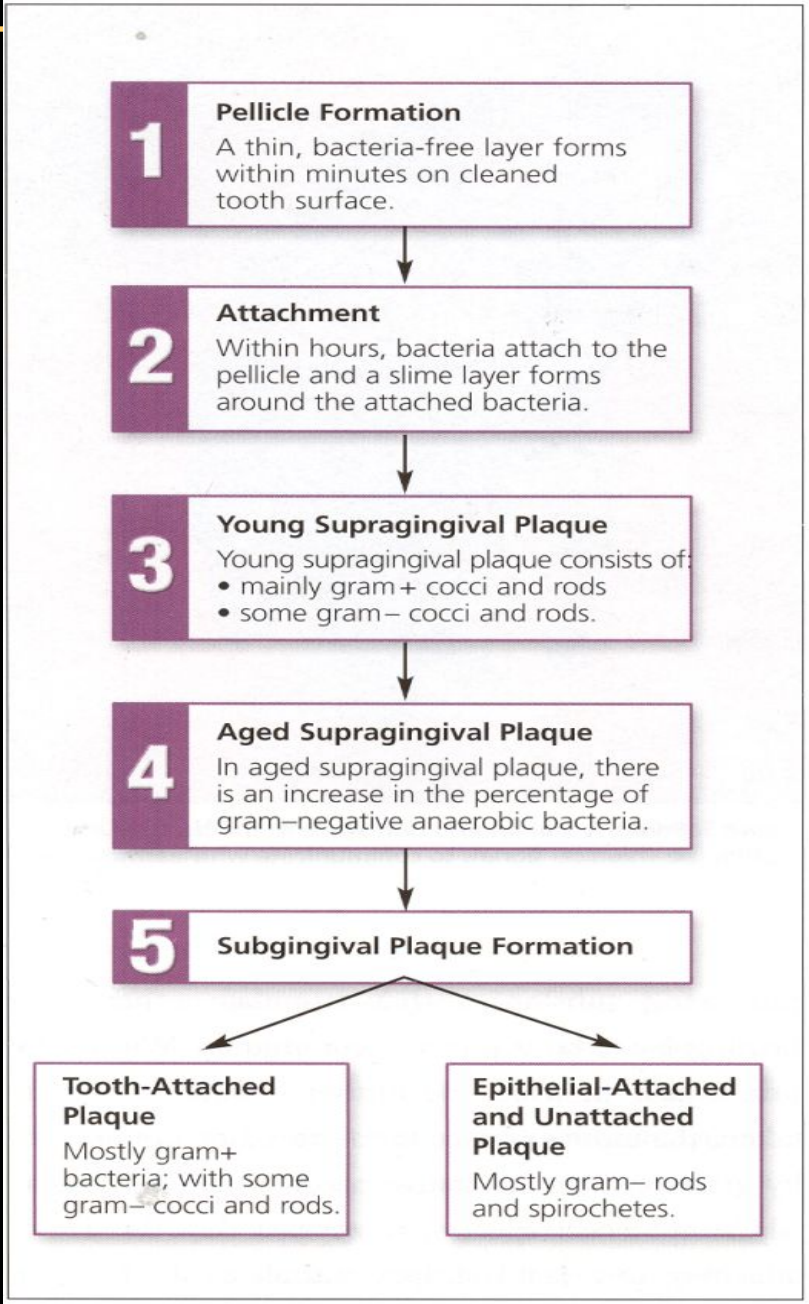
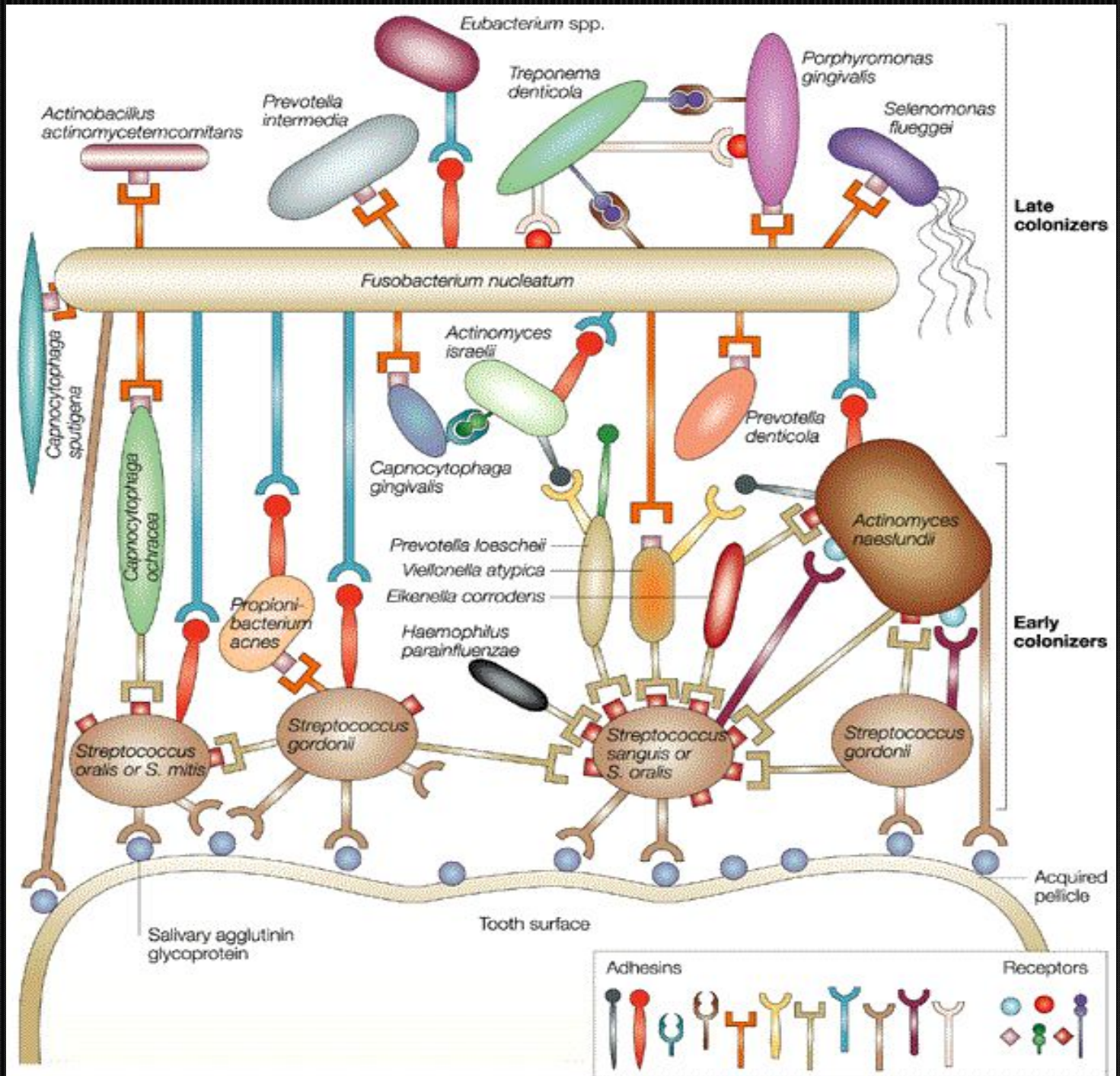
