Oral microbiology

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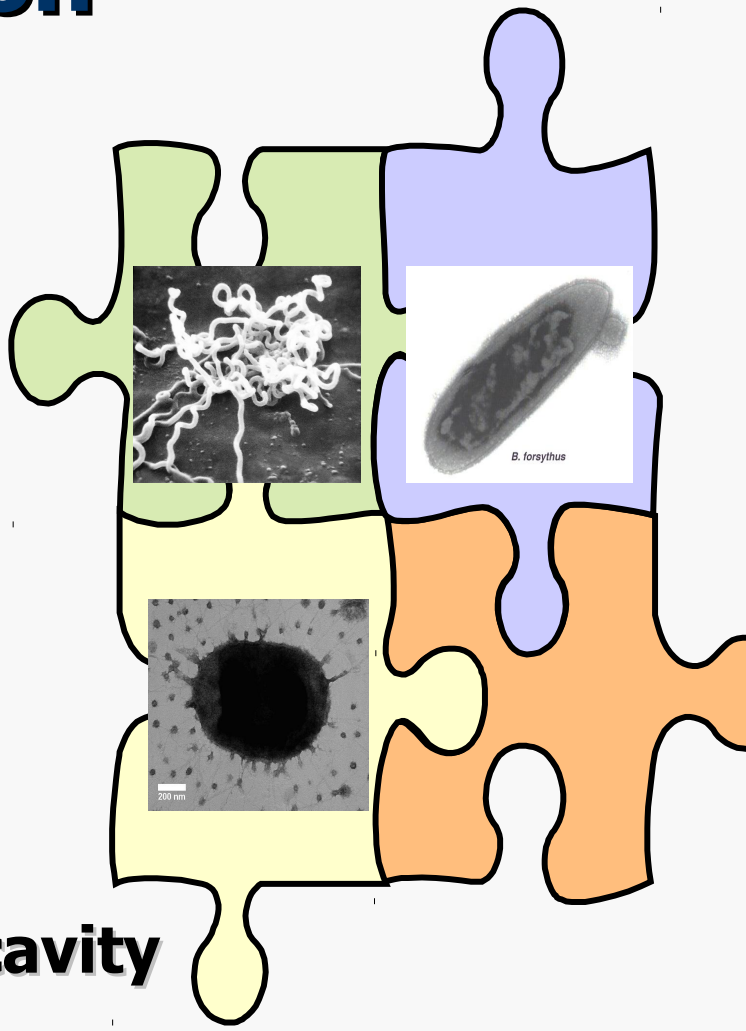
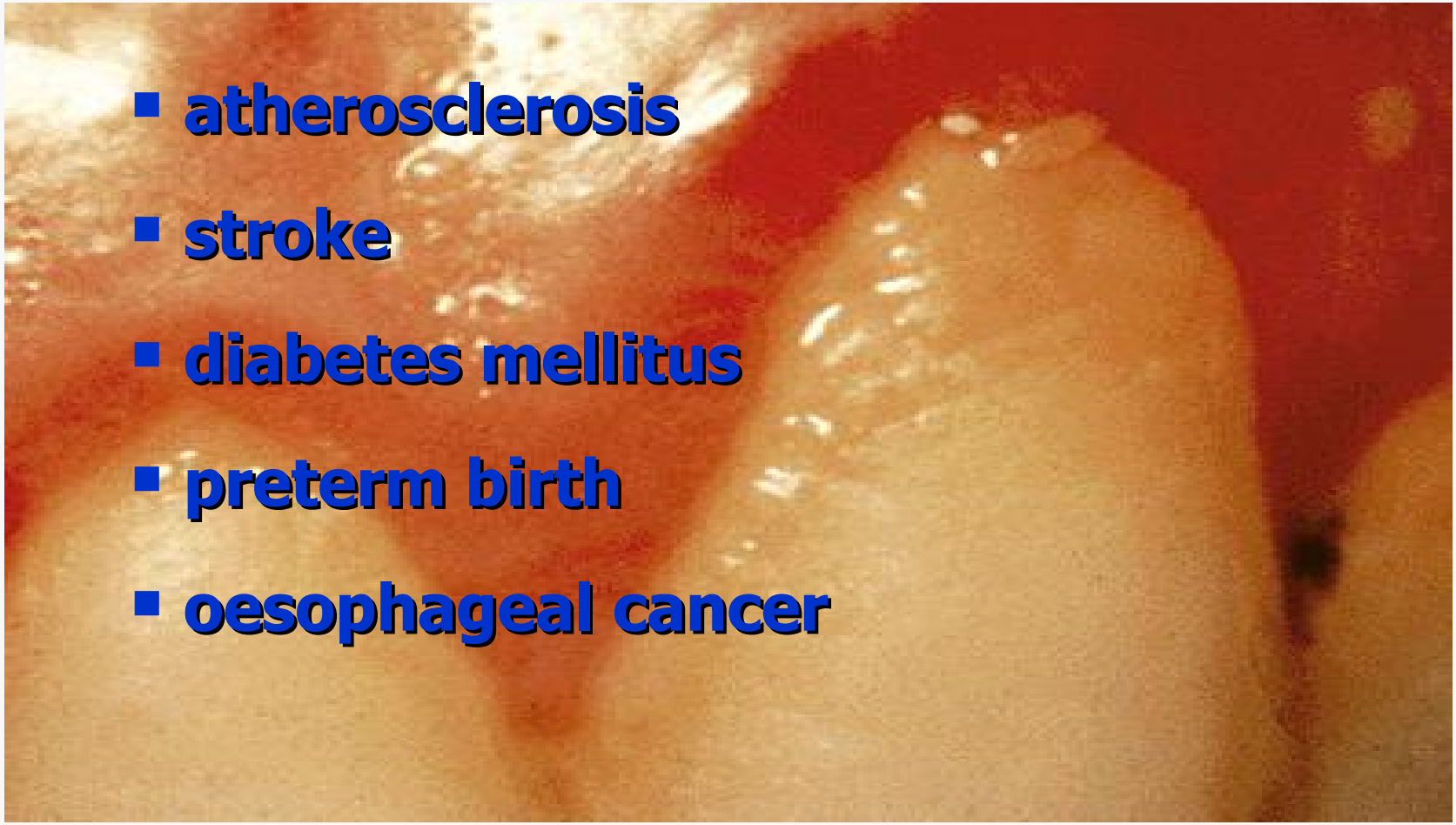
Lectures - Dentistry / spring 2014

Introduction

- The resident oral microflora
- Dental plaque
- Dental caries
- Periodontal diseases
- Infectious diseases in the oral cavity

