Human microbiome

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Human microbiome

- Microflora
  - Microbes, that are present on/in our body and have some relationship with it
- Microbiome
  - Is the aggregate of all microbiota along with the corresponding anatomical sites
  - Includes:
    - skin
    - mammary glands
    - seminal fluid
    - uterus
    - ovarian follicles
    - saliva
    - oral mucosa
    - conjunctiva biliary tract
    - gastrointestinal tract

Development of microbiome

- In uterus
- Delivery, newborn
- Breastfeeding
- Solid nutrition
- First teeth
  - Ca 1 yr – adult-like micorbiota
- Necrobiota

GIT microbiome

- Gastrointestinal tract
  - One of the largest microbiomes
  - 1150 different species, each individual at least 160 different species
  - About 1-2 kilograms of cells
  - *Bacteroides fragilis, Bacteroides melaninogenicus, Bacteroides oralis, Enterococcus faecalis, Escherichia coli, Enterobacter sp., Klebsiella sp., Bifidobacterium bifidum, Staphylococcus aureus, Lactobacillus sp., Clostridium perfringens, Proteus, Clostridium tetani, Clostridium septicum, Pseudomonas aeruginosa, Faecalibacterium prausnitzii, Peptostreptococcus sp., Peptococcus sp.*
- Most gut bacteria - normal commensal colonists of the gut; some always pathogenic, some opportunistic pathogens
- Many functions
  - Pathogen interactions
  - Modulation of immune system
  - Digestion and absorption of nutrients
  - Autoimmune diseases
  - Irritable Bowel Syndrome
  - Mood & depression
  - Obesity
  - Increasing cancer risk for the host

Functions of GIT microbiome

- Pathogen interactions
  - Prevents colonization
- Modulation of immune system
  - Capable of modulating host immune systems
  - Required for tonic and reactive stimulation of the immune system
- Effects of Microbiota on Immune Mechanisms
  - Innate immune system
    - Growth and maturation of intestinal lymphoid follicles
    - Production of mucus
    - Induction of neutrophil bactericidal activity
    - Induction of macrophages
    - Induction of innate lymphoid cells
  - Adaptive immune system
    - Growth and maturation of intestinal lymphoid follicles
    - Activate dendritic cells
    - Activate differentiation of T cells in the lamina propria
    - Activate B cells in the lamina propria

Functions of GIT microbiome

- Digestion and absorption of nutrients
  - Vitamins (biotin and vitamin K), aminoacids (tryptophan), nutrients - fermenting unused energy substrates
- Autoimmune diseases
  - Systemic lupus erythematosus & an alteration of the intestinal flora
  - Changes to the gut and periodontal disease & rheumatoid arthritis
  - Other autoimmune diseases - also modifications of the microbiome
- Irritable Bowel Syndrome
  - In IBS patients - elevations in cortisol, postprandial serotonin levels etc.

Functions of GIT microbiome

- Mood & depression
- Obesity
- Therapeutic approaches
  - Probiotics - live microorganisms
  - Prebiotics - food compounds not digestible by the host
  - Symbiotic - probiotics and prebiotics simultaneously
  - Faecal microbiota transplantation
- Host Factors that affect microbiota composition and function
  - Antibiotic usage
  - Diet
  - Bowel preparations
  - Gut mucosal integrity
  - Expression of epithelial receptors
  - Functional immune cell populations
  - Expression of antimicrobial peptides…

UGT microbiota

- Key role in the health of the UGT
- Lactobacilli
  - production of lactic acid
  - antimicrobial agents
  - suppress infection
- In women – most of microbes in vagina, but other places, formerly believed to be sterile, also colonized, and similarly in men
  - Vagina, uterus, ovarian follicles, seminal fluid in men

UGT microbiota

- Vagina
  - Anaerobes:
    - *Lactobacillus, Peptostreptococcus, Clostridium, Propionibacterium, Eubacterium, Bifidobacterium, Prevotella, Bacteroides, Fusobacterium, Veillonella,* diphtheroids, *Actinomycetales*
  - Aerobes:
    - *Staphylococcus aureus, CoNS, Group B Streptococcus, Enterococcus faecalis, Actinomyces israelii, Actinomyces neuii, Escherichia coli, Klebsiella, Proteus, Enterobacter, Acinetobacter, Citrobacter, Pseudomonas, Candida*
- Vaginal microbiota changes
  - Age of woman
  - Pregnancy
  - Antibiotic therapy
  - No link between taking oral probiotics and maintaining normal microbiota populations of lactobacilli in vagina

UGT microbiota

- Uterus
  - Over 278 genera
- Ovarian follicle
  - Culture techniques – Lactobacilli sp., Propionibacterium, Actinomyces
- Male reproductive tract
  - Acute prostatitis
  - Chronic prostatitis
  - Infertility
  - Pseudomonas, Lactobacillus, Prevotella

Skin microbiome

- pH of the skin - pH 4-4.5
- Salinity
- Antimicrobial peptides
  - *Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus aureus, alpha-haemolytic Streptococcus sp., Acinetobacter sp., Bacillus sp., Corynebacterium sp., Cutibacterium acnes, Micrococcus sp., Peptostreptococcus sp., Propionibacterium sp., Sarcina sp., Candida albicans, Candida parapsilosis*
- Acne vulgaris
- Atopic dermatitis
- Psoriasis vulgaris
- Rosacea

Oral microbiome

- One of the largest microbial communities
- Resident & transient
- Ecological system with many niches
- Biofilm formation
- Important for health
- Relation to etiology of dental caries, parodontitis, halitosis…

Oral health consequences

- Aterosclerosis of coronary vessels
- Stroke
- *Diabetes mellitus*
- Pre-term delivery
- Low birth weight
- Aesophagal carcinoma

Ecosystem of oral cavity

- Very specific environment by its composition
- Mucosal surfaces
- Sulcar liquid
- Communicates with outer environment

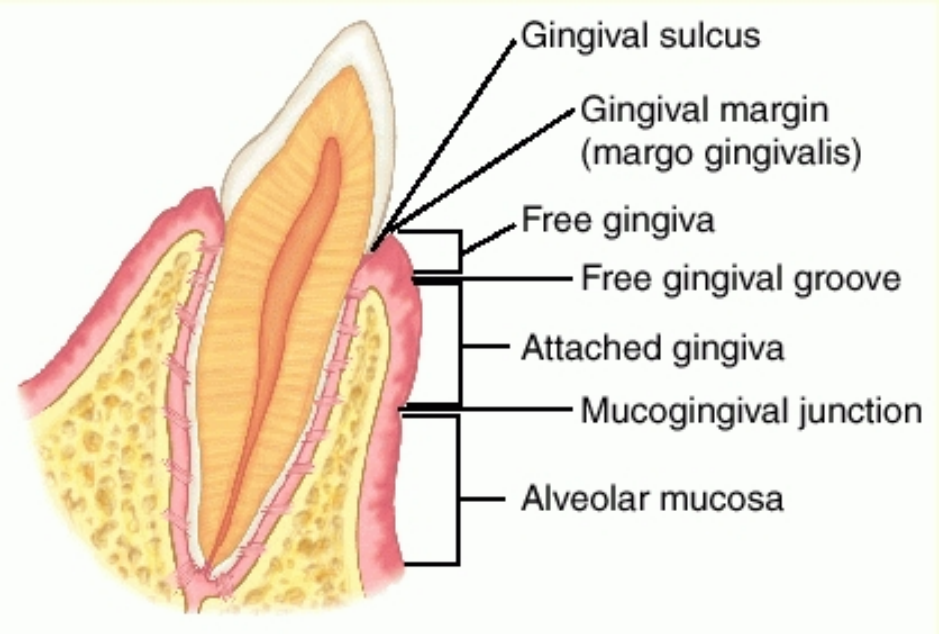
Oral cavity as microbial biotop

- Lips
- Buccal mucosa & soft palate
- Tongue
- Supragingival teeth surfaces
- Sulci gingivales

Particular surfaces in oral cavity

- Lips
- Buccal mucosa & soft palate
- Surface of the tongue
- Teeth
- Artificial teeth and dental implantates
- Mucous membrane of sulcus gingivalis

Sulcus gingivalis



- Mix of aerobic and anaerobic species – SRSP, *Actinobacillus*, *Fusobacterium*, *Treponema* sp., *Wollinella* sp., RED COMPLEX bacteria…

Streptococci in the oral cavity I.