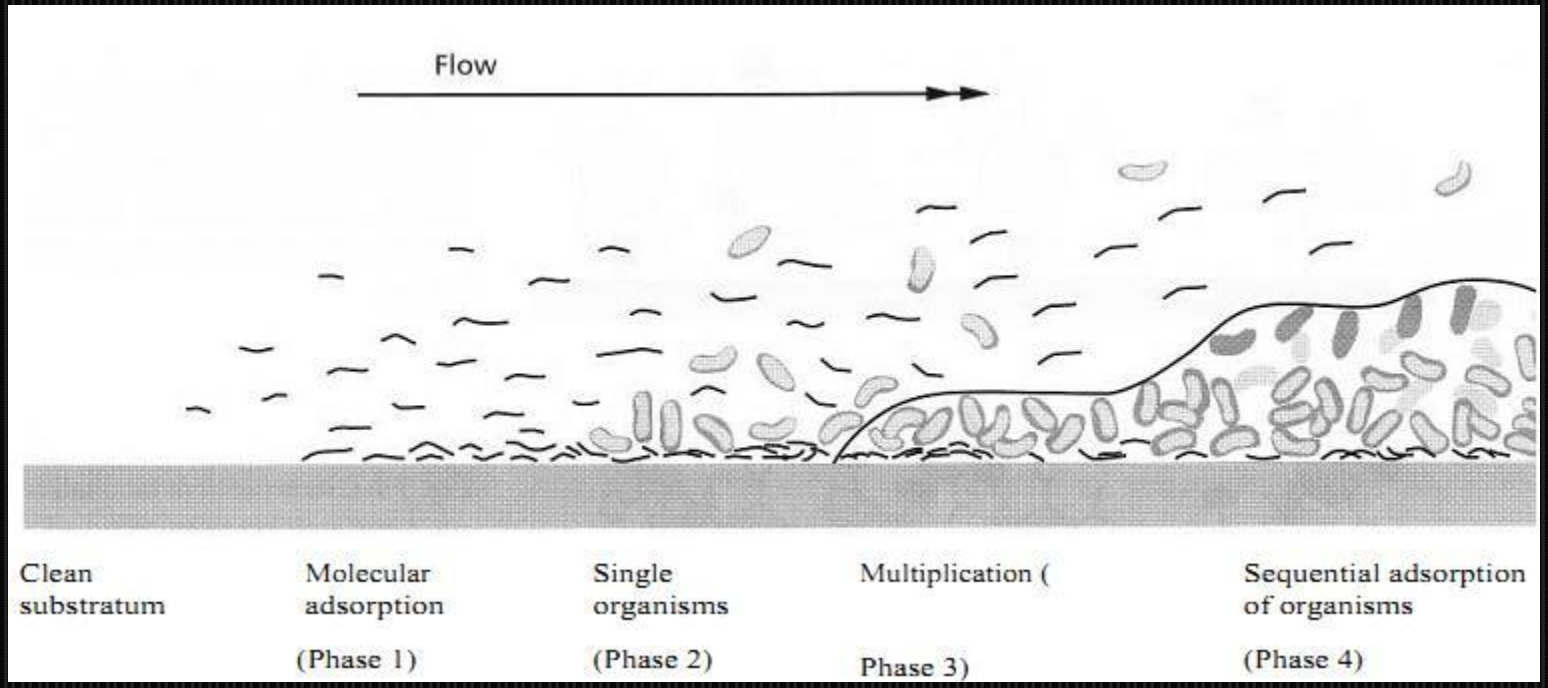