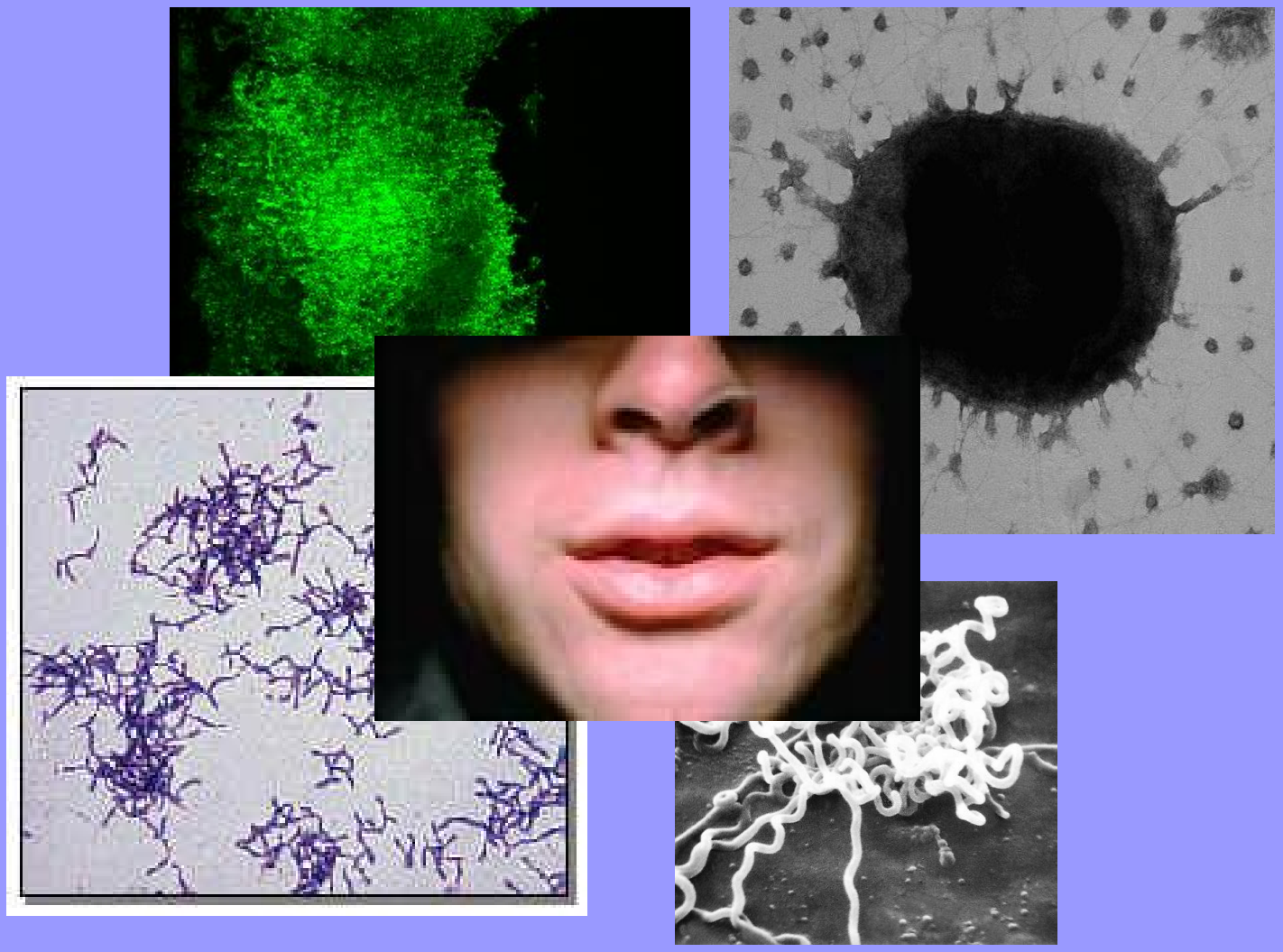


Consequences

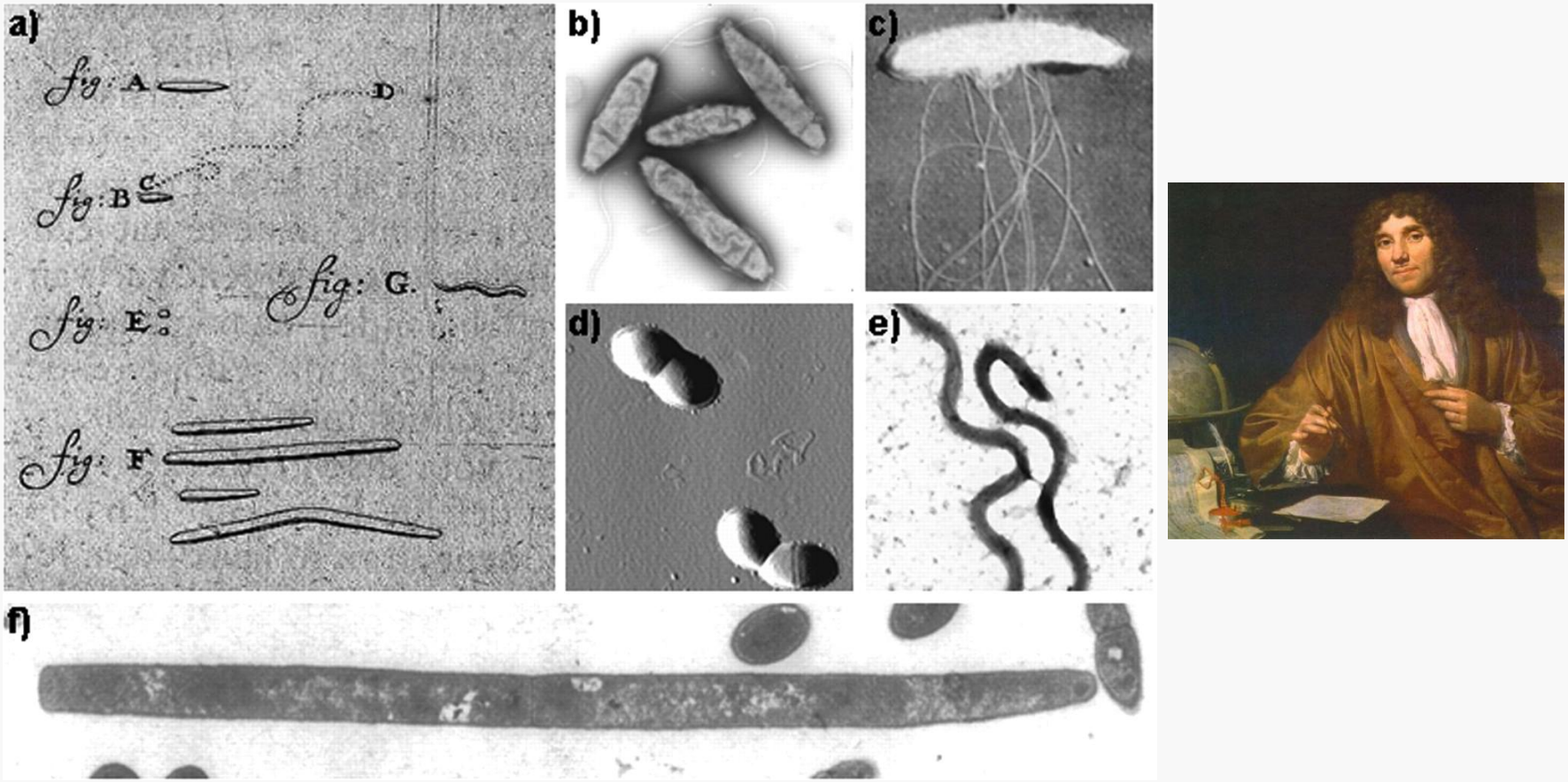


- atherosclerosis
- stroke
- diabetes mellitus
- preterm birth
- oesophageal cancer

I. The resident oral microflora



Leeuwenhoek 1632 –1723



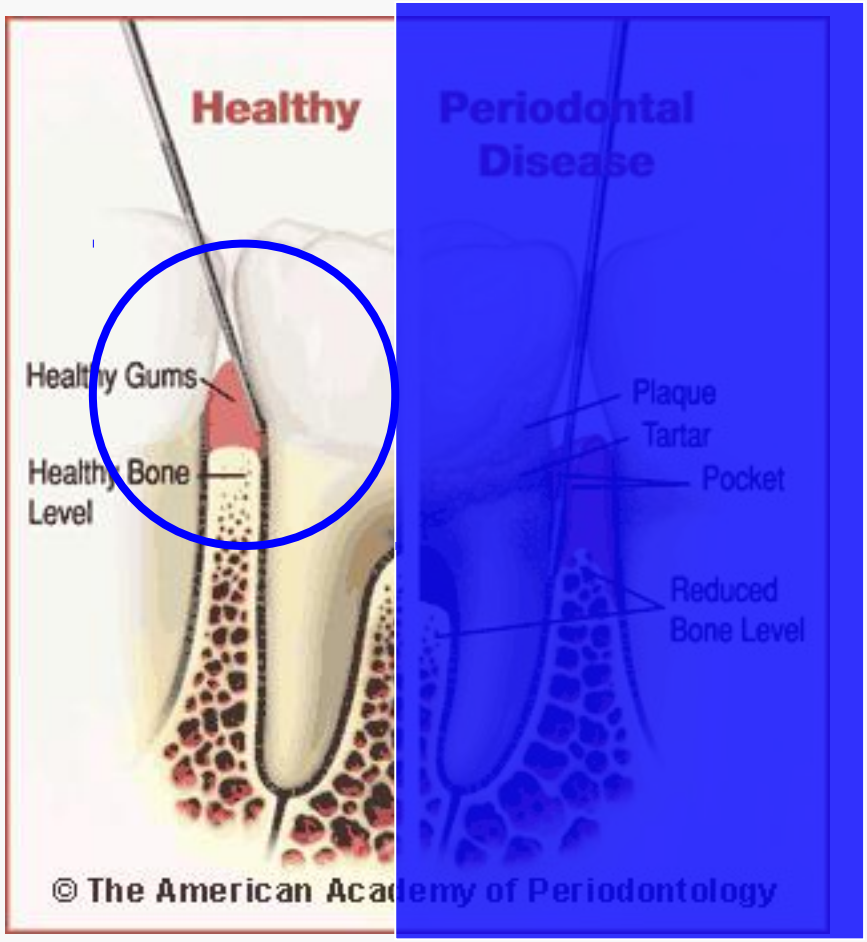
(a) Original drawing A.L., (b) *Campylobacter rectus,* (c) *Selenomonas sputigena* , (d) oral cocci (e) *Treponema denticola,* (f) *Leptotrichia buccalis*

The resident oral microflora

- One of the broadest microbial communities, over **700 genera**, some were not still described
- Resident – commensal, or transient
- Ecological system
- **Biofilm formation**
- Influential factor of human health (both local and in general)
- Etiology of **dental caries and parodontitis**

Sulcus gingivalis

Colonizing bacteria – the key factor in development of parodontal diseases, **anaerobic environment**



Sulcus gingivalis - microflora

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*

Streptococcus

- α-hemolytic streptococci, divided into the following groups:

- **S. mutans group:**

  S. mutans - the MOST FREQUENT, less often *S. sobrinus, S. cricetus,* and *S. rattus* (rare), make acids from saccharides

- **S. salivarius group:**

  S. salivarius, S. vestibularis - in saliva and on the tongue surface, growth in mucous colonies, can cause endocarditis.

- **S. mitis group:**

  Subacute bacterial endocarditis (SBE)

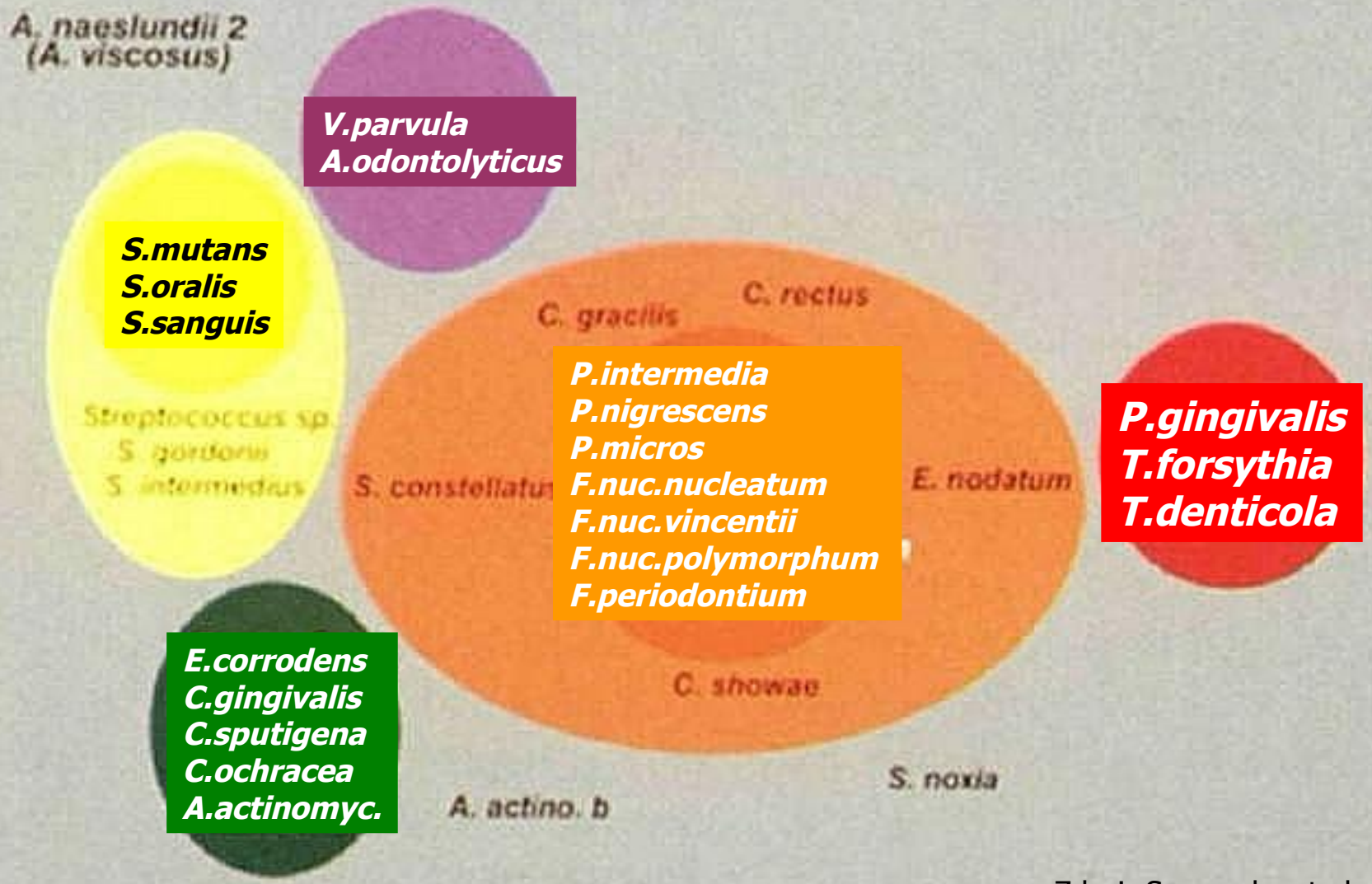
  S. mitis, S. oralis a *S. peroris* – on mucous membranes and dental **plaque** - the causative agent of SBE (*S. mitis* exemption)

  S. sanguinis and *S. gordonii* – the **tongue**, buccal mucous membranes, dental **plaque**. *S. sanguinis* cleaves secretorial IgA.

- **S. anginosus group** - growing in tiny colonies - Dentoalveolar and endodontal infections

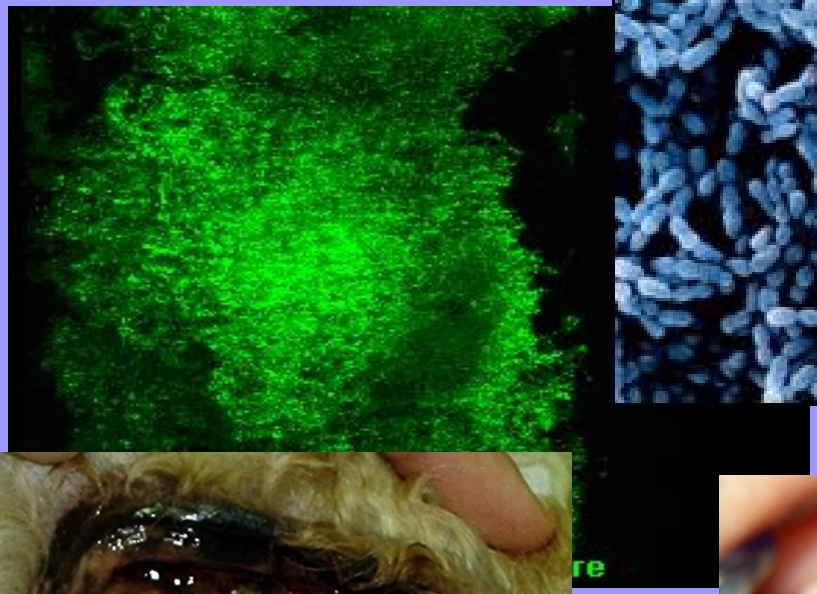
  S. anginosus (*S. milleri* in British texts), *S. constellatus* and *S. Intermedius,* in nasopharynx, sulci gingivales