- A-haemolytic streptococci

- ***S. mutans* group**
  - *S. mutans*, less frequent *S. sobrinus*, rare *S. cricetus* and *S. rattus*

- ***S. salivarius* group**
  - *S. salivarius, S. vestibularis*
  - Can cause endocarditis

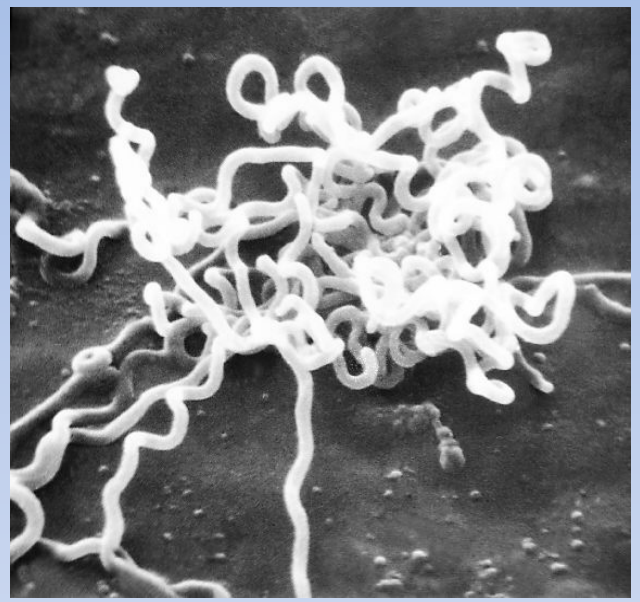
Streptococci in the oral cavity II.

- ***S. mitis* group**
  - *S. mitis , S. oralis, S. peroris*
  - *S. sanguinis* and *S. gordonii*
  - Subacute bacterial endocarditis

- ***S. anginosus* group**
  - *S. anginosus* (*S. milleri*), *S. constellatus* – *S. c. constellatus* and *S. c. pharyngis*, and *S. intermedius*

Treponema denticola

- Spirochaetae
- Close relationship to *P. gingivalis*
- Adhesins, invasins
- Haemolysins

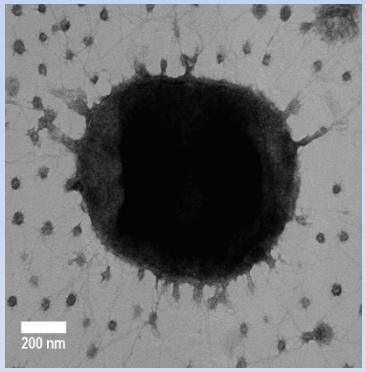


wikipedia.org/wiki/Treponema

- Inflammatory starters – starts secretion of cytokins & chemokins
- Protease – degradation of barriers, cells and protective macromolecules

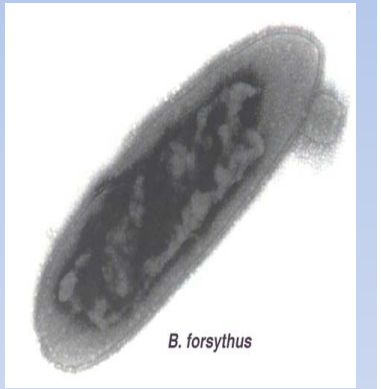
Porphyromonas gingivalis

- Highly proteolytic
- Proteolytic microorganism – quickly resides *sulcus gingivalis*
- Crossfeeding
- Releases outer membrane vesicles
- Fimbriae
- Vesicles
- Contains black pigment

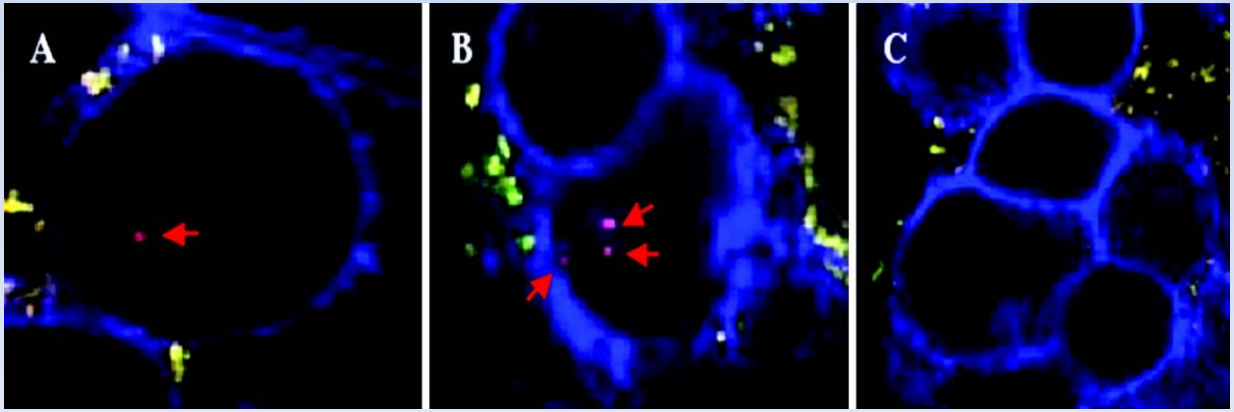


Tannerella forsythia

- Interaction between *T. forythia* and *P. gingivalis*
- Implicated in periodontal diseases
- Red complex



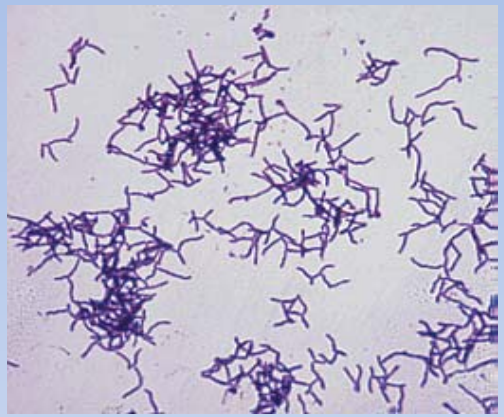
Invasion of *T. forsythia* into cells, Inagaki 2006, confocal laser microscopy



Other G+ and G- bacteria

- *N. subflava, N. sicca, N. mucosa*
- Staphylococci, micrococci
- *Eikenella*
- *Veillonella*
- Other genera – *Propionibacter, Enterococcus*

Mycoplasmas, actinomyces, lactobacilli



- *Actinomyces* sp.
- *Lactobacillus* sp.
- *Mycoplasma pneumoniae, M. hominis, M. salivarium*

Interactions of microorganisms

- Products of metabolism

- *P. gingivalis*

  X

- *Fusobacterium nucleatum*

- Bacteriocins

Protection against immunity system

- Susceptibility to phagocytosis
- Rescue before immune response
- *A. actinomycetemcomitans* and bacteria of red-complex more resistant

- Microbes from oral cavity can cause metastatic infections

Parasites and fungi in oral cavity

- *Entamoeba gingivalis, Trichomonas tenax*
- *Candida* sp.

Dental plaque

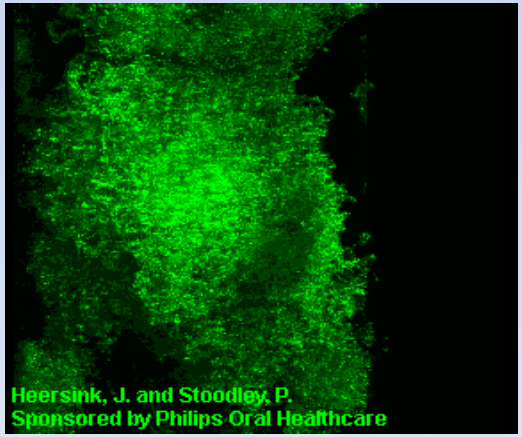
- Dental plaque is an adherent microbial layer on the tooth surface = live and dead bacteria + their products + host compounds (from saliva)
- Can not be washed
- According to the location
- Sometimes also classified as coronary, fissural, supragingival and subgingival

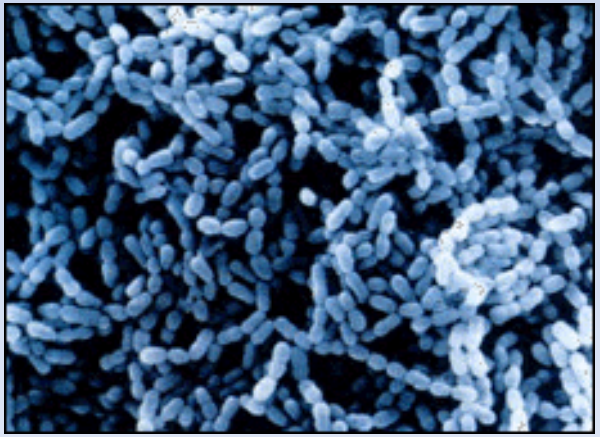
Definition of biofilm

- Sessile microbial community
- Its cells are irreversibly attached to a substratum or interface or to each other
- They are embedded in a matrix of extracellular polymeric substances that they have produced