Figure 6. Phases of Plaque Formation. The phases of plaque formation are: pellicle formation, attachment, young supragingival plaque, aged supragingival plaque, and subgingival plaque formation.

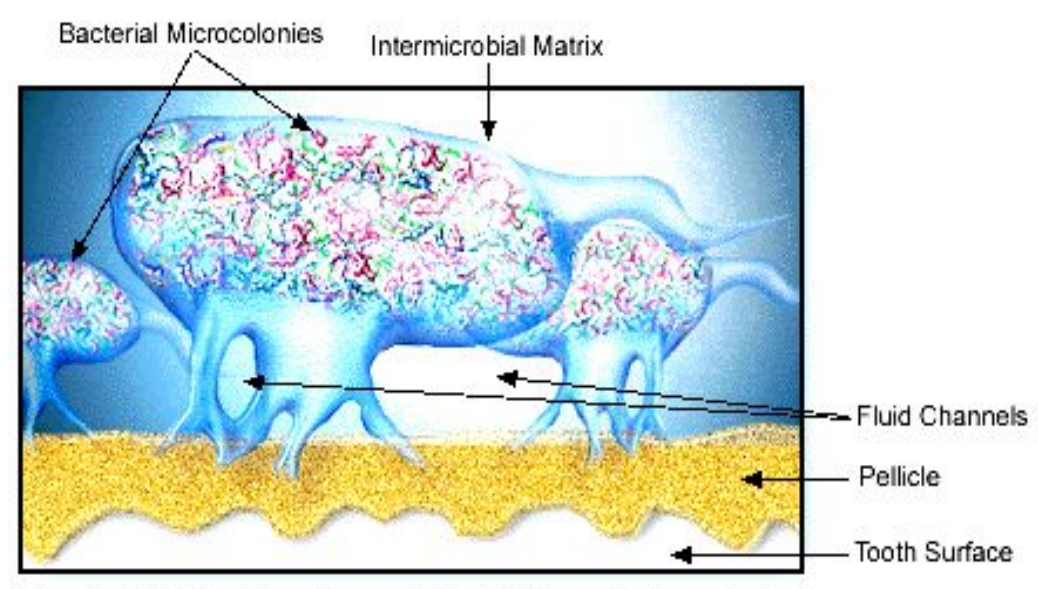

Eubacterium spp.
Porphyromonas gingivalis
Treponema denticola
Prevotella intermedia
Actinobacillus actinomycetemcomitans
Selenomonas flueggei
Late colonizers
Fusobacterium nucleatum
Actinomyces israelii
Capnocytophaga sputigena
Capnocytophaga ochracea
Capnocytophaga gingivalis
Prevotella denticola
Actinomyces naeslundii
Prevotella loescheii
Veillonella atypica
Eikenella corrodens
Propioni-bacterium acnes
Haemophilus parainfluenzae
Early colonizers
Streptococcus oralis or S. mitis
Streptococcus gordonii
Streptococcus sanguis or S. oralis
Streptococcus gordonii
Acquired pellicle
Salivary agglutinin glycoprotein
Tooth surface
Adhesins
Receptors


Flow
Clean substratum
Molecular adsorption
(Phase 1)
Single organisms
(Phase 2)
Multiplication (
Phase 3)
Sequential adsorption of organisms
(Phase 4)

BIOFILM

Organized structure composed of microcolonies of bacterial cells non randomly distributed in a glycocalyx matrix.

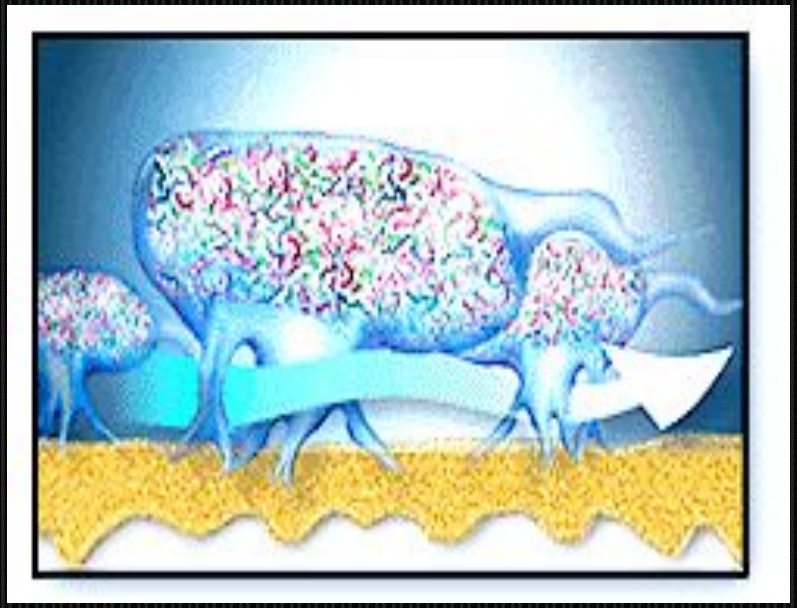
PLAQUE AS BIOFILM



Lower layers are dense with microbes bound together in polysaccharide matrix with organic and inorganic materials.

On top of the lower layer, a loose layer appears that is often irregular.

- Open fluid filled channels runs through the plaque mass, which permit passage of nutrients and other agents. Act as a primitive circulatory system
- The bacteria exist and proliferate in the intercellular matrix