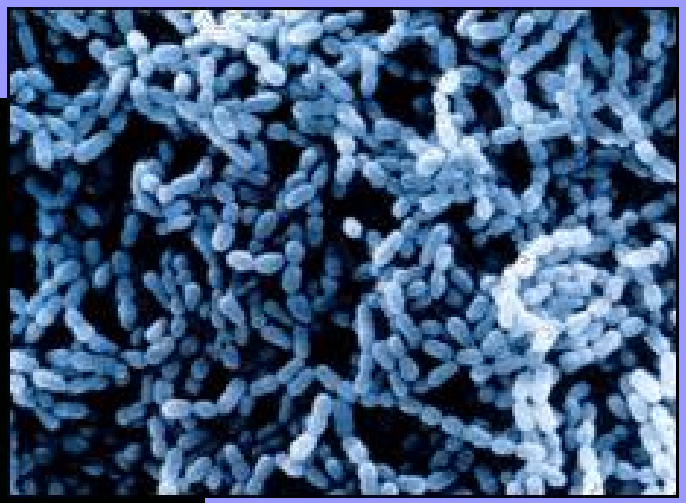
Bacterial communities in periodontitis

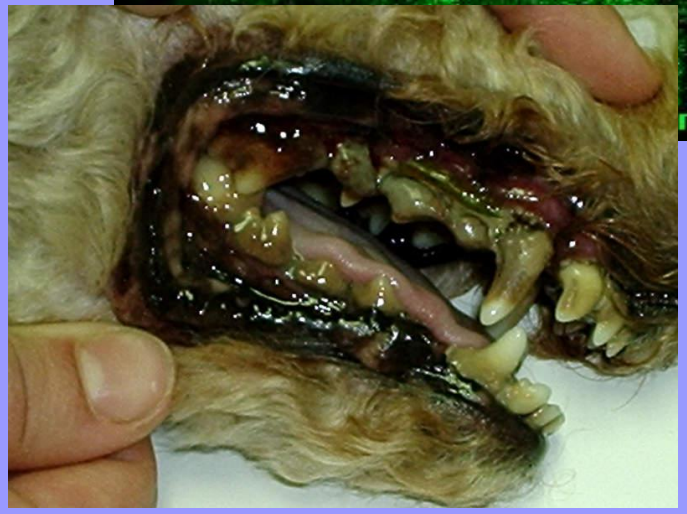


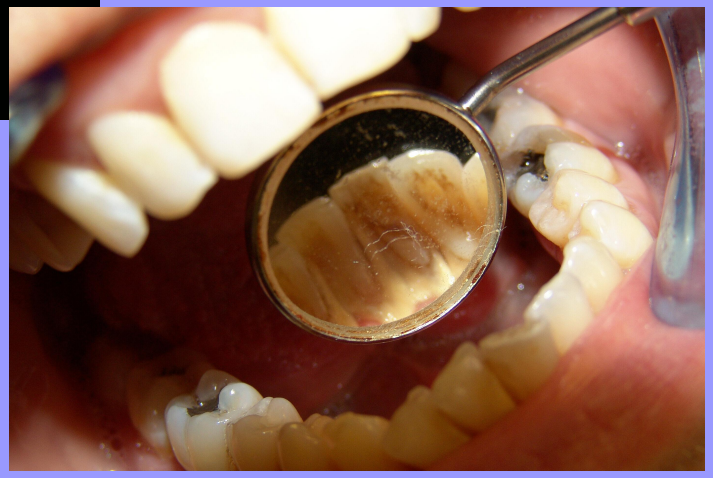
Zdroj: Socransky et al. 1998

II. Dental plaque









Dental plaque - biofilm

- **Adherent microbial layer on the tooth surface = live and dead bacteria + their products + host compounds (from saliva)**

- It can NOT be washed, can be removed only mechanically (tooth brushing)
- Composition dependent on its location and age

Location:

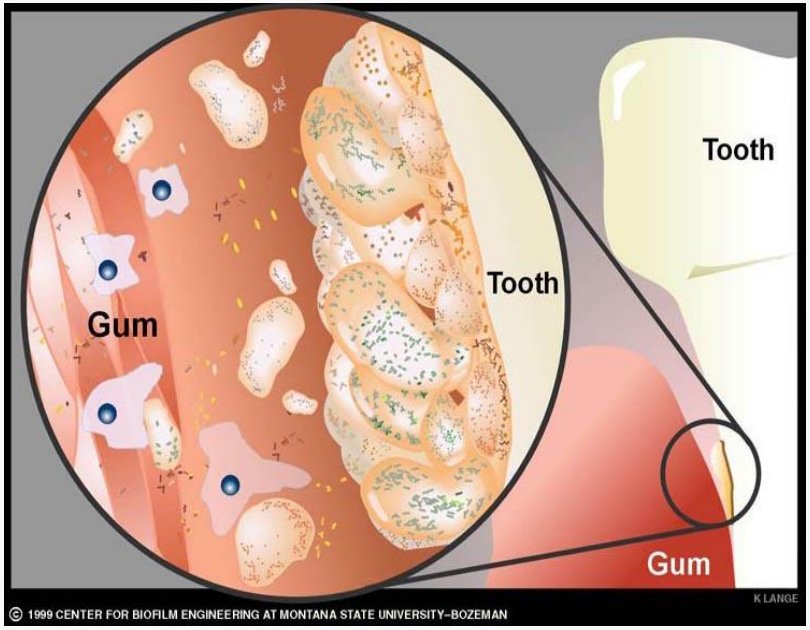
- **Supragingival plaque**
- **Subgingival plaque**

Subgingival plaque

- **Plaque of two types** - adherent and non-adherent one

- **Adherent plaque** – adherent to the dental root, similar to supragingival plaque = i.e. G+ rods, filaments (actinomycetes), and G+ cocci

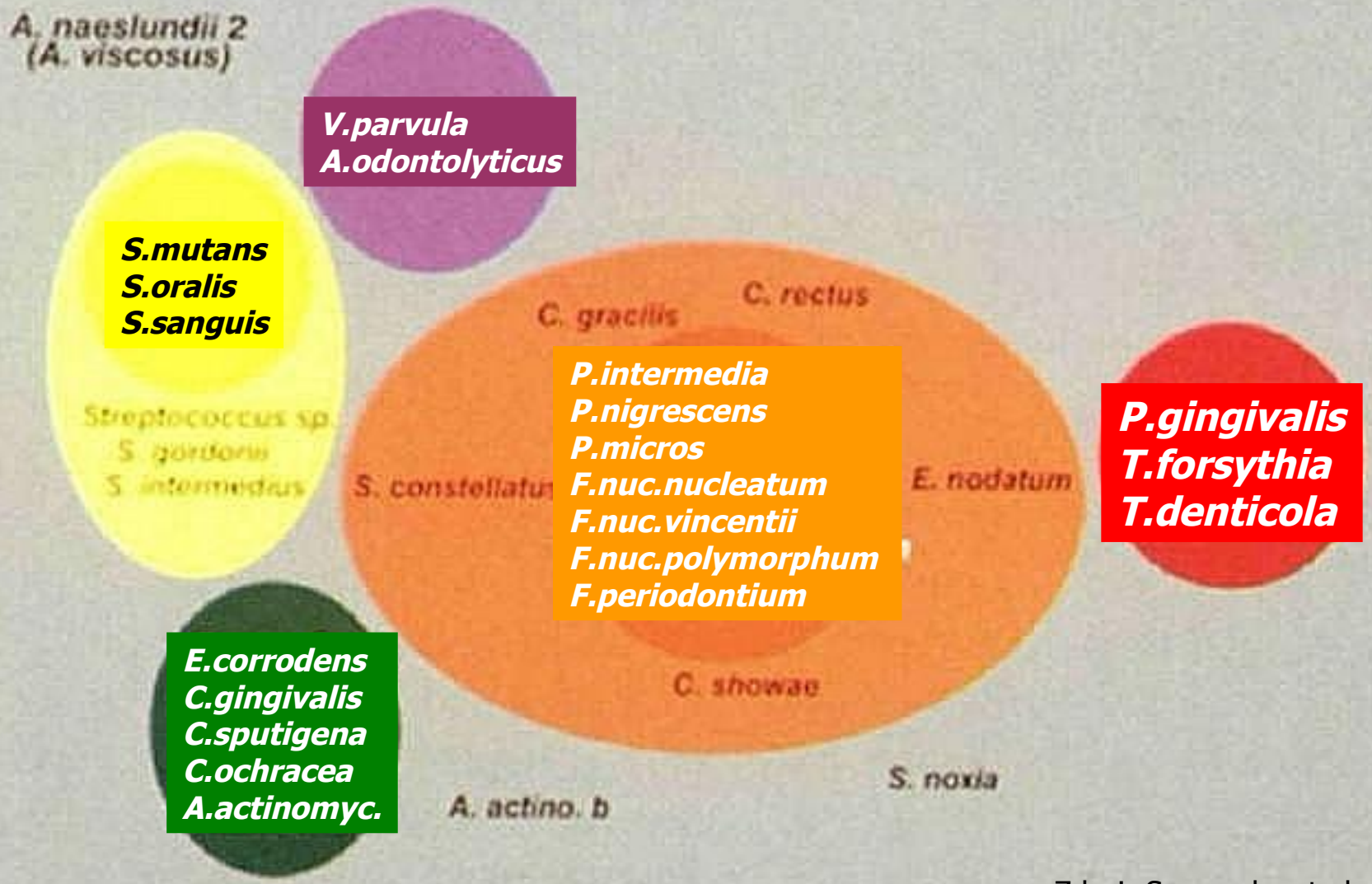
- **Non-adherent plaque** – between adherent plaque and gingival surface = G- motile anerobes



Distribution of microorganisms

- **Actinomyces sp.** is the most frequent genus in both supra- and subgingival plaque
- **Supragingival plaque** – significantly higher amount of some actinomyces sp., neisseriae, streptococci, and bacteria of **"green" and "purple"** complex
- Periodontal pathogens can be occasionally found in supragingival plaque
- Supragingival plaque – reservoir of infection in the subgingival area
- **Subgingival plaque** - significantly higher amount of *Prevotella* sp., *Tannerella forsythia* and *P. gingivalis*, i.e. the **"red" a "orange"** complex (Ximénez-Fivye et al., 2000)

Bacterial communities in periodontitis

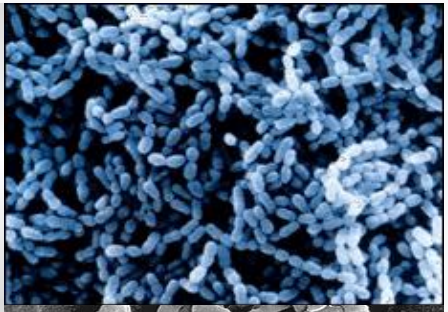


Zdroj: Socransky et al. 1998

Development of dental plaque

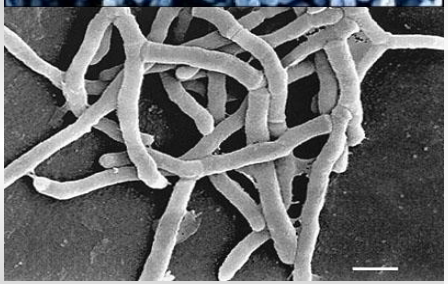
Less than 24 hours

Streptococci of *mutans, sanguis,* and *mitis groups are prevalent in suprag. plaque*