Dental plaque

- It is composed of numerous bacteria
- Change in disease
- Dental plaque has open architecture similar to other biofilms, with channels and voids





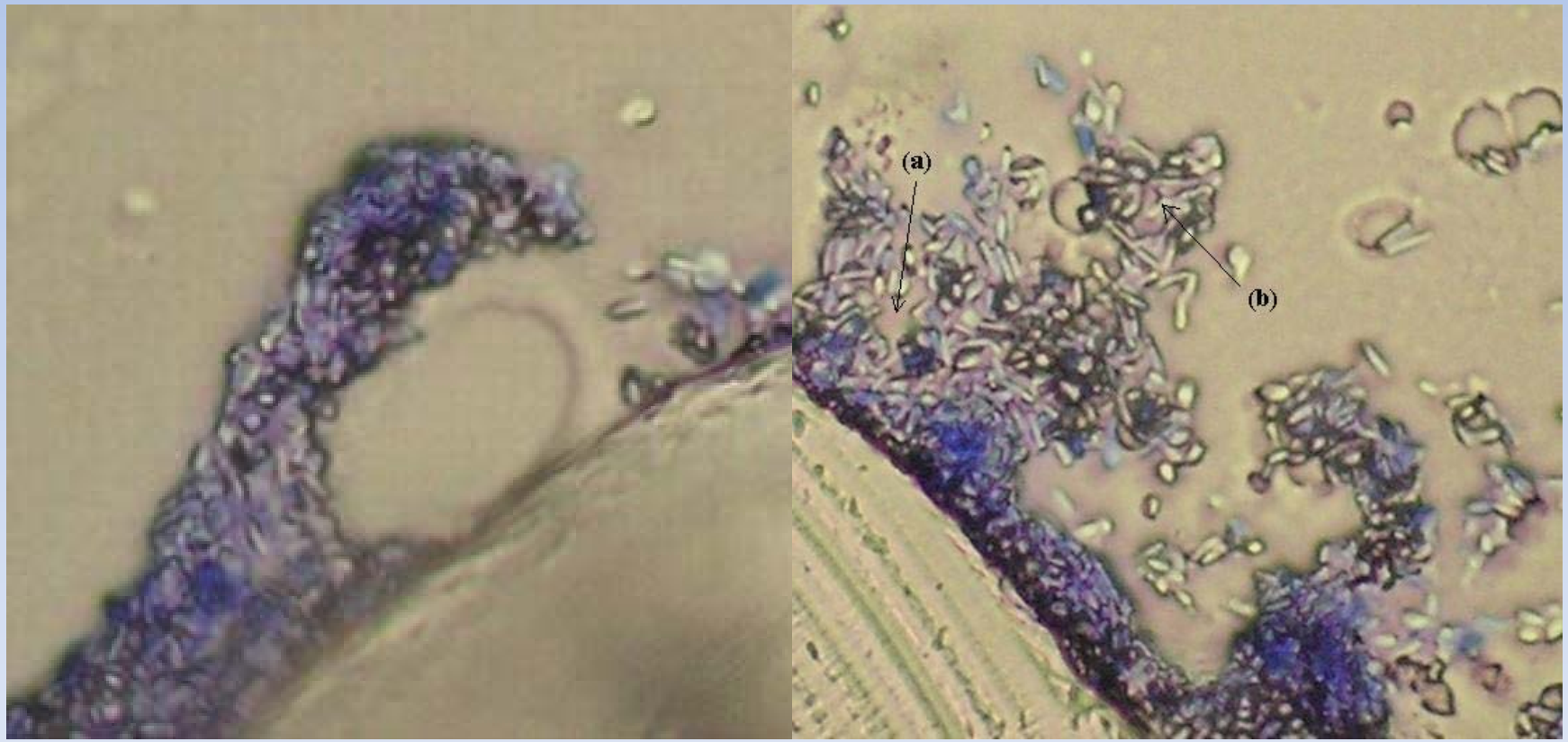
Biofilm architecture

- Microcolonies embedded in extracellular matrix form fungus-like structures interwowen with system of channels and voids

Oral microflora

(a) channel

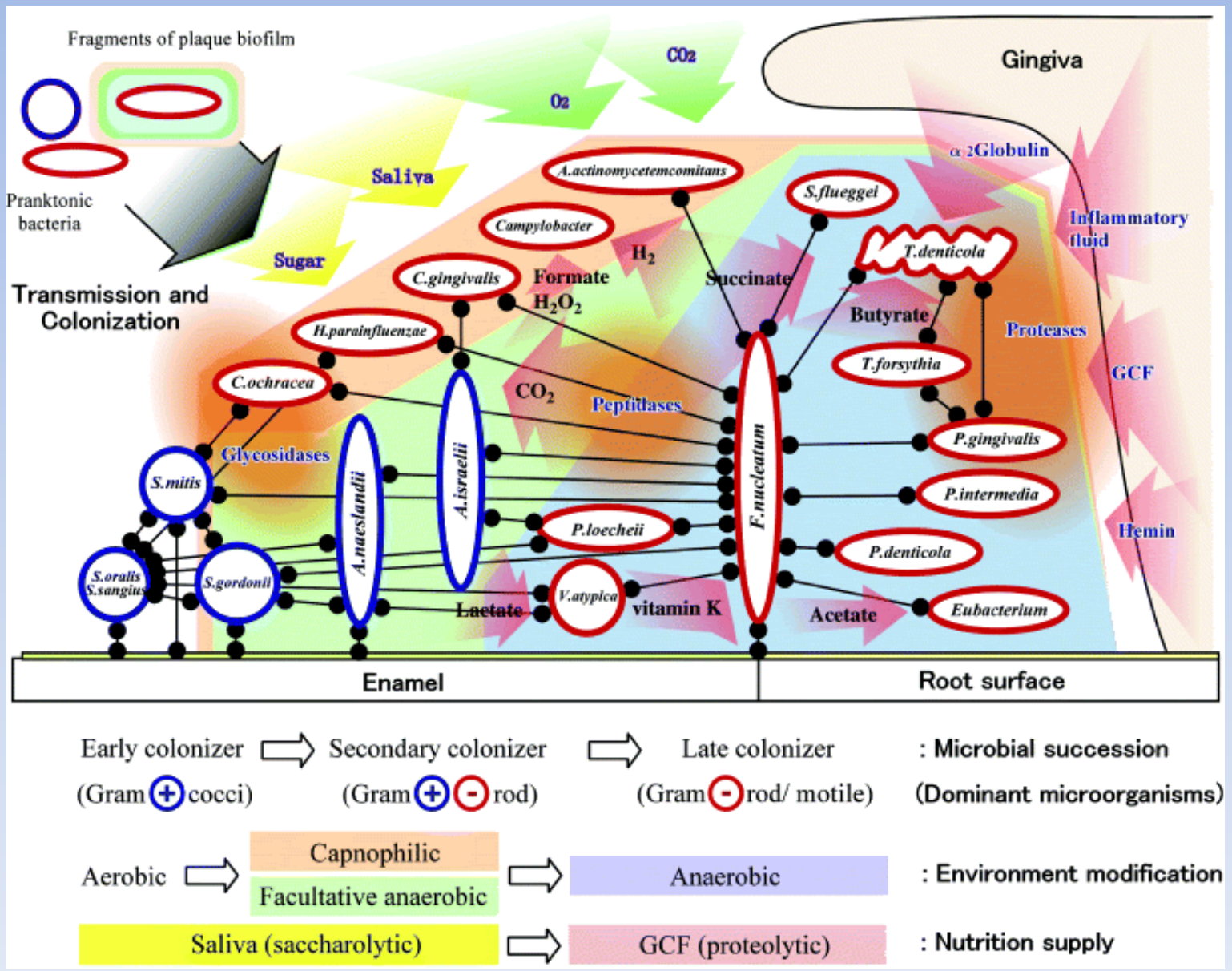
(b) fungoid structure



Mutual relations between biofilm bacteria

- Bacteria in plaque communicate mutually
  - Through coaggregation and coadhesion
  - Through conventional metabolic interactions
  - Via small diffusible signalling molecules