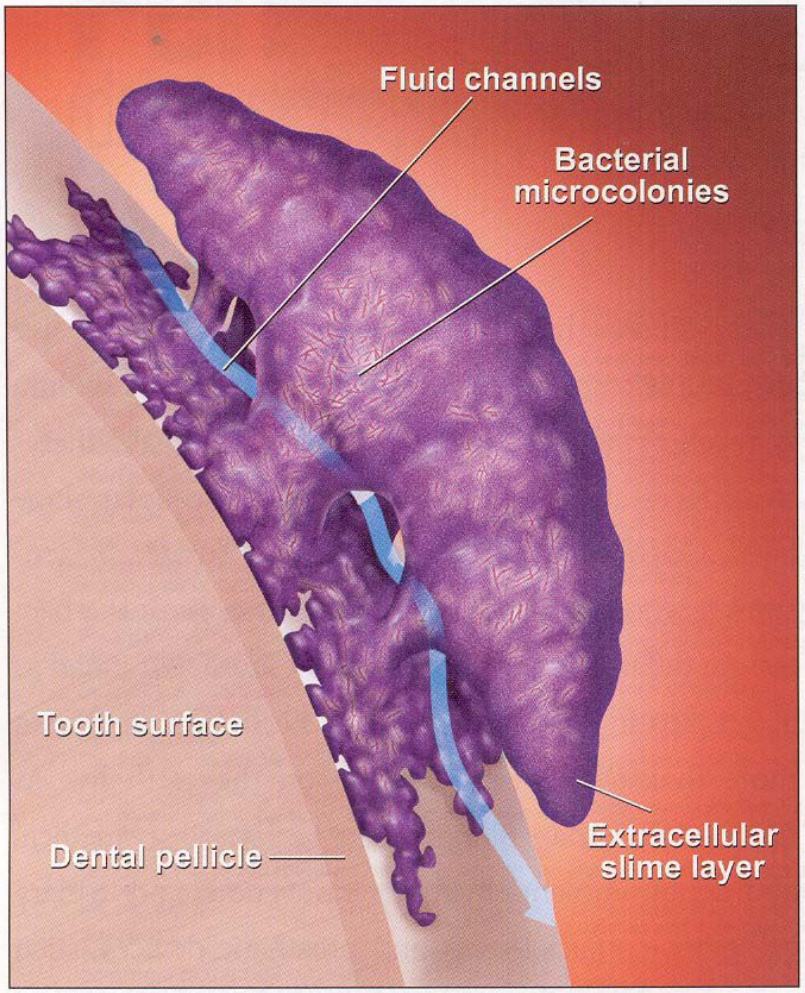


Figure 2. Dental Plaque Biofilm. Conceptual illustration of the biofilm structure.

Properties

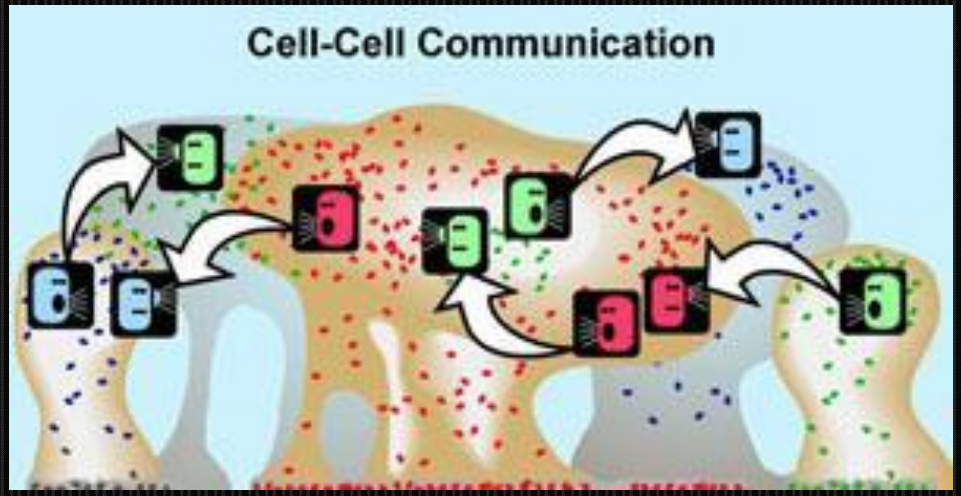
- The matrix confers a specialized environment – distinguishes bacteria that exist in the biofilm from those that are free floating (bacteria in saliva, GCF)
- Biofilm matrix- function as a barrier
- Antibiotic resistance - 1000 – 1500 times

Antibiotic resistance

- Slower rate of growth - makes them less susceptible
- Extracellular enzymes may be trapped and concentrated in the ECM, thus inactivating some antibiotics.
- Biofilm matrix is a barrier to diffusion of antibiotics.

"Super resistant" bacteria with multidrug resistant pumps can extrude antimicrobial agents, offers protection against antibiotics.

Quorum sensing



Seems to play a role in

- *expression of genes for antibiotic resistance,*
- *encouraging the growth of beneficial bacteria in the bf*
- *discouraging the growth of competitors*

Association of plaque microorganisms with periodontal disease

- Two hypothesis have been put forward. They are:
- Non-specific plaque hypothesis
- Specific plaque hypothesis

NON - SPECIFIC PLAQUE HYPOTHESIS

- Periodontal disease results from the elaboration of noxious products by the entire plaque flora
- Less plaque – the noxious products are neutralized by the host
- More plaque – more noxious products – overwhelm the host defense mechanism leading to disease
- Control of PD depends on control of plaque accumulation

- The disease results from the shear mass of organisms present and once this exceeds a certain threshold, disease will occur.
- The quantity of plaque, rather than quality, is considered to be the deciding factor in the development of disease

Contradictions for this theory

- Some individuals with considerable amount of plaque and calculus never develops periodontitis
- Site specificity - some sites affected, some unaffected. Advanced destruction adjacent to normal sites
- This concept is discarded

Specific plaque hypothesis

- Loesche, 1976
- Only certain plaque is pathogenic
- Pathogenicity depends on the presence or increase of specific microorganisms
- Quality of the bacteria rather than quantity
- Specific forms of periodontal disease have specific bacterial etiologies eg LAP – A.ac