Days

G+ rods and filamentous microorganisms (lactobacilli, actinomycetes) accumulate

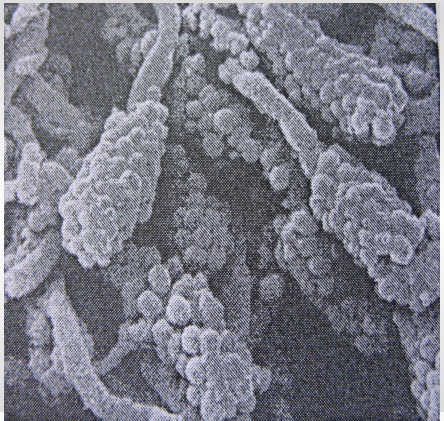


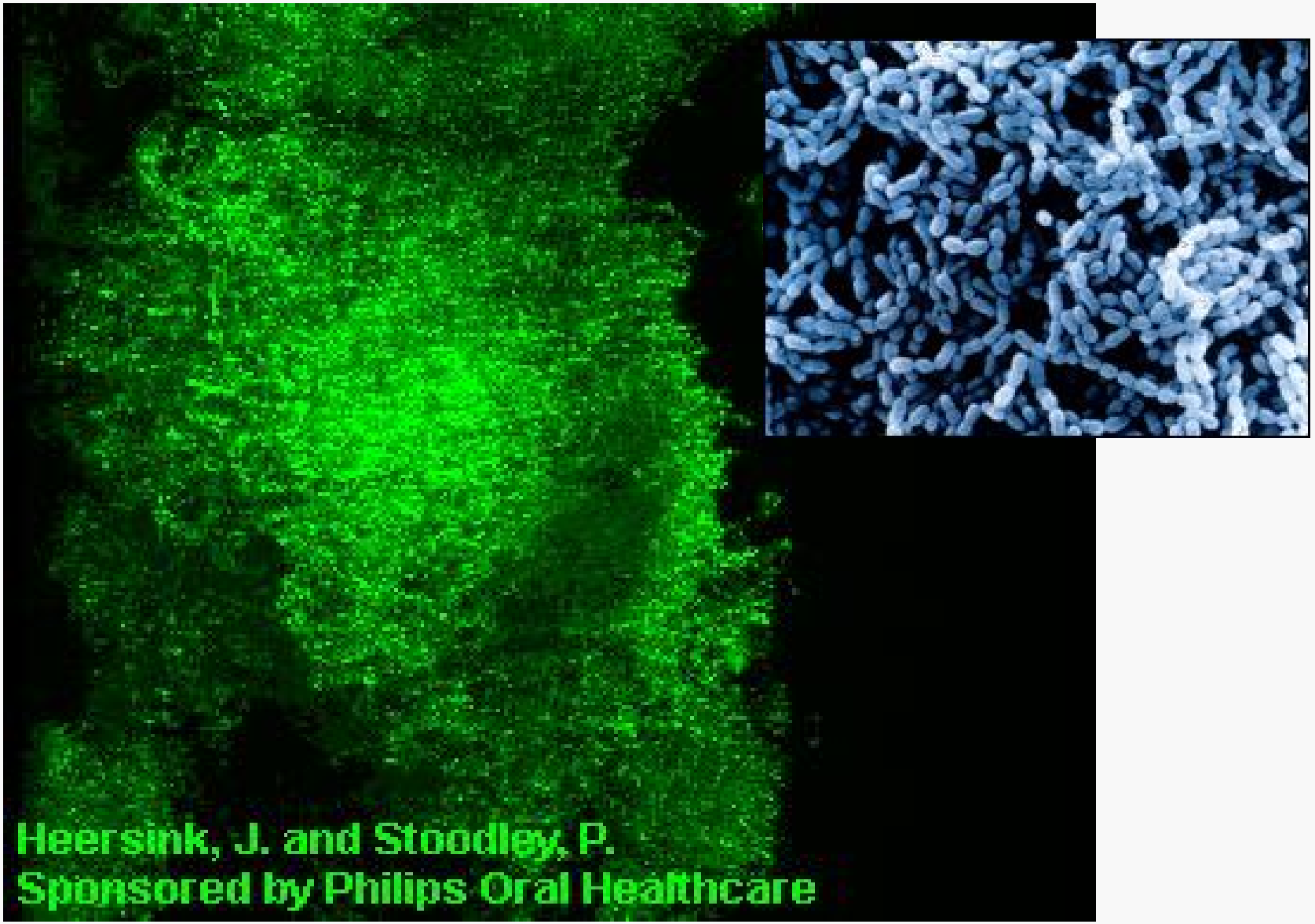
Week

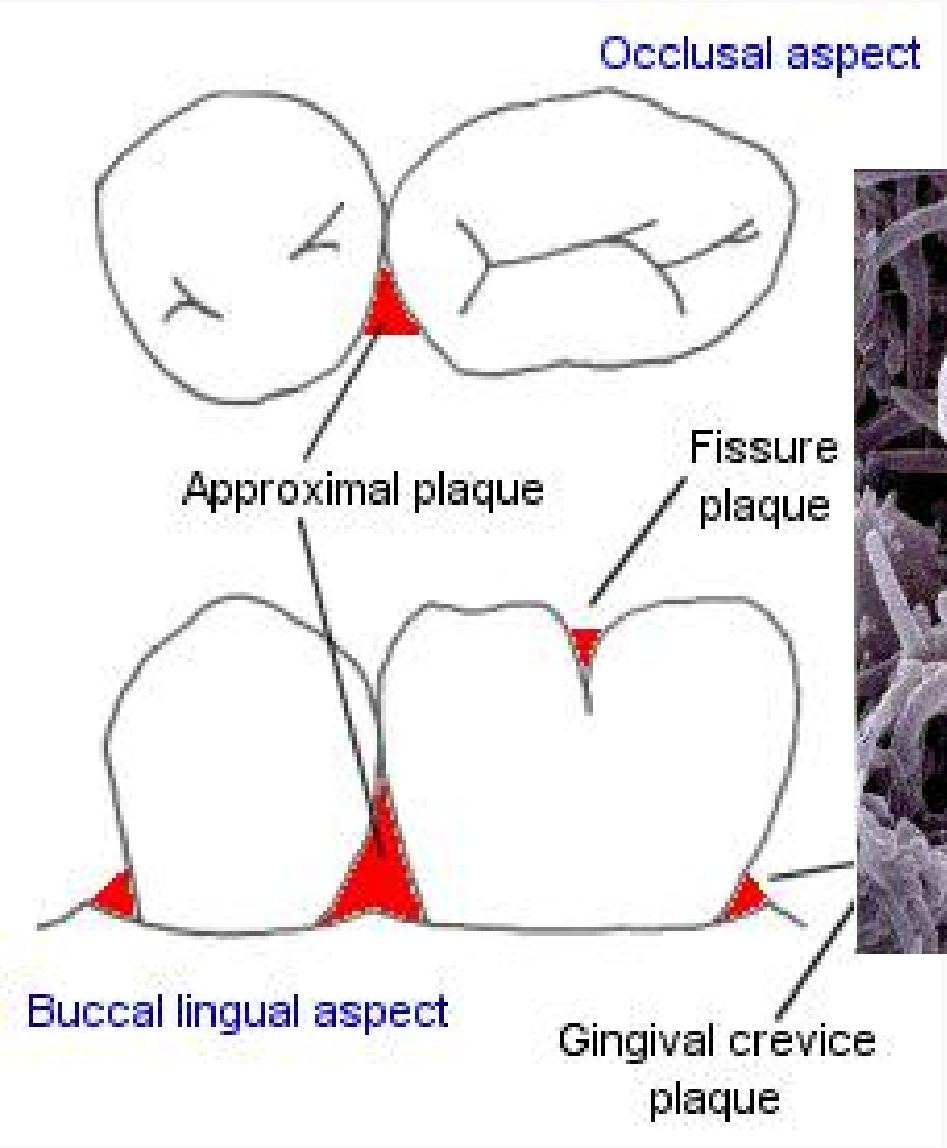
Columns/microcolonies of coccoid microbes – rods and filamentous microbes get attached on their surface

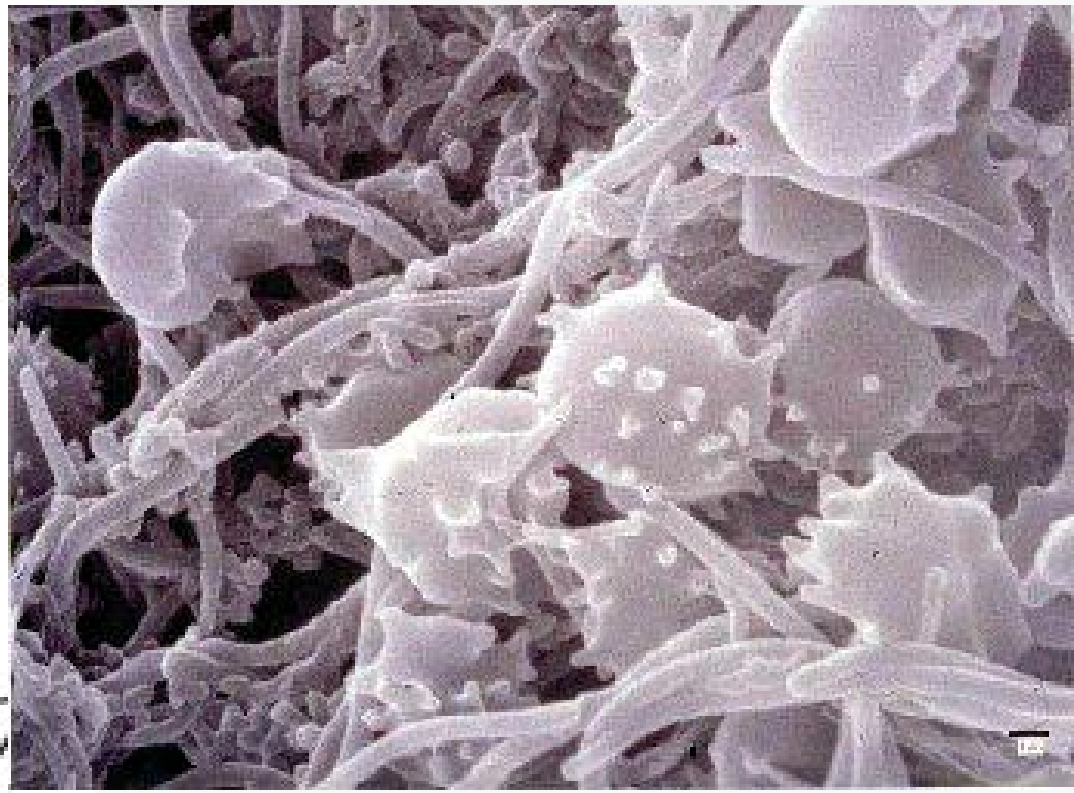
Three weeks

filamentous microbes are prevalent,
„corn-cob" formation:
a central filament (*Eubacterium yurii*)
is encompassed by G+ cocci