Relations of bacteria in the plaque

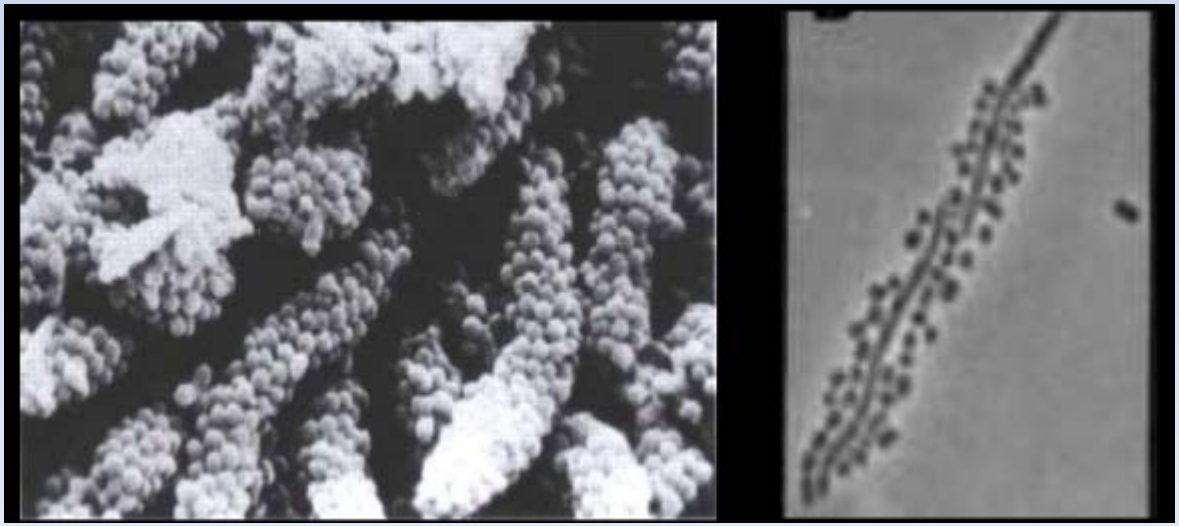


http://dentalplaque.wikispaces.com/Formation

Coaggregation in plaque

- E.g. anaerobic *Fusobacterium nucleatum*
  - Early colonizers of the tooth surface

    and
  - Anaerobic late colonizers



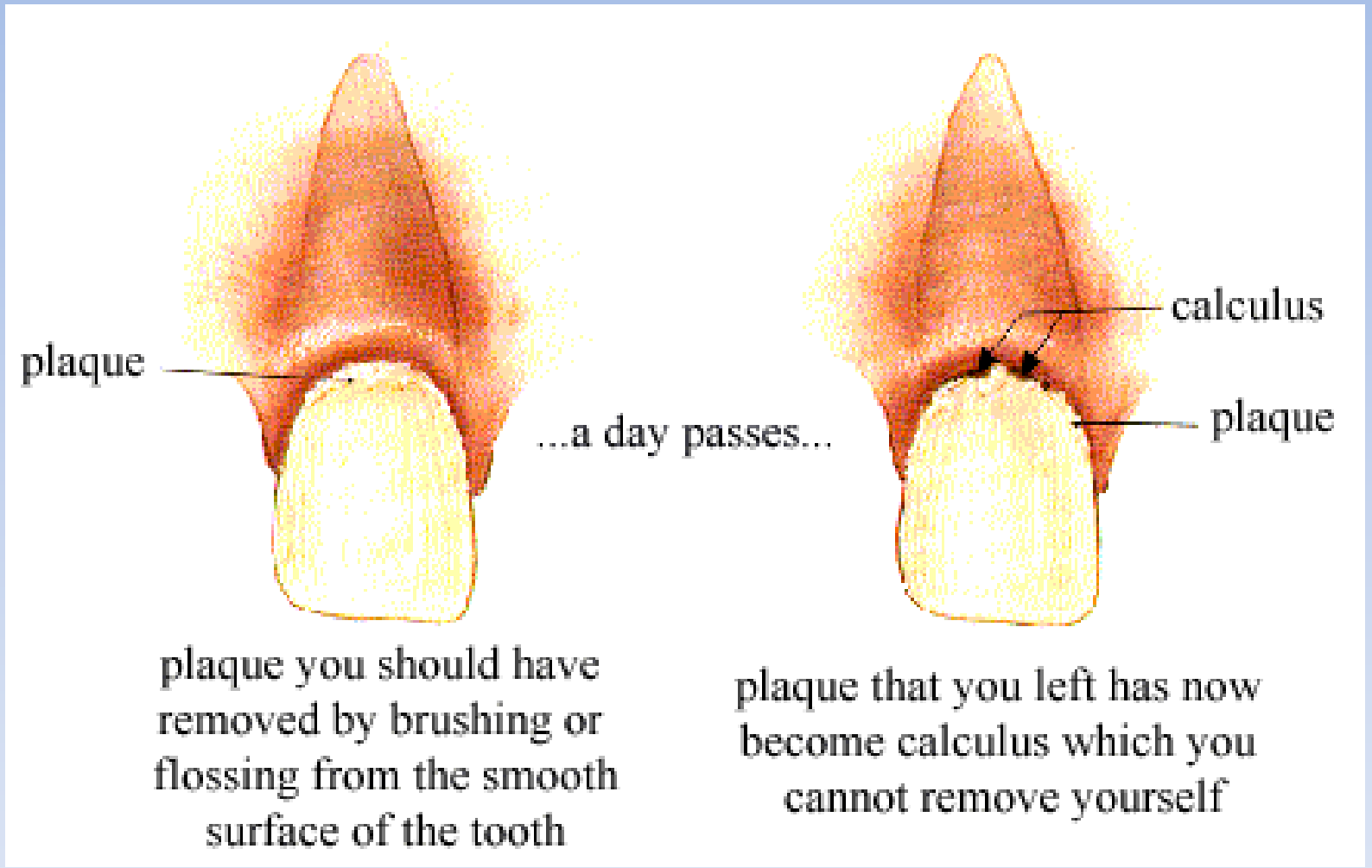
Dental plaque development mechanisms I.

- Starts by a thin layer – pellicula
- Receptors for adhesion of G+ cocci and rods
- Exopolysaccharid production
- Bacterial metabolism in plaque
- Plaque development is accelerated by sucrose

Dental plaque development mechanisms II.

- In bottom layers the plaque is mineralised
- Influence of bacterial metabolism
- Subgingival calculus (concrement)
- Calculus porous

Dental tartar



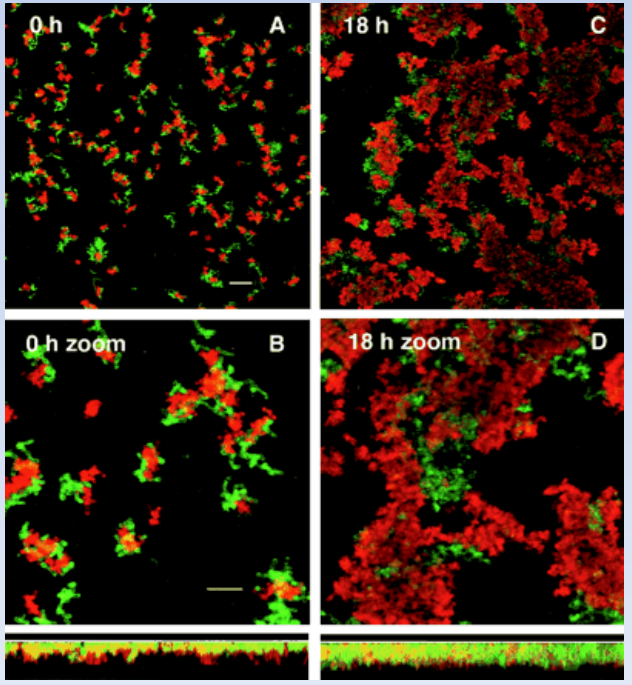
http://www.intelligentdental.com/2011/12/03/what-are-the-causes-of-tartar-on-human-teeth/