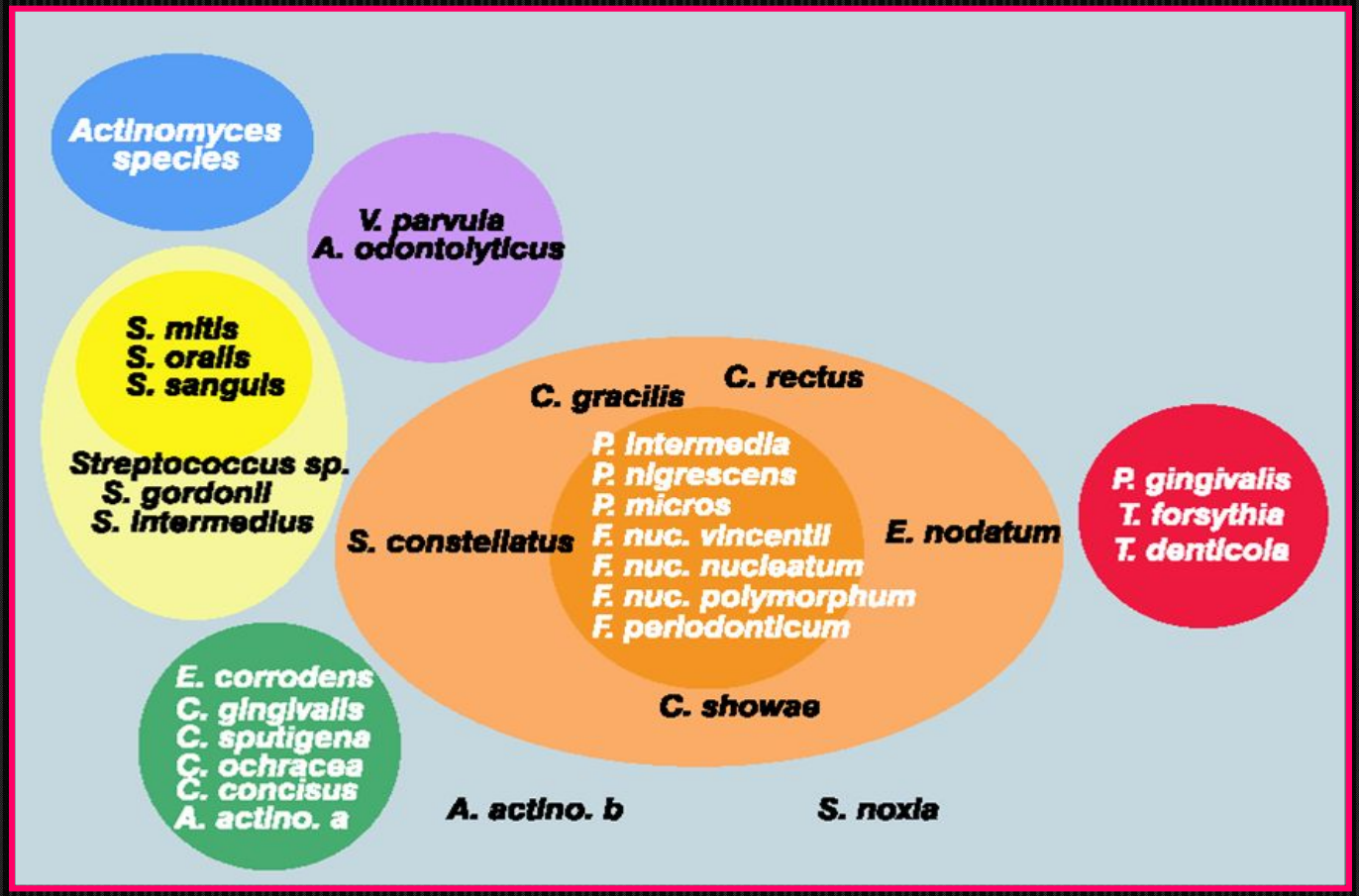
Ecological plaque hypothesis

- a change in the environmental conditions of the oral cavity could change the composition of microflora from healthy to diseased.

Socransky's criteria - by which periodontal microorganisms may be judged to be potential pathogens

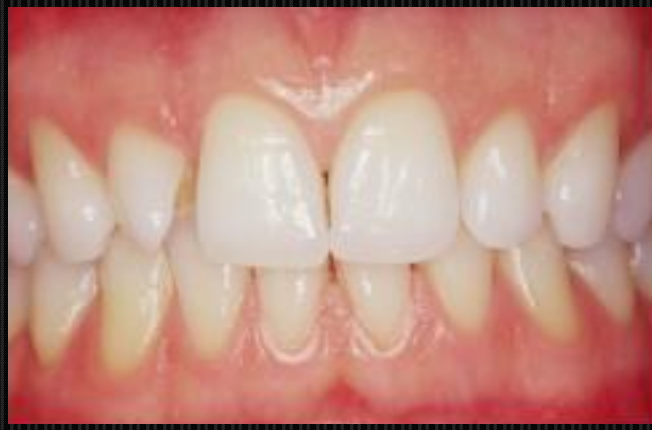
- Must be associated with disease
- Must be eliminated or decreased in sites that demonstrate clinical resolution of disease with treatment
- Must demonstrate a host response
- Must be capable of causing disease in experimental animal models
- Must demonstrate virulence factors