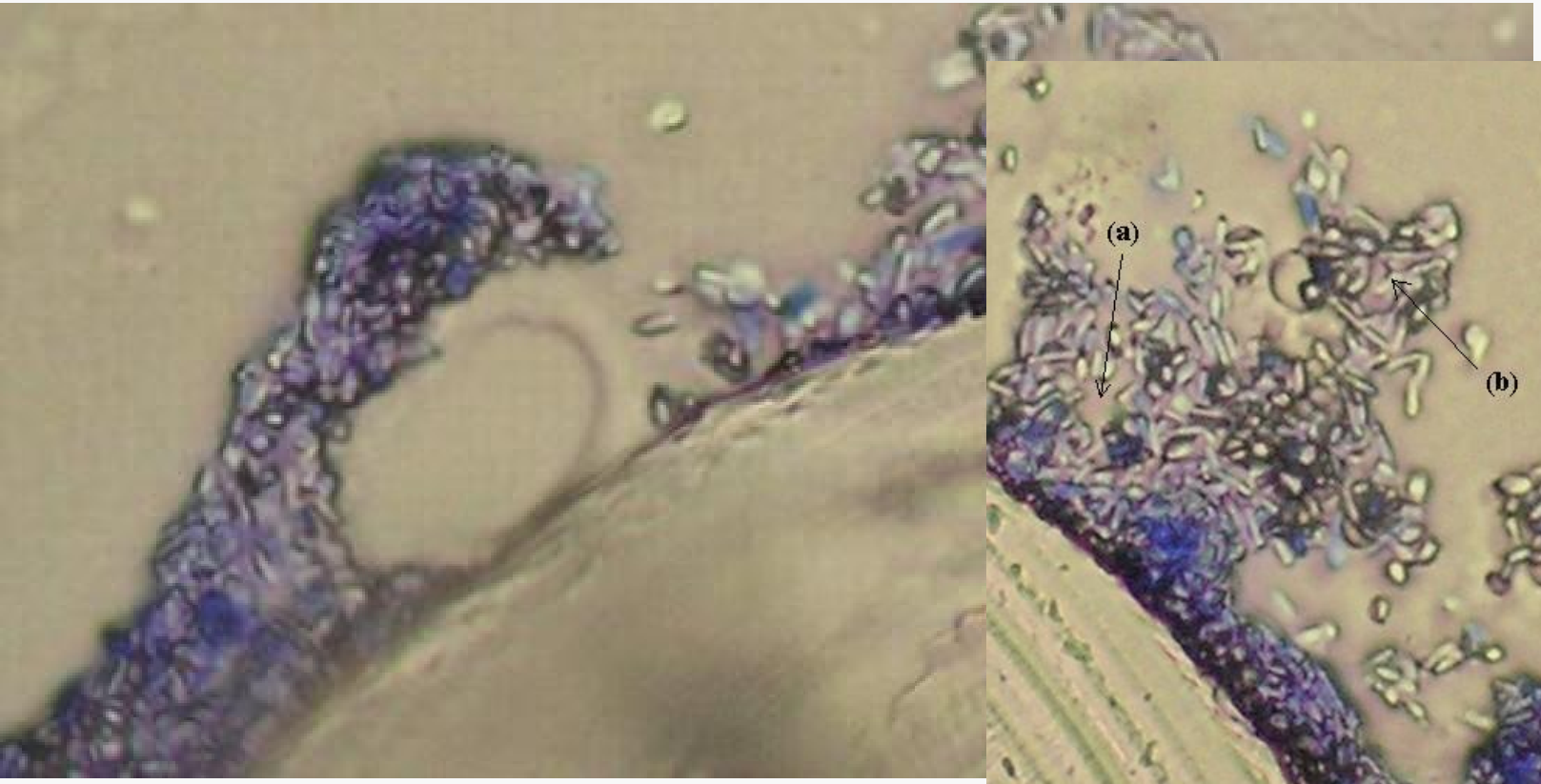



Heersink, J. and Stoodley, P.
Sponsored by Philips Oral Healthcare





Source: www.bact.wisc.edu
www.ncl.ac.uk

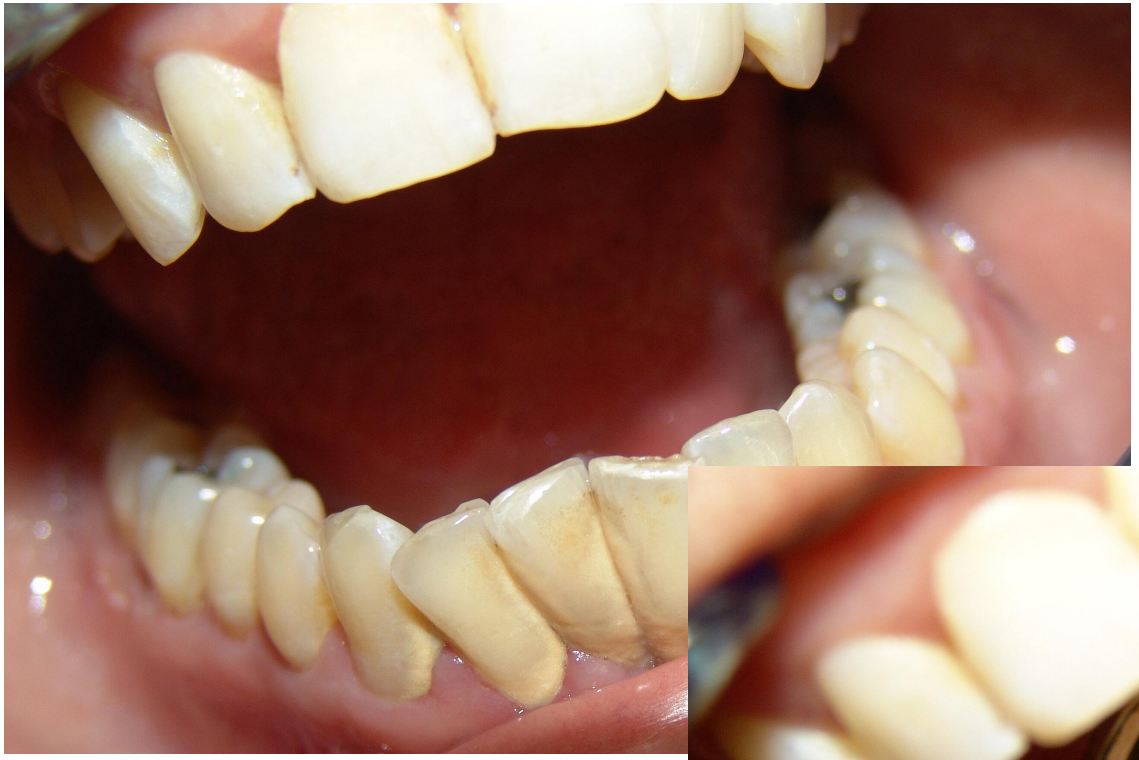


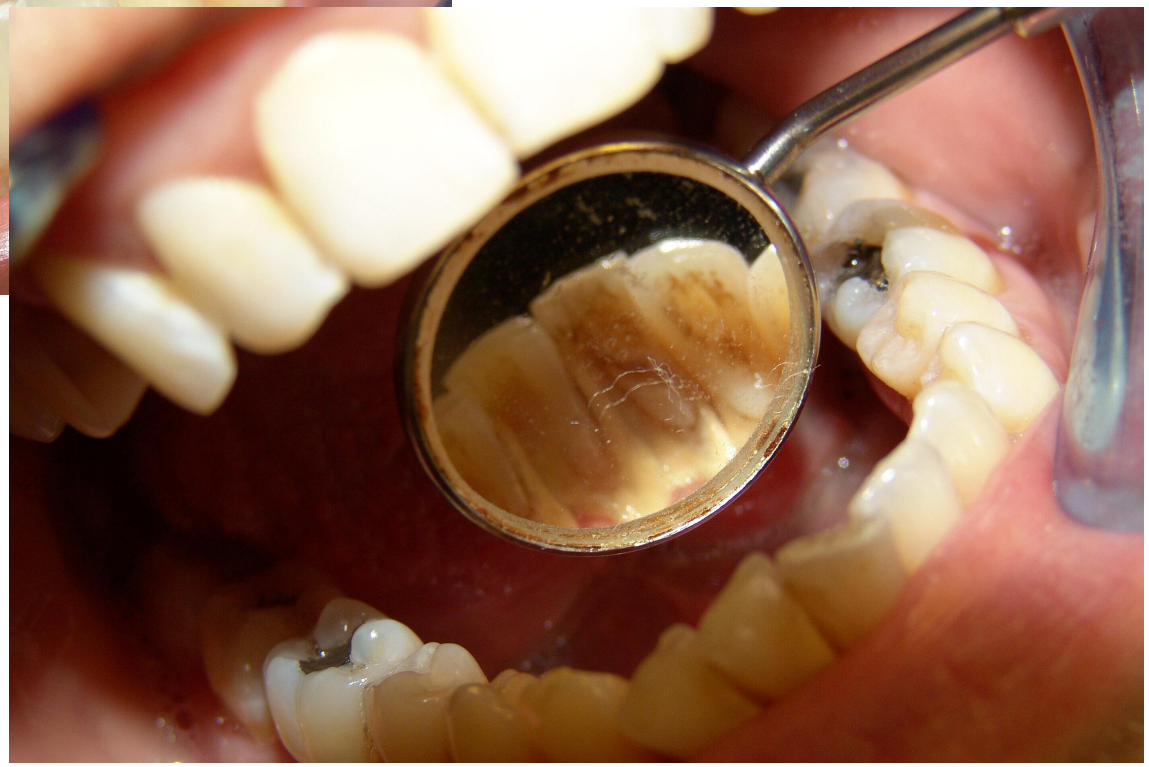
Biofilm on a catheter (stafylococci and candida):
a) - canaliculus, b) - porous structure

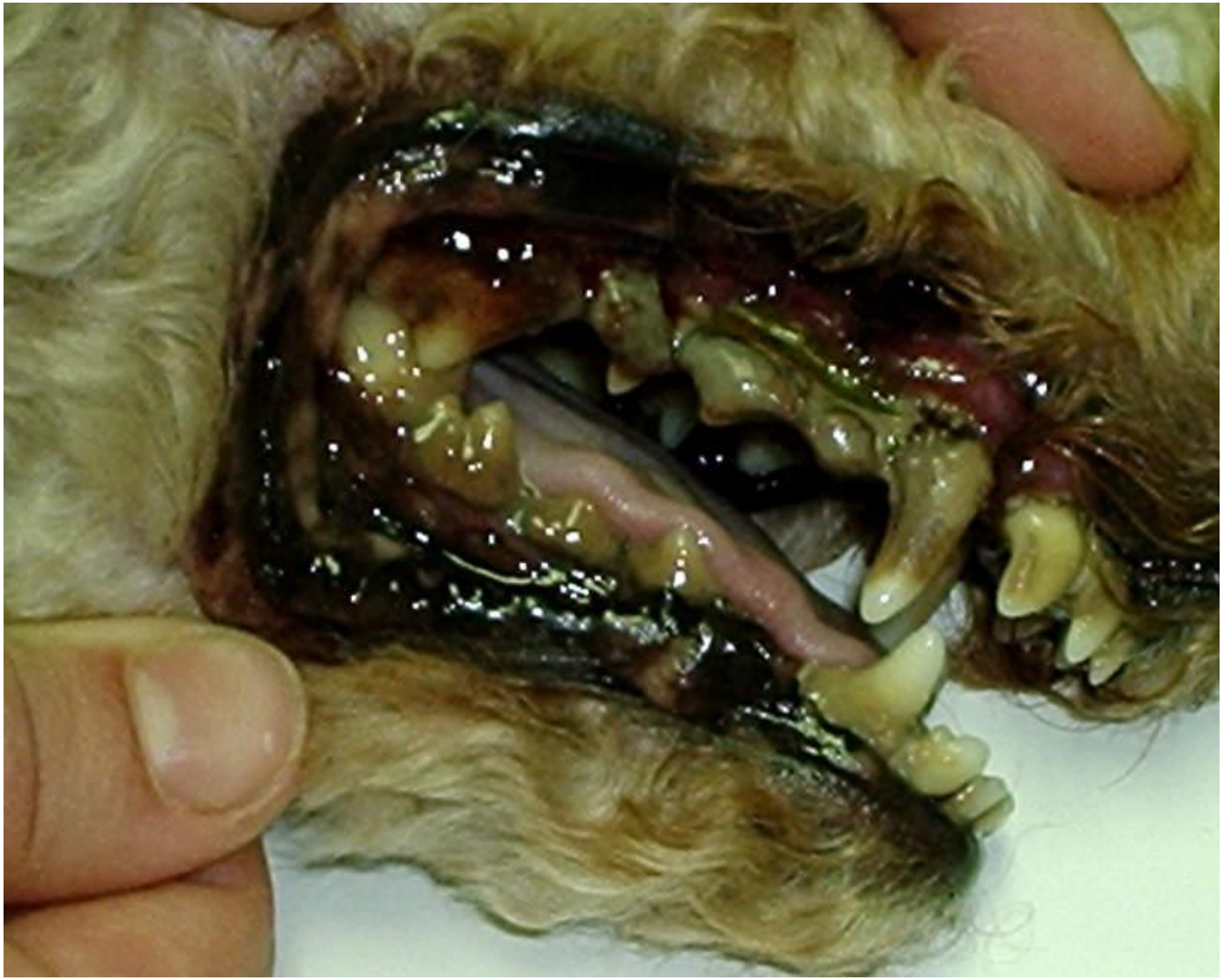
Photo: Dr. Veronika Holá, MÚ

Dental plaque development

- Glykoproteins – **pellicula** – receptores for G+ cocci and rods
- **Exopolysacharid** production – the main part of the intercellular matrix
- Bacterial metabolism in plaque – other species involvment, development quicker in a presence of **sacharose**
- In bottom layers **calculus (tartar)** is being formed– calcified dental plaque - 80 % minerals
- **pH** decreases as a result of bacterial metabolism to < 5.5 – enamel demineralisation
- **Subgingival calculus** - G - mikroorganisms
- Calculus is porous – filamentous bacteria on the surface – deposits of microbial compounds - **toxic for parodontal tissues**