Development of dental plaque

- Less than 24 hours: Streptococci of groups *mutans*, *sanguis*, and *mitis*
- Days: G+ rods and filamentous microorganisms (lactobacilli, actinomycetes)
- Week: Columns/microcolonies of coccoid microbes
- Three weeks: filamentous

microbes are prevalent,

„corn-cob“ formation

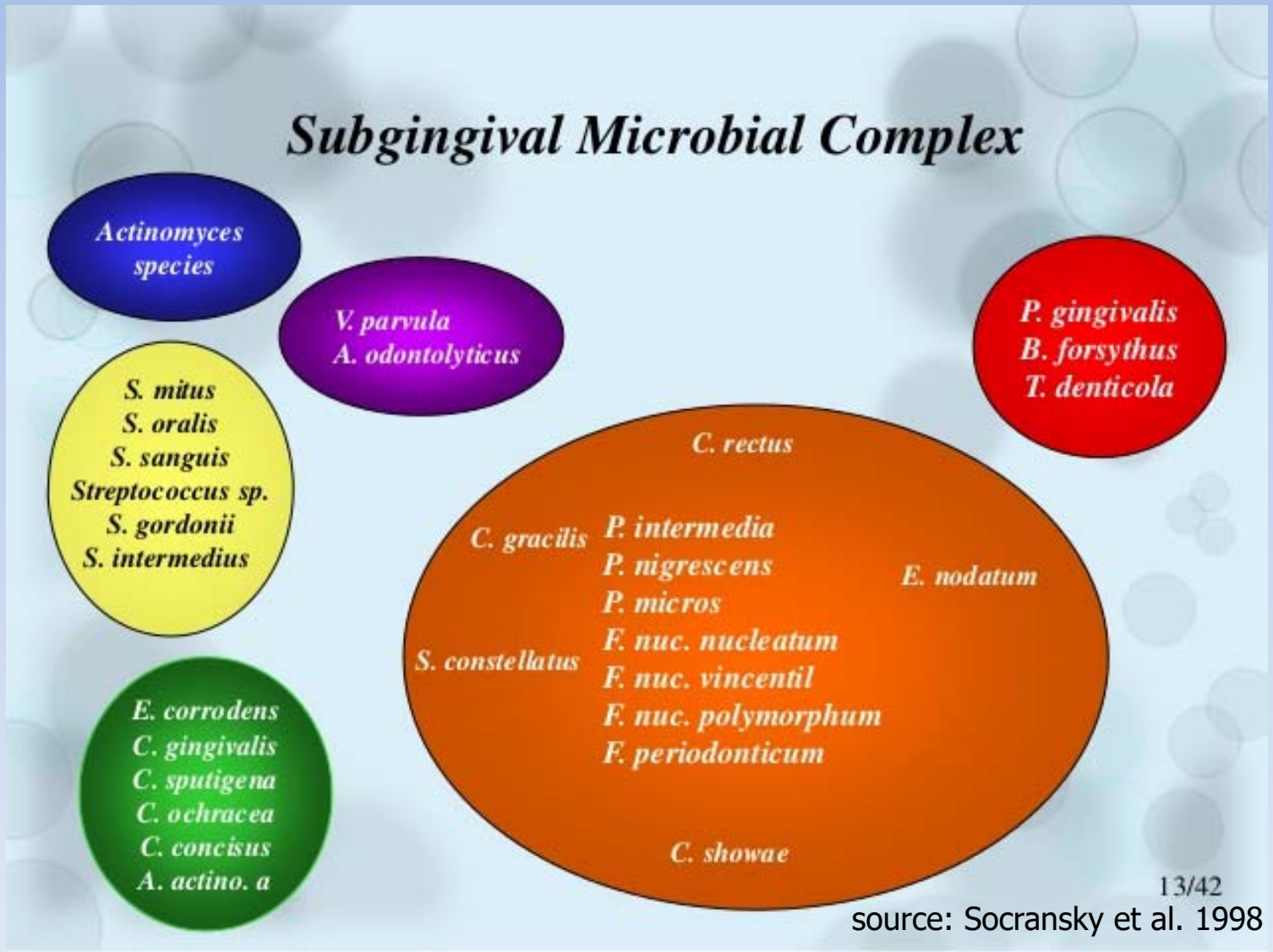


Confocal laser mikroscopy – two-species biofilm, Kolenbrander et al., 2002

Distribution of microorganisms

- *Actinomyces* sp. in both
- Supragingival plaque – *Actinomyces* sp., neisseriae, streptococci, "green" and "purple" complex
- Subgingival plaque – *Prevotella* sp., *Tannerella forsythia* and *Porphyromonas gingivalis* ("red„), and "orange" complex
- Supragingival plaque - reservoir