Microbial complexes



Microorganisms associated with specific periodontal diseases

PERIODONTAL HEALTH



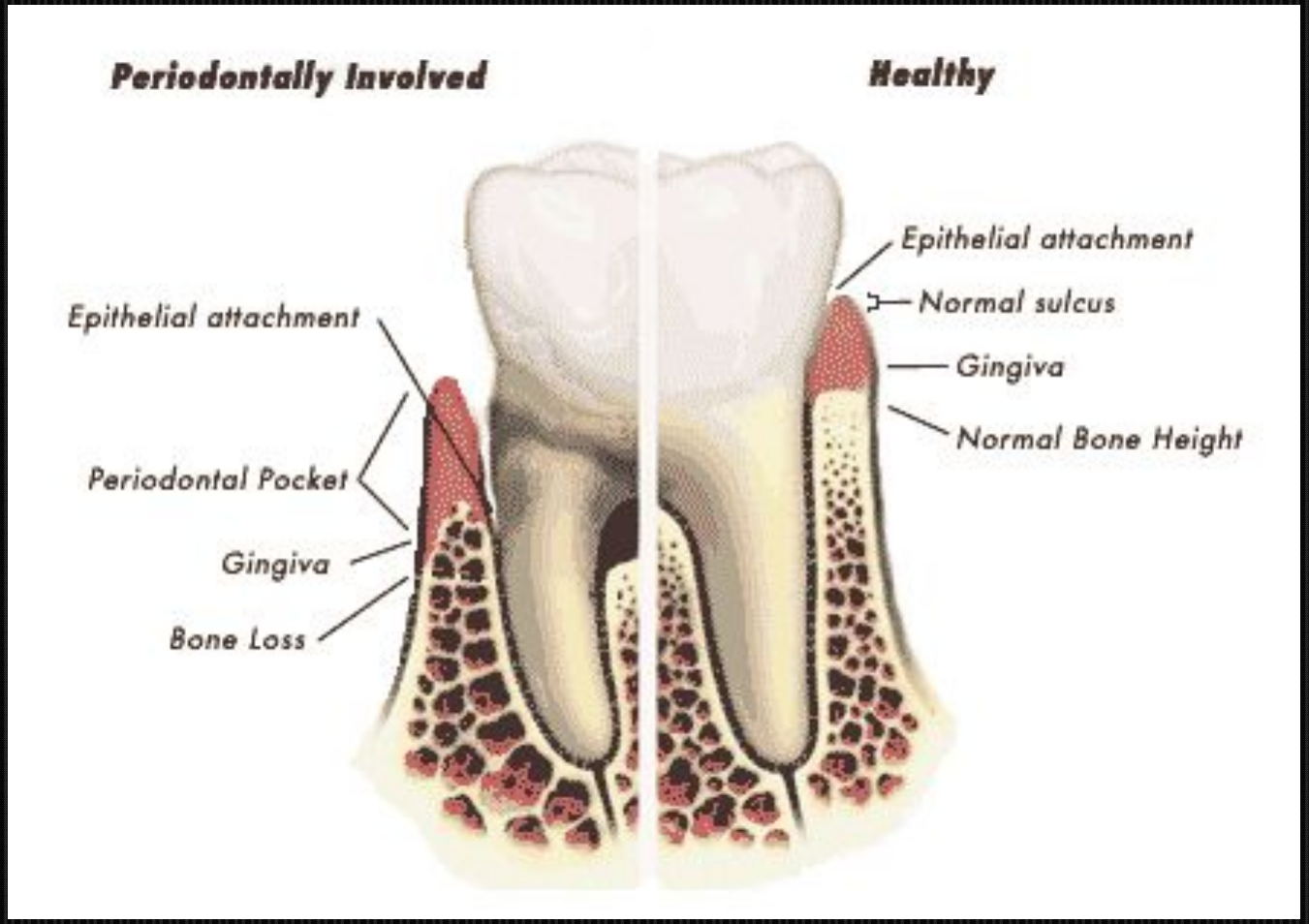
- Primarily Gm +ve facultative rods and cocci
- *S.sanguis, S.mitis, A.viscosus, A. naesulundii.*
- Small proportions of Gm –ve species [*P.intermedia, F.nucleatum, Capnocytophaga, Nisseria, Vellionella]*
- *Gram –ve rods - health(13%) - gingivitis (40%) - adv. periodontitis (74%)*

- Protective and beneficial to the host: *S.sanguis, V.parvula, C.ochraceus*

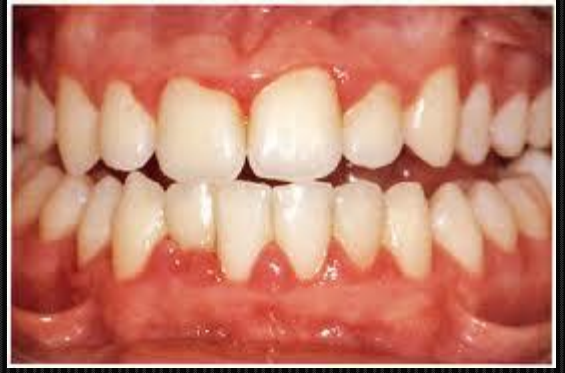
- > Inactive sites
- < Active periodontal destruction
- Prevent colonization or proliferation of pathogenic m.o

Mechanism:

- Production of H_2O_2 by *S.sanguis* – lethal to *A.a.*

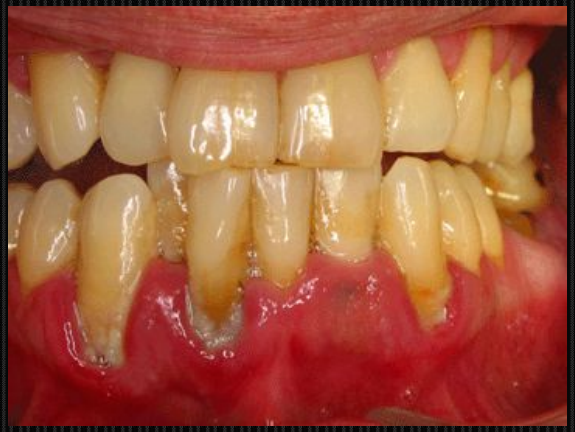

Periodontally Involved
Healthy
Epithelial attachment
Periodontal Pocket
Gingiva
Bone Loss
Epithelial attachment
Normal sulcus
Gingiva
Normal Bone Height