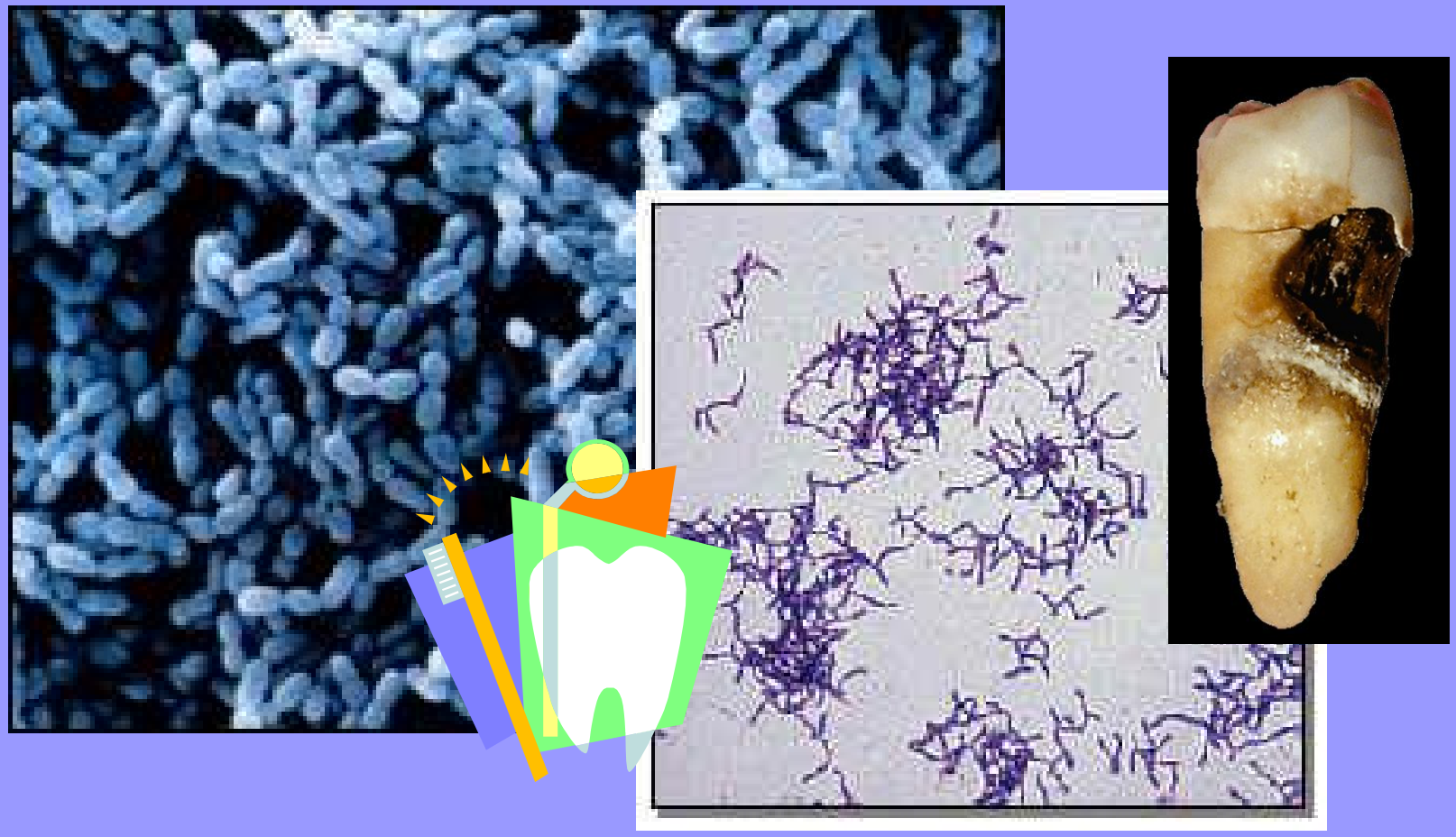




Dental plaque on dental plates

- Area close to the mucous membranes - streptococci *mutans* and *sanguinis* prevalent + *Candida sp.* found very often
- Anaerobes - G+ rods *Actinomyces israelii* and veillonelae
- often staphylococci, especially *Staphylococcus aureus*

III. Dental caries



History

- Archeological findings conclude that dental caries is very old disease
- Increase in number of caries lesions during neolite was a result of increaseing volume of saccharides in a diet
- In the beginning of rice-growing in South Asia as a results dental caries increase was reported
- Sumerian texts about 5000 years B.C. describe „dental worms" causing dental caries

(wikipedia.org)

Microbiology of caries

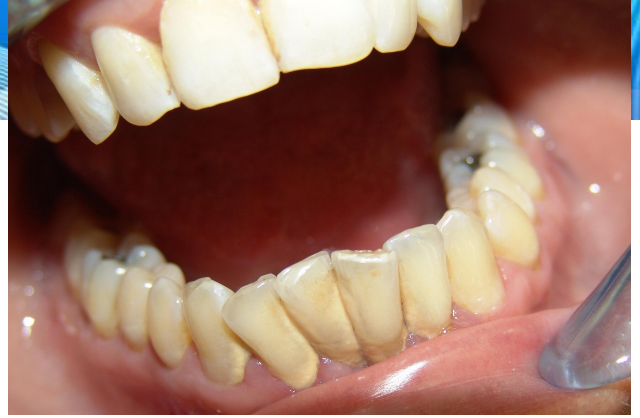
- Dental caries – the most frequent current disease
- Definition - local destruction of the tooth tissue
- Microbiological point of view – chronic infection caused by normal oral flora
- Destruction is a result of demineralisation of the tooth caused by acids producted by microorganisms in the dental plaque during metabolism of saccharides from food

Course of caries

- Primary lesion of enamel (whitish spot) is reversible, it can remineralise
- After destruction of enamel, the process spreads to dentin and causes inflammation and necrosis
- Also development of periapical acute or chronic inflammation

Dental caries = multifactorial disease

1. endogenous factors
2. food
3. microbes in the dental plague



Endogenous factors:

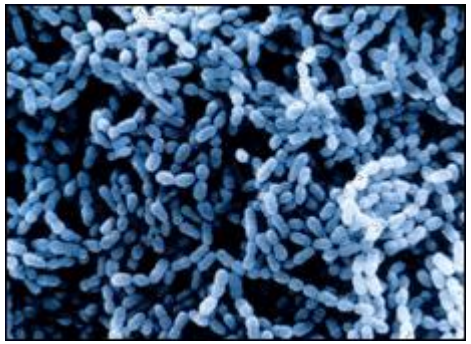
- Tooth shape
- Enamel structure
- Saliva – volume, flow and composition (buffer)

Nutritious factors:

- saccharides intake
- Saccharose is the most cariogenic sugar
  - Excellent solubility, diffund to the plaque easily - cariogenic streptococci change it to insoluble glucan
  - glucan enables initial adhesion of microbes on the tooth surface, is a source of nutrients and takes place in intercellular matrix development

Role of microbes

- almost all microbes in the dental plague have cariogenic effect thanks to their biochemical features
- the most important in caries development - streptococci of the mutans group, lactobacilli, and actinomycetes
- also combination of other microbes can start the cariogenic process



Streptococcus

- α-hemolytic streptococci, divided into the following groups:

- **S. mutans group:**

  S. mutans - the MOST FREQUENT, less often *S. sobrinus, S. cricetus,* and *S. rattus* rarely, produce acids from saccharides

- **S. salivarius group:**

  S. salivarius, S. vestibularis - in saliva and on the tongue, growth in mucous colonies, can cause endocarditis

- **S. mitis group:** *S. mitis, S. oralis* a *S. peroris* – on mucous membranes and in the dental plaque - the causative agent of sepsis lenta (*S. mitis* is an exemption*)*

  S. sanguinis and *S. gordonii* – on the tongue, buccal musous membranes, dental plaque. *S. sanguinis* cleaves secretorial IgA

  Both species are important cause of subacute bacterial endocarditis (*sepsis lenta*)

- **S. anginosus group** - tiny colonies - *S. anginosus* (*S. milleri* in British texts)*, S. constellatus* with two subspecies, *constellatus* and *pharyngis*, and *S. intermedius*.
- In nasopharynx, sulci gingivales, dentoalveolar and endodontal infections

Caries and *mutans* group streptococci I

In man usually: *S. mutans* (serotypes c, e, and f)
S. sobrinus (serotypes d and g)

Some strains seems to be more cariogenic.

Ethiological role - facts:

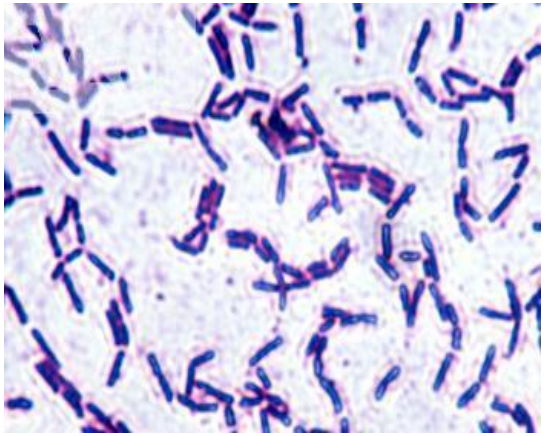
- Numberes in the plaque and saliva correlates with caries prevalence and incidence
- Isolated from the tooth surface immediately before caries
- Immunisation of animals with *S. mutans* specif. serotypes decreases caries incidence

Caries and *mutans* group streptococci II

- lesion progression and *S.mutans* numbers correlates
- are attached to the tooth surface and together by glucanes formed from saccharose
- are the most efficient microbes in making caries in lab animals
- able to form acids and multiply in low pH
- reach pH needed to enamel demineralization quicker than other bacteria
- form reserves e.g. glycogen (in case of low levels of saccharides in food)