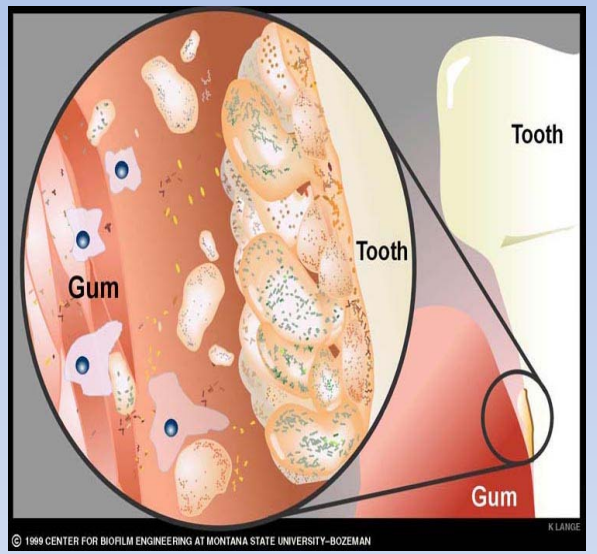
Bacterial complexes in the oral cavity



source: Socransky et al. 1998

Subgingival plaque

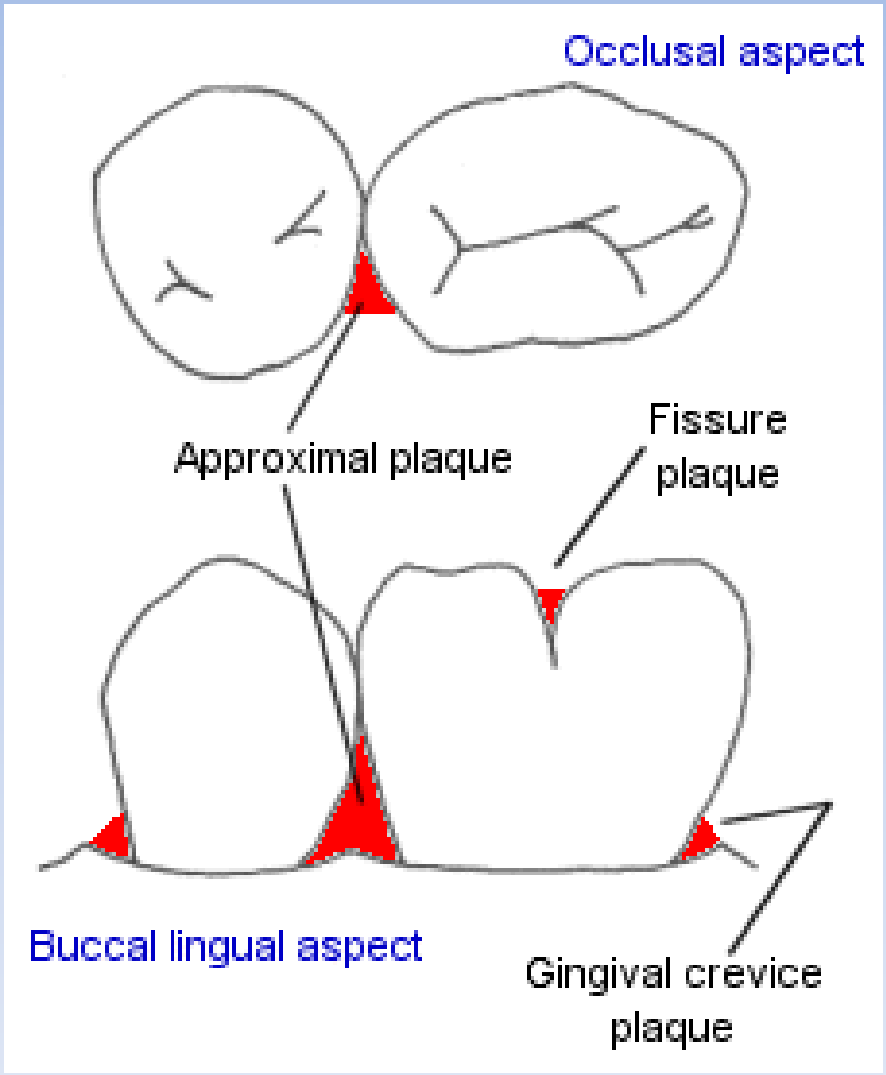
- Adherent plaque
- Non-adherent plaque

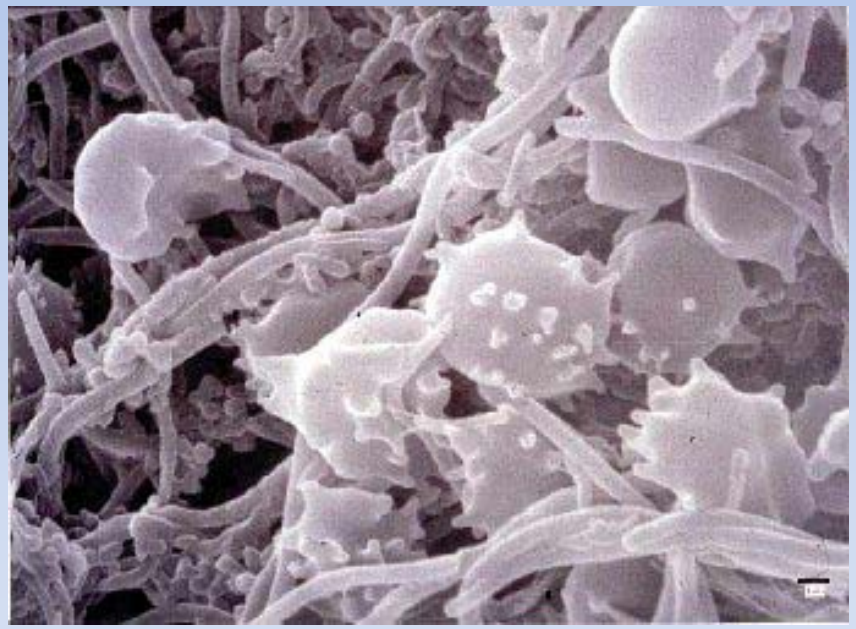


Subgingival plaque

Adherent part	Non-adherent part
Rather Gram-positive	Rather Gram-negative
Rather non-motile	Rather motile
Rather facultative anaerobes	Rather strict anaerobes

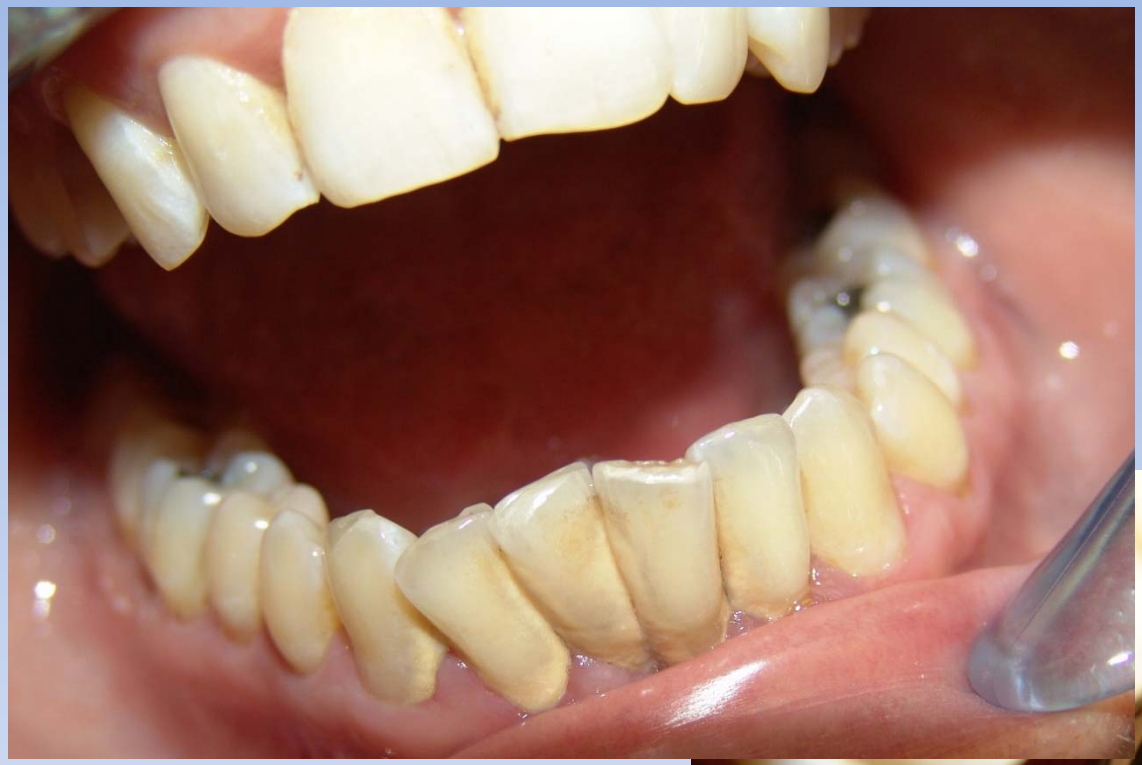
Supragingival plaque

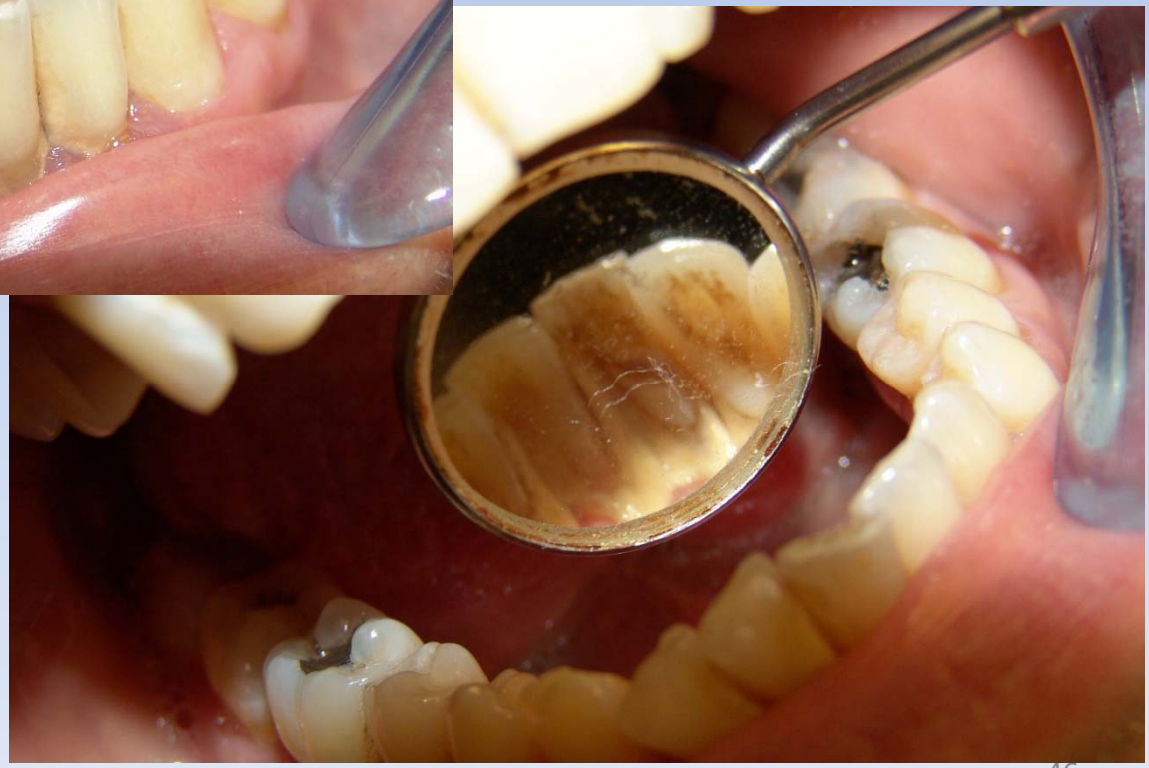




Supragingival plaque

- *Actinomyces* sp.
- Supragingival plaque – significantly higher amount of some *Actinomyces* sp., neisseriae, streptococci, and bacteria of "green" and "purple" complex
- Periodontal pathogens





Dental plaque on dental plates

- Different and fluctuating composition
- Area close to the mucous membranes

- *Candida* sp.