GINGIVITIS



- Almost equal proportions of Gram +ve, Gram –ve, as well as facultative and anaerobic m.o.
- Gm +ve: *S. sanguis, S. mitis, S. intermedia, S. oralis, A. viscosis,*
- Gram –ve*: F. nucleatum, P. intermedia, V. parvula, Haemophilus, Capnocytophaga*.
- Pregnancy – associated gingivitis – increase in *P. intermedia*

CHRONIC PERIODONTITIS



- Elevated proportions of Spirochetes.
- High % of anaerobic and Gm –ve bacterial species.
- *P.gingivalis,T.forsythia, P.intermedia, C.rectus, E.corrodens, F.nucleatum, A.actinomycetemcomitans, Treponema.*

- Recent studies: Association between Herpes viruses and chronic periodontitis – mainly HCMV, EBV -1

Microbial Shift during disease

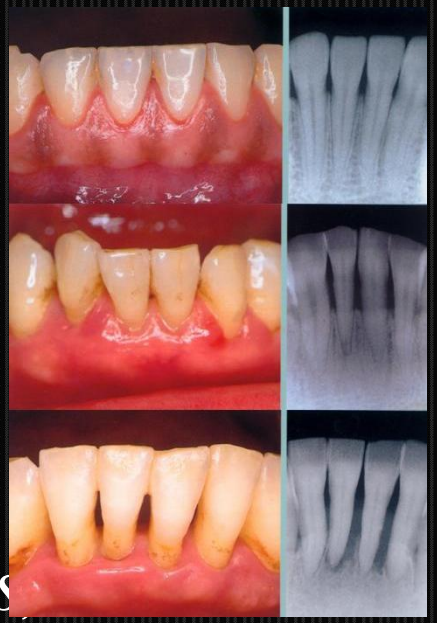
Gm+ve → Gm-ve

Cocci, rods → Spirochetes

Nonmotile → Motile

Facultative anaerobes → Obligate anaerobes

Localised Aggressive Periodontitis



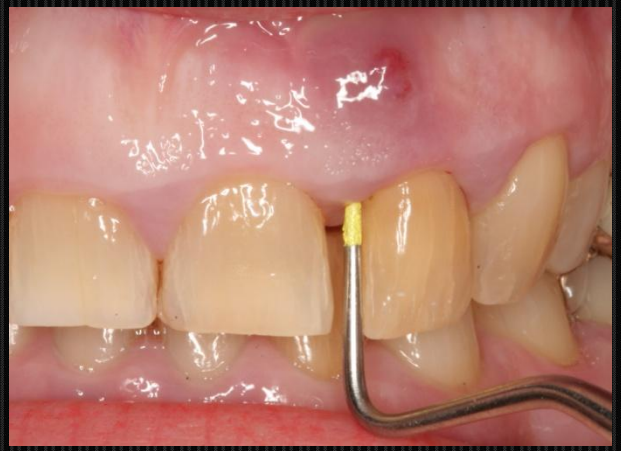
- Gm –ve, anaerobic rods.
- 90% - *A.actinomycetemcomitans*.
- Others: *P.gingivalis, E.corrodens, C.rectus,*

  F.nucleatum, Caphnocytophaga and Spirochetes.
- *EBV-1, HCMV*.

Necrotizing Periodontal Diseases

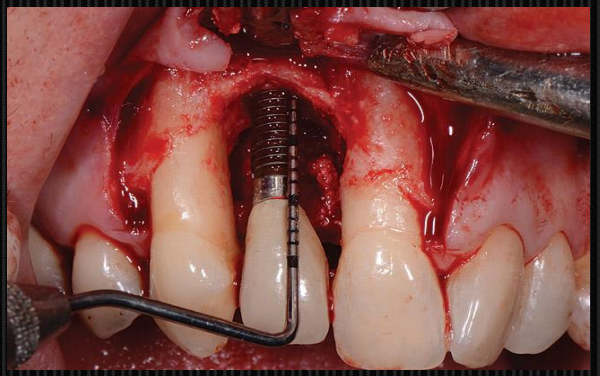
- High levels of *P.intermedia* and Spirochetes.
- *Spirochetes* are found to penetrate necrotic tissue and unaffected CT.

Abscess of periodontium



- *F.nucleatum,P.intermedia, P.gingivalis, P.micros, T.forsythia*

Periimplantitis



- High proportion of anaerobic gm-ve rods, motile organisms and spirochetes.
- *A.actinomycetemcomitans, P.gingivalis,T.forsythia, P. micros, C.rectus, Fusobacterium.*

Key Characteristics of specific Periopathogens

Major Periodontopathogens

- *Aggregatibacter actinomycetemcomitans*
- *Porphyromonas gingivalis*
- *Prevotella intermedia & Prevotella nigrescens*
- *Campylobacter rectus*
- *Fusobacterium nucleatum*
- *Tannerella forsythia*
- *Spirochetes*

Aggregatibacter actinomycetemcomitans

- Small, short, straight or curved rod with round ends.
- Serotypes: a-e based on diff. in polysaccharide composition
- **Pathogenicity: endotoxin**, leukotoxin, **collagenase and** protease.