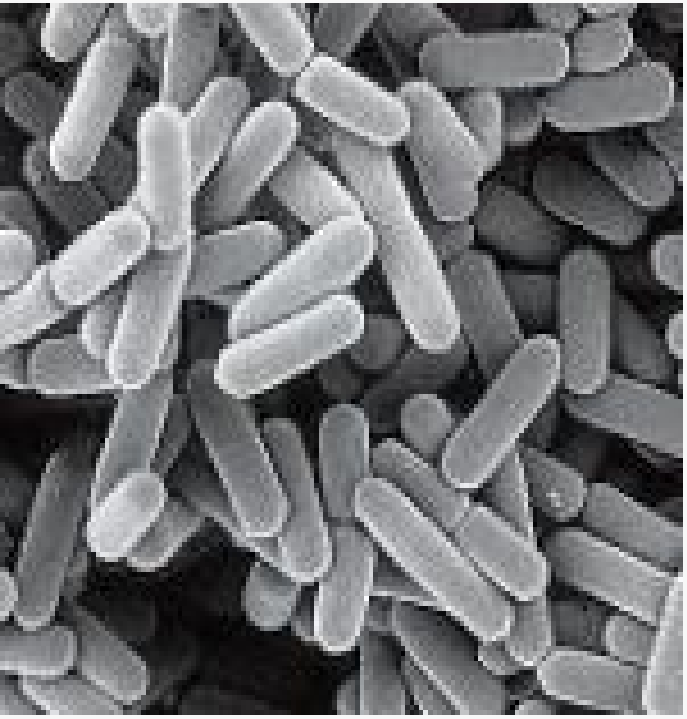
Dental caries and other microbes I

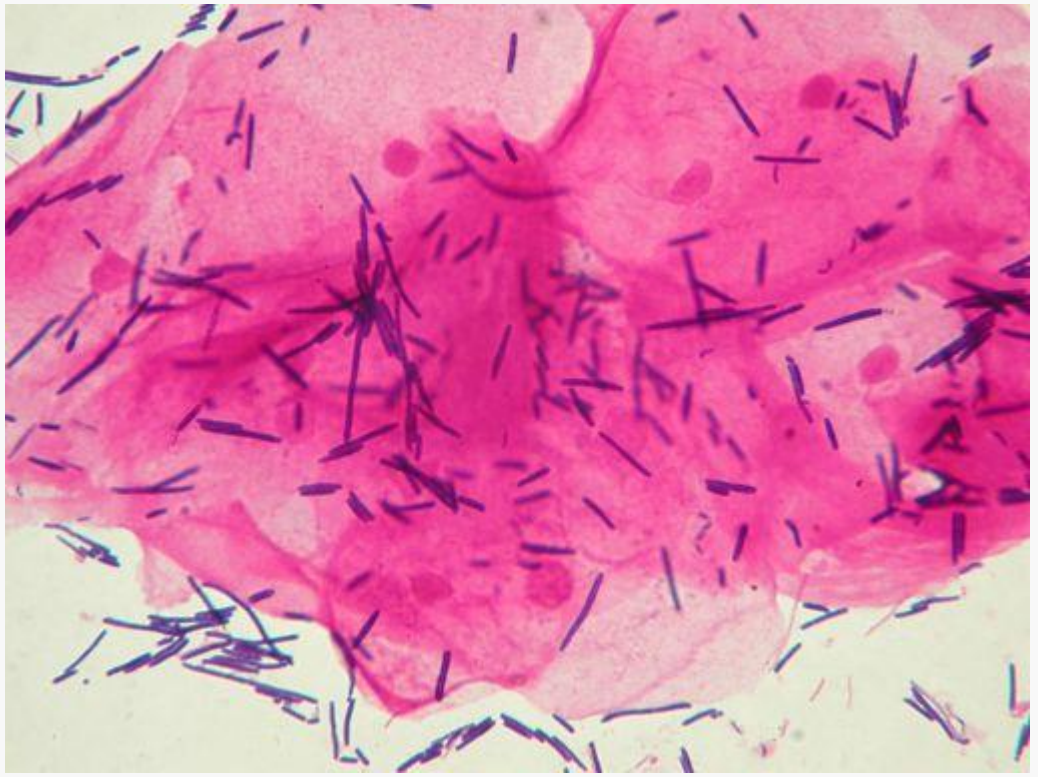
Lactobacilli

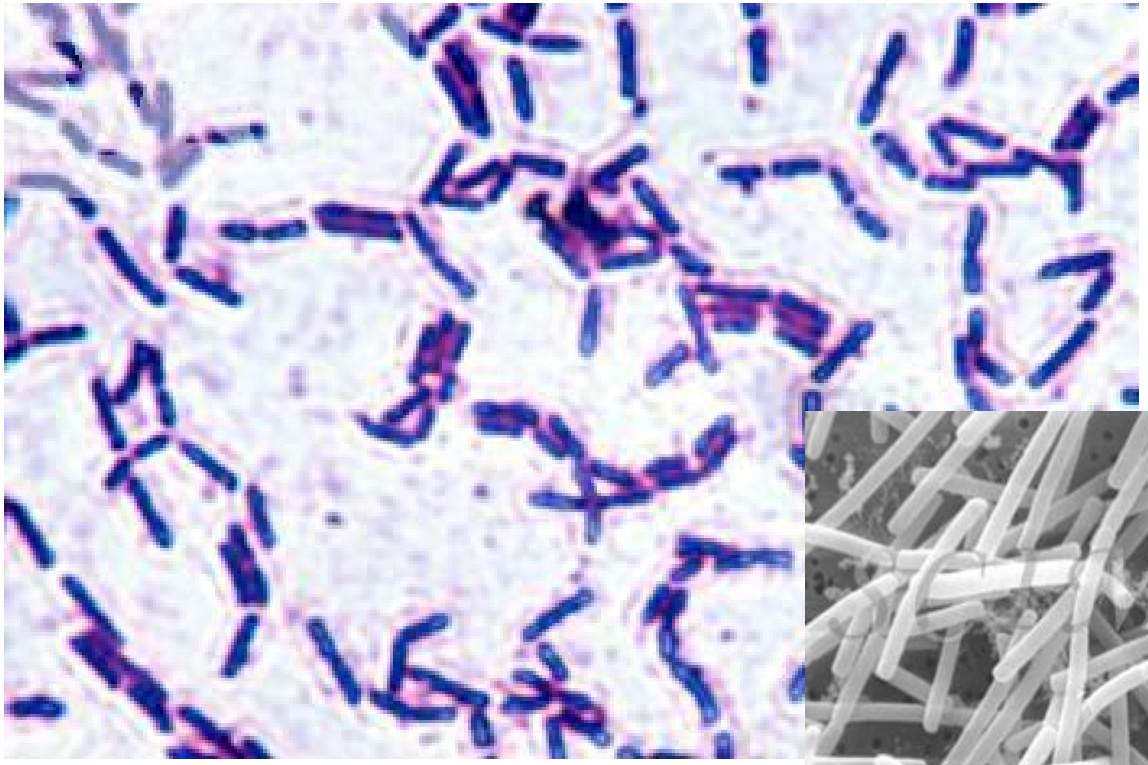


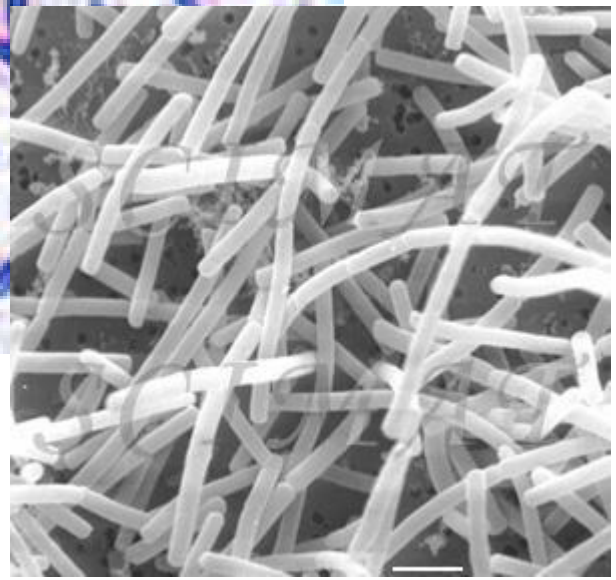
- in high numbers in dental caries
- their numbers in saliva (and dental plaque) and caries activity correlate
- growth in pH lower than 5 + develop lactate
- biochemically active - extracellular and intracellular polysaccharides from saccharose
- some strains cause caries in microbe-free animals
- in healthy teeth – low numbers of lactobacilli

Lactobacilli







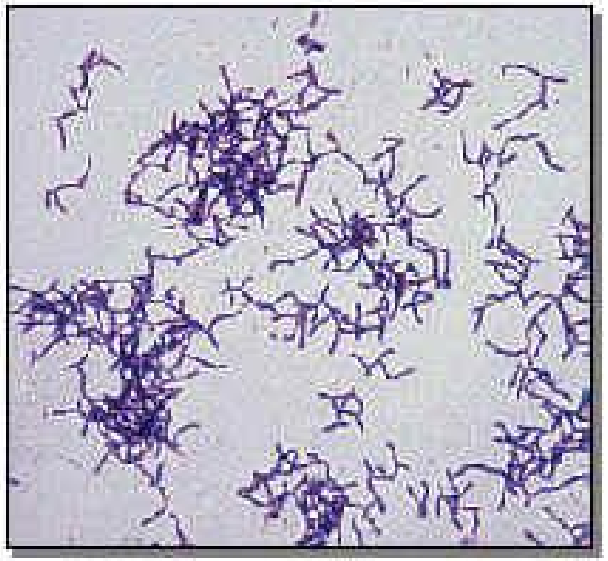


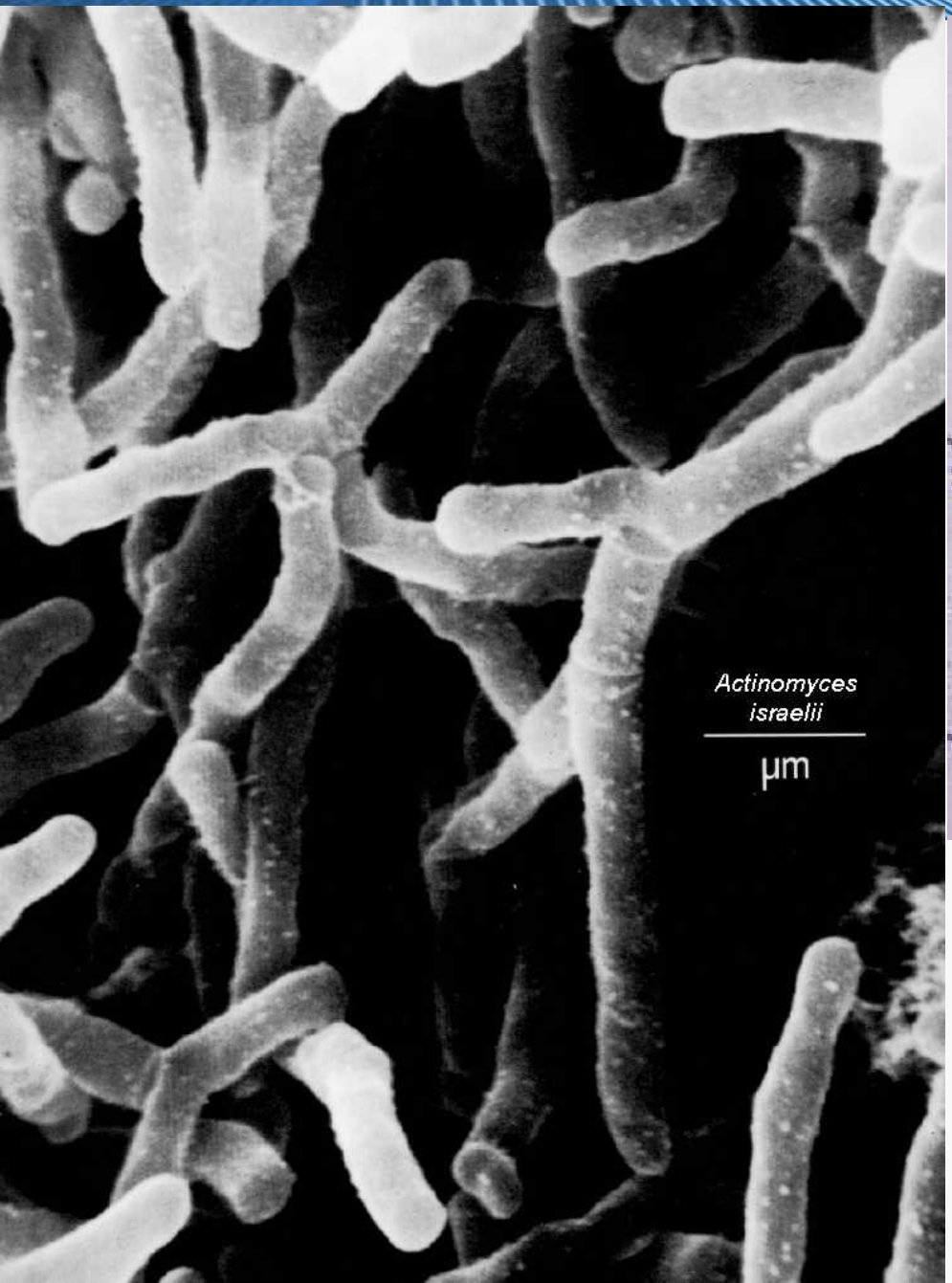
Lactobacilli

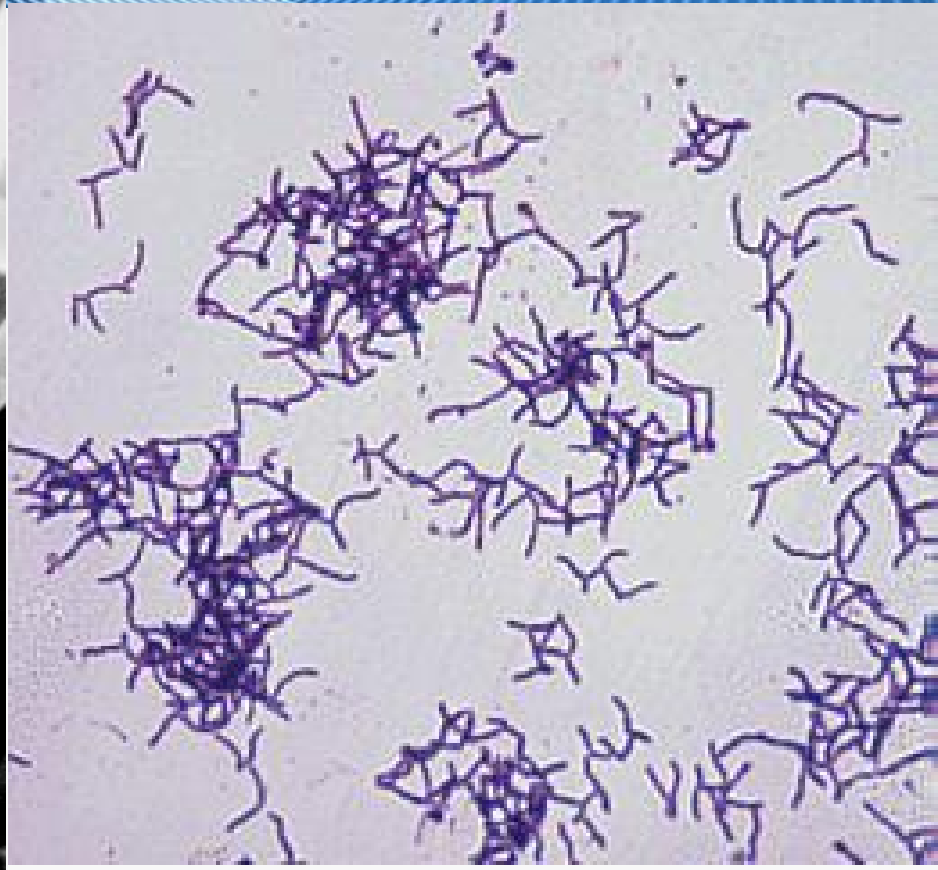
Dental caries and other microbes II

Actinomycetes

- Related to root caries – especially *Actinomyces viscosus*
- The role of actinomycetes in caries development is not elucidated completely