- Anaerobes G+ rods incl. *Actinomyces israelii* , but also G- cocci - *Veillonella* sp.
- Commonly also staphylococci, mostly STAU

Microbiology of dental caries

- Most common civilisation illness
- Bounded destruction of tooth tissue
- Multifactorial illness
  - Endogenous factors
  - Nutrition
  - Microbial factors
- Affection
  - Demineralization by acids
  - Microbial metabolism of saccharides

Endogenous factors

- Tooth profile
- Structure of enamel
- Saliva

Nutrition

- Saccharides intake x caries formation
- Saccharose
- Consumption
- Also glucose, galactose, lactose, soluble amyls

Microbial factors

- Specific plaque hypothesis
- Unspecific plaque hypothesis
- Most of microbes present have biochemically cariogenic potential

S. mutans group

- Most commonly *S. mutans* c, e & f and *S. sobrinus* d & g
- Some of them more cariogenic
- Correlation of their numbers and progression of caries lesion
- Glucans formation
- Animal models
- Survive and multiply in low pH
- Create low pH environment very rapidly - deminalization
- Form glycogen for time with low or no saccharide intake
- Immunization of animals

Lactobacilli

- High numbers in caries lesions
- Correlation with caries activity
- Multiply in low pH
- Form lactic acid
- Animal models
- In plaque of healthy teeth low numbers

BUT

- In the initial caries lesions commonly not present

Actinomycetes

- Esp. *A. viscosus* relation to dental root caries
- Role not clear

Veillonela

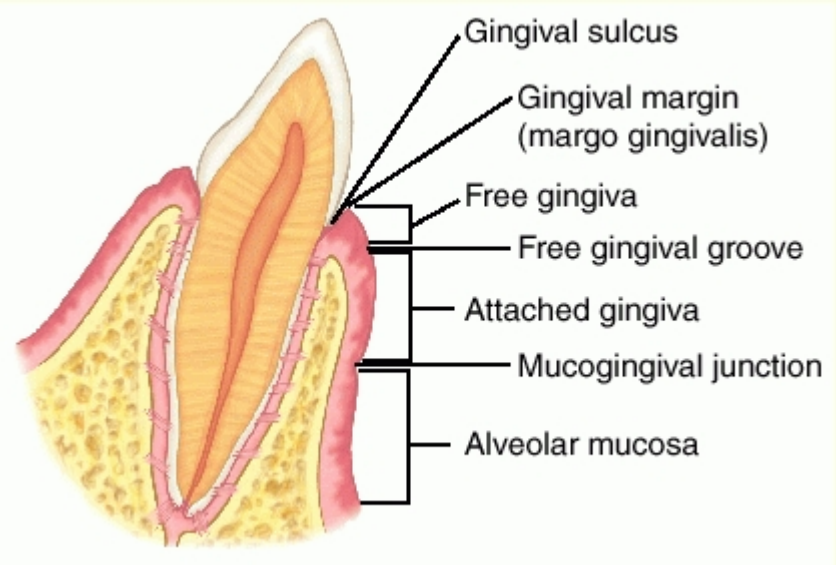
- In most of supragingival plaques
- Lactate from other microbes for their growth

Dental root caries

- *A. naeslundii, A. odontolyticus, A. gerensceriae*
- Low numbers of *S. mutans* group & lactobacilli
- Higher ratio of G- species (*P. nigrescens, Capnocytophaga* sp., *Campylobacter* sp., *Leptotrichia* sp.)

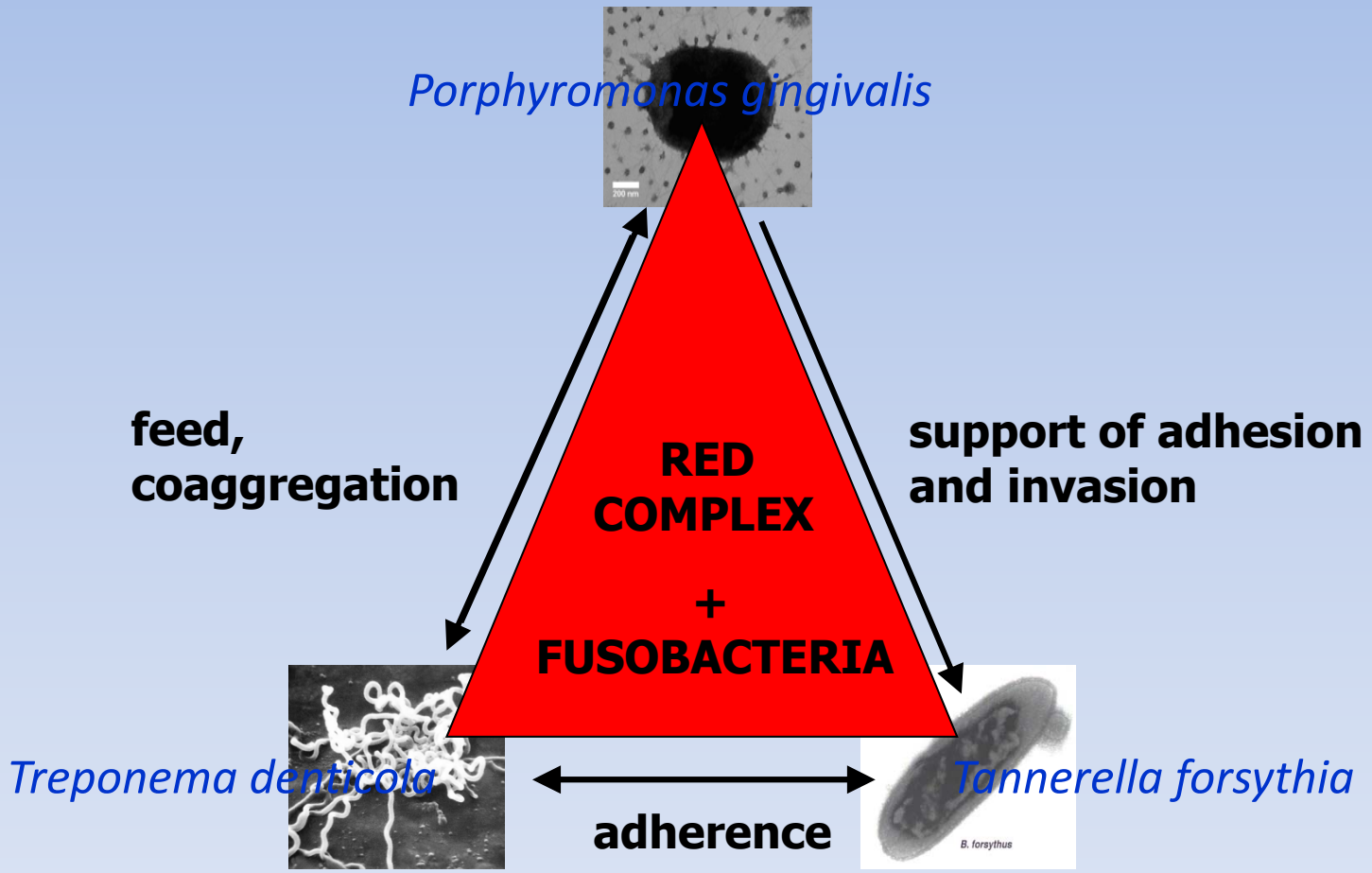
Microbiology of parodontitis

- **Sulcus gingivalis I.**
- Anaerobic environment
- Sulcar fluid
- Rich in nutrients
- Bacteria are important for development of parodontic illness



- Mix of aerobic and anaerobic species – SRSP, *Actinobacillus*, *Fusobacterium*, *Treponema* sp., *Wollinella* sp., RED COMPLEX bacteria…