Porphyromonas gingivalis

- **Nonmotile, rod and gm-ve obligate anaerobe.**
- **Pathogenicity: An aggressive pathogen.**
- **Produces many proteases [destroys Ig], hemolysin, collagenase.**
- **Inhibit migration of neutrophils across an epithelial barrier.**
- **Can invade soft tissues**

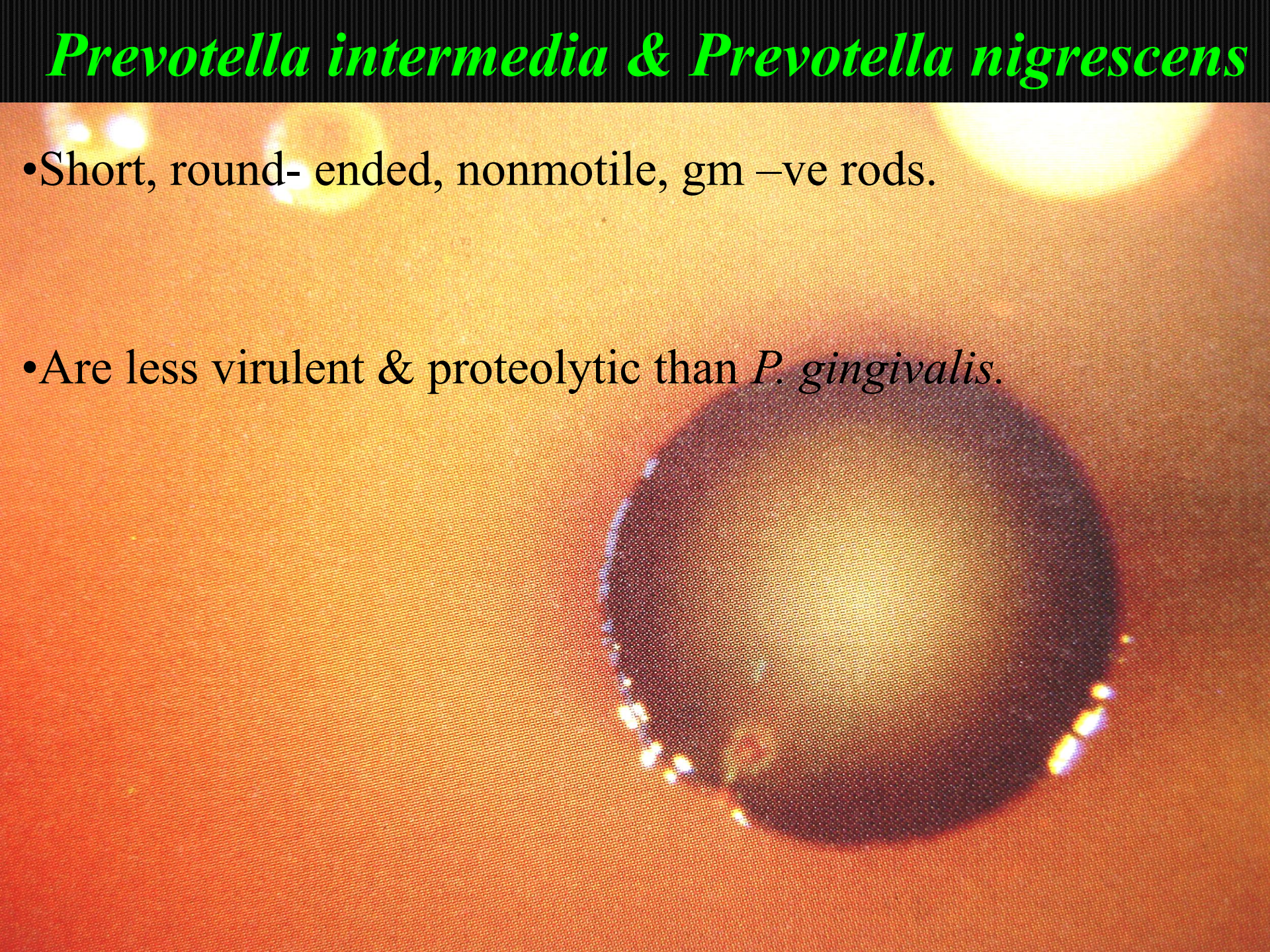
Tannerella forsythia

- Nonmotile, spindle shaped rod and gm-ve obligate anaerobe.
- Virulence: severly proteolytic, destroys Immunoglobulins.
- Induces apoptic cell death.

Prevotella intermedia & Prevotella nigrescens

- Short, round- ended, nonmotile, gm –ve rods.

- Are less virulent & proteolytic than *P. gingivalis*.



Campylobacter rectus

- **Rare motile organisms involved in periodontitis.**
- **Gm-ve, short rod, curved [vibrio] or helical.**
- **Motility from flagellum.**
- **Virulence: produces leukotoxin.**

Fusobacterium nucleatum

- **Gm –ve, cigar shaped bacillus with pointed ends.**
- **Virulence: Can induce apoptic cell death in PMN's.**
- **Co- aggregates with most bacteria.**
- **Bridging microorganism between primary & secondary colonisers.**

Spirochetes

- Diverse group of spiral, Gm –ve, motile organism.
- Types: *Treponema.denticola, T.vincenti, T.socranskii*.
- Pathogenecity : Travels through viscous environment, enables to migrate within GCF.
- Penetrate both the epithelium and CT
- T denticola produces enzymes that can destroy Igs

Future advances in periodontal microbiology

- Advanced molecular biology techniques
- Offers remarkable adv in time and cost savings compared to culture
- Increased sensitivity
- Recognition of beneficial bacteria opens up new strategies for periodontal therapy (probiotics)


Thank You. . .