Red complex bacteria

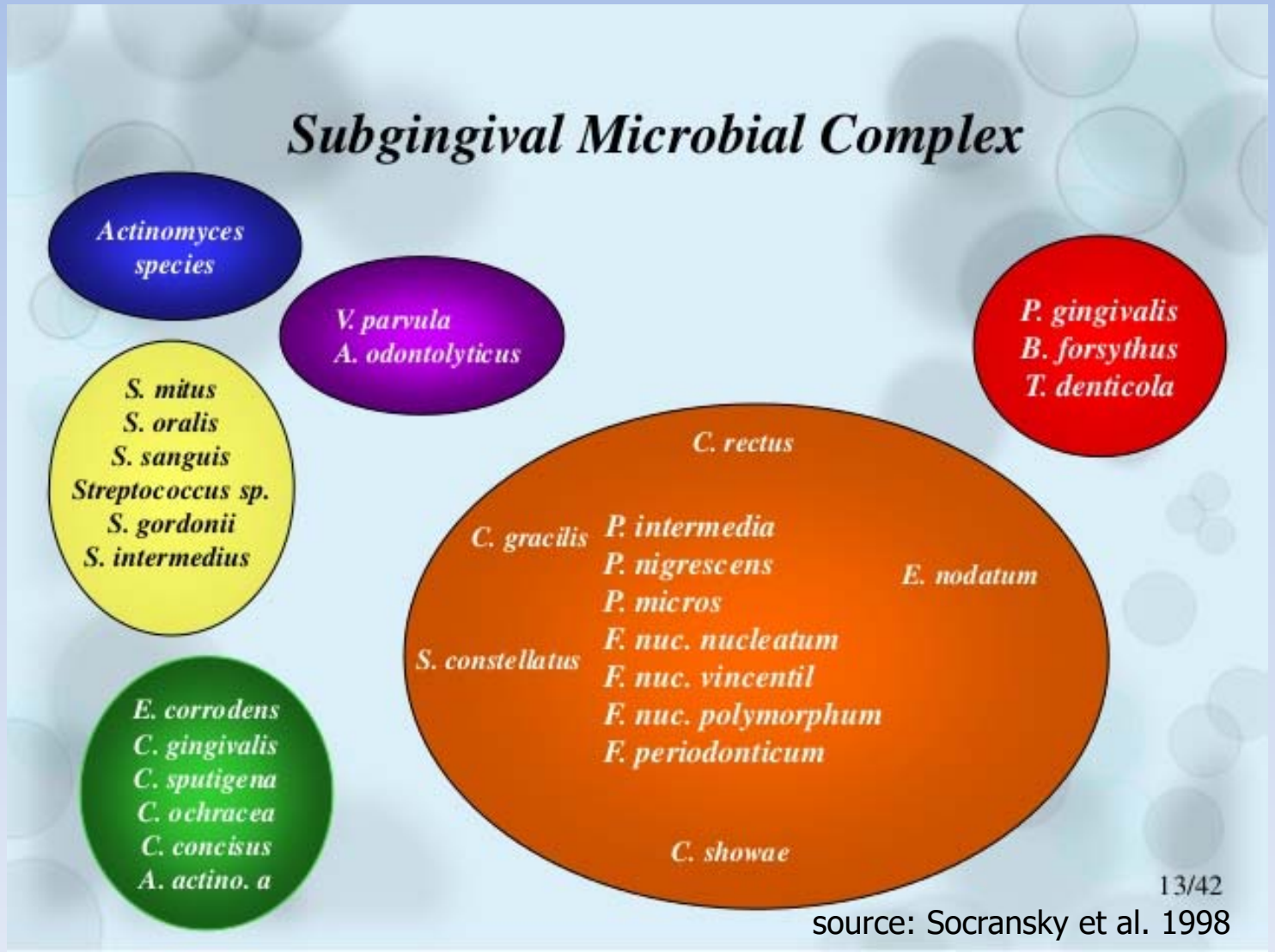


Responsibility for parodontitis

Sulcus gingivalis II.

ANAEROBES	*Aggregatibacter (Actinobacillus) actinomycetemcomitans* *Actinomyces – A. gerencseriae, A. georgiae* *Fusobacterium – F. nucleatum, F. alocis, F. sulci* *Prevotella nigrescens* *Porphyromonas gingivalis, P. endodontalis* *Treponema denticola, T. vincentii, pectinovarum, socranskii* *Tannerella forsythia* *Wolinella succinogenes* *Selenomonas sputigena*
AEROBES	*Streptococcus anginosus, Streptococcus constellatus* subsp. *constellatus, Streptococcus constellatus* subsp. *pharyngis*, *Streptococcus intermedius*

Relationship of bacterial communities in parodontitis



source: Socransky et al. 1998

Subgingival microbial complexes I.

- **The red complex**
  - *Porphyromonas gingivalis*
  - *Tannerella forsythia*
  - *Treponema denticola*
- Association with severe forms of periodontal disease
- Very strong relationship with pocket depth
- Sites with none of the species exhibited the shallowest mean pocket depth, while sites harboring all 3 showed the deepest

Subgingival microbial complexes II.

- **The orange complex**
  - *Fusobacterium nucleatum* - *Eubacterium nodatum*
  - *Prevotella intermedia* - *Campylobacter showae*
  - *Prevotella nigrescens* - *Campylobacter gracilis*
  - *Peptostreptococcus micros* - *Campylobacter rectus*
  - *Streptococcus constellatus*
- Appears related to the red complex
- Closely associated with one another
- *P. intermedia* + *F. nucleatum* - deep pockets
- Significant association with increasing pocket depth
- MTZ decreased their levels

Subgingival microbial complexes III.

- **The yellow complex**
  - *Streptococcus sanguis*
  - *Streptococcus oralis*
  - *Streptococcus mitis*
  - *Streptococcus gordonii*
  - *Streptococcus intermedius*
- Not directly associated with periodontal disease

Subgingival microbial complexes IV.

- **The purple** and **green complexes**
- Not significantly associated with periodontal diseases
- The **purple complex**
  - Not directly associated with gingivitis or periodontitis
- Prepares the way
- **The blue complex**
- Not associated with progression of periodontal diseases

Systemic infections related to the oral microflora

- Affection of many systemic illnesses
  - Metastatic infections
  - Metastatic injury
  - Metastatic inflammation

Metastatic infection

- Metastatic infection from oral cavity via transient bacteremia
  - Subacute infective endocarditis
  - Acute bacterial myocarditis
  - Brain abscess
  - Cavernous sinus thrombosis
  - Sinusitis
  - Lung abscess/infection

Metastatic injury

- Metastatic injury from circulation of oral microbial toxins
  - Cerebral infarction
  - Acute myocardial infarction
  - Abnormal pregnancy outcome
  - Persistent pyrexia

Metastatic inflammation

- Metastatic inflammation caused by immunological injury from oral organisms
  - Behçet's syndrome
  - Chronic urticaria
  - Uveitis
  - Inflammatory bowel disease
  - Crohn's disease

Bacteraemia

- *Propionibacterium acnes*
- *Peptostreptococcus prevotii*
- *Fusobacterium nucleatum*
- *Prevotella intermedia*
- *Saccharomyces cerevisiae*
- *Actinomyces israelii*
- *Streptococcus intermedius*
- *Streptococcus sanguis*

Other consequences

- *P. gingivalis* has been linked to rheumatoid arthritis
- *T. forsythia* has been identified in atherosclerotic lesions

Subacute infectious endocarditis

- Endocarditis
  - Besides presence of microbe in blood stream
  - Also more narrow relationship to the endocardium, especially in damage by previous disease or treatment
- Commonly - *S. sanguis* and *S. gordonii, S. oralis, S. peroris,*
- Rarely - *S. salivarius, S. vestibularis*
- Difficult and long-term treatment

Risk of IE I.

- ***Low***
  - Defect of heart atrium septum
  - Implantation of pacemaker
  - After bypass surgery
- ***Medium***
  - Other congenital heart defects
  - Provisional surgery of heart defects
  - Rheumatoid valve defects
  - Prolaps MI valve with MI insufficiency
  - Hypertrophic obstruction cardiomyopathy (HOCM)
  - After surgical correction of cardiovascular defect (later than ca 6 mths)

Risk of IE II.

- ***High***
  - Heart valve replacement
  - After IE
  - After surgical correction of cardiovascular deffect (until ca 6 mths)

Therapy recommendation

- Consultation and cooperation with dentists & other specialists
- ATB prophylaxis
- Medium risk
  - Adults: AMC 2g in 1 dose 1 hr prior to intervention
  - Children: AMC 50mg/kg, max. 3g in 1 dose 1 hr prior to intervention
- High risk
  - Adults: AMC 2g in 1 dose 1 hr prior to intervention, followed by 750 mg/6 hrs (7 doses)
  - Children: AMC 50mg/kg, max. 3g in 1 dose 1 hr prior to intervention, followed by 15 mg/kg/6 hrs (7 doses)

Therapy recommentation

- In PNC allergy alternatively CLI (600mg, children 15 mg/kg 1 hr prior to intervention)

- In high risk continue with 300 mg/6hrs (7 doses), children 7,5 mg/kg/6hrs (7 doses)