Source: www.bact.wisc.edu

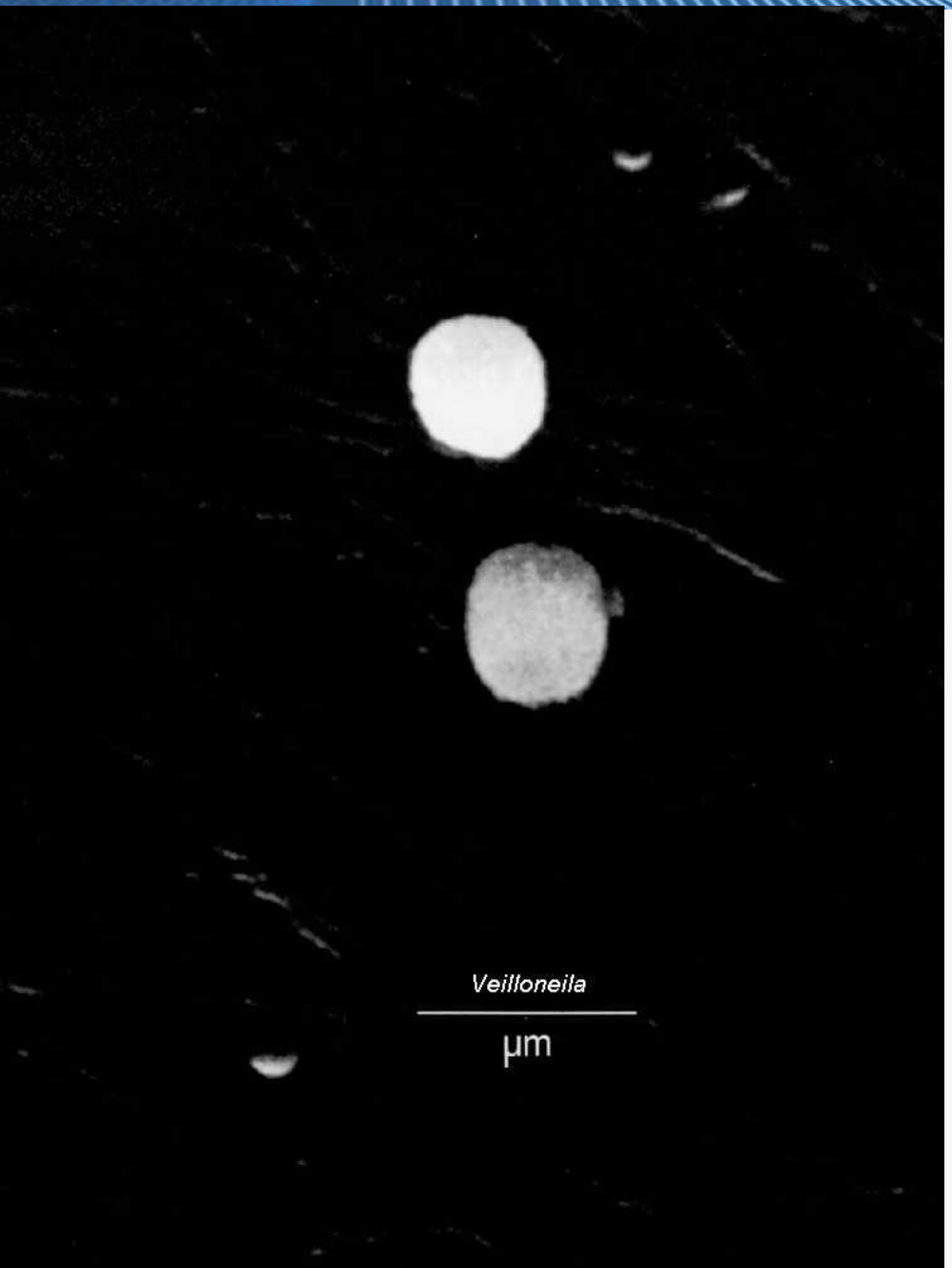
Caries and other microbes

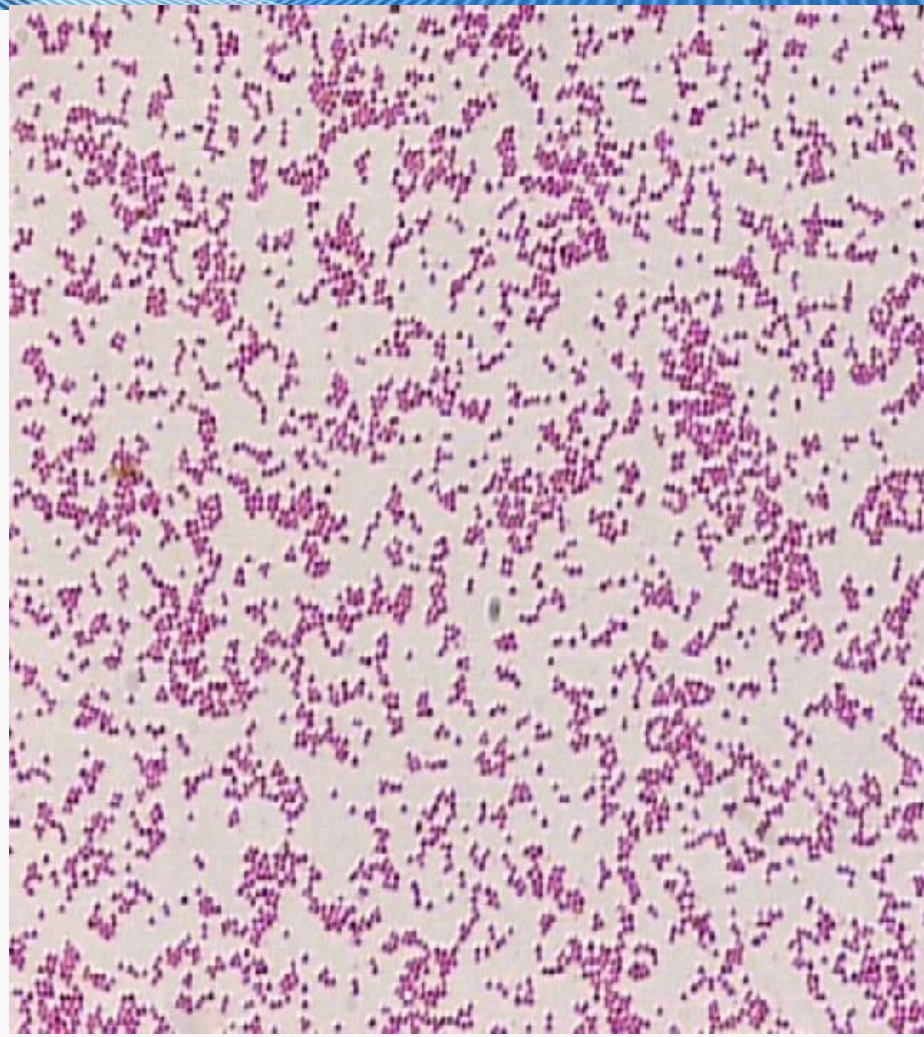
Veillonela sp.

- in high numbers in supragingival plague of most people
- need lactate, are NOT able to use saccharides and use lactate developed by other microbes – transform it to less cariogenic organic acids

…… positive outcomes……?

Ecological plaque hypothesis


Veilloneila
µm



Veillonelae in people with (A) and without (B)dental caries

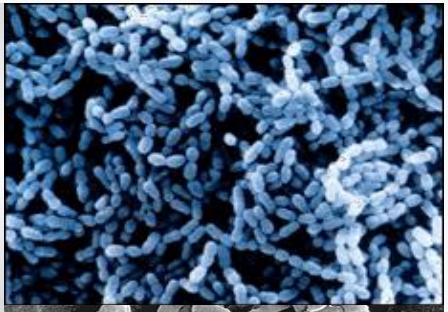
- similar numbers (unsignificant differences), BUT:
- veillonellae in A less diverse, in B more diverse
- *V. parvula, V. dispar, V. atypica* in both groups
- *V. denticariosa* only in caries lesions
- ***V. rogosae*** only in people without dental caries
- in A highly probable finding of one predominant V. species
- average number of genotypes in lesions lower than in fissurs or buccal location

(Source: Arif, J Dent Res, 2008)

Dental plaque development

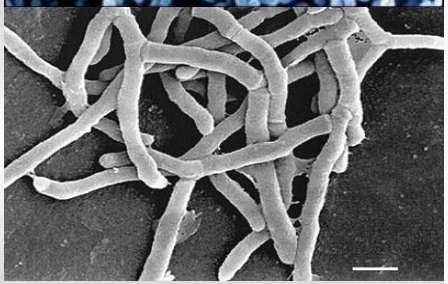
Less than 24 hours

Streptococci of *mutans, sanguis,* and *mitis groups are prevalent in suprag. plaque*



Days

G+ rods and filamentous microorganisms (lactobacilli, actinomycetes) accumulate

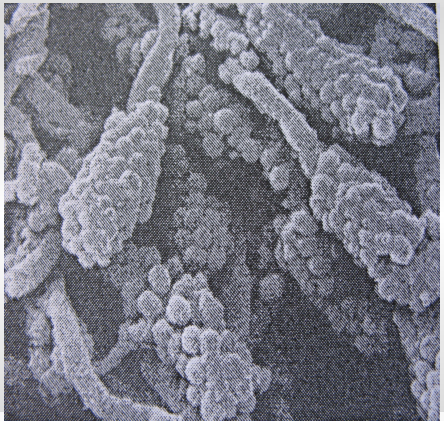


Week

Columns/microcolonies of coccoid microbes – rods and filamentous microbes get attached on their surface

Three weeks

filamentous microbes are prevalent,
„corn-cob" formation:
a central filament (*Eubacterium yurii*)
is encompassed by G+ cocci



Microbiological testing of people at risk

- Saliva sample is taken
- *S.mutans* a Lactobacillus sp. numbers assessed by cultivation

- **High risk** patients > 10^6 S.m. or/and L. 10^5
- Low risk patients < 10^5 S.m. or/and L. 10^4

Preventive factors

- **Milk, diary products, milk proteins** - buffer, increase of pH thanks to decarboxylation of aminoacids from casein
- **Milk casein** – adsorbtion on the tooth surface, casein layer prevents S.*mutans* adhesion
- **Calcium phosphate** from casein boost enamel remineralization
- **Fluorides** – boost tooth mineralization, diminish glykolyse, impair CM, and inactivate enzymes
- **Xylitol** – inhibition of bacterial growth

Treatment and prevention

- Standard treatment =

  ablation of impaired tooth tissue, preparation of cavity and filling

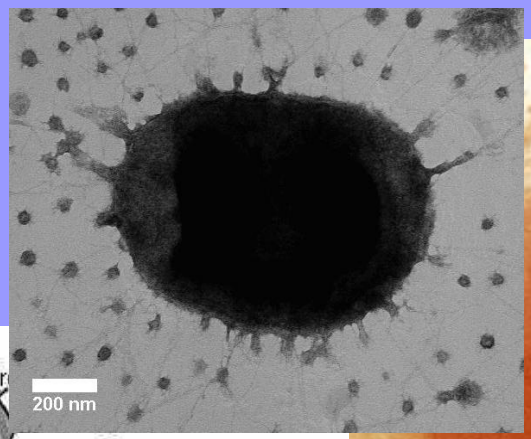
- Preventive measures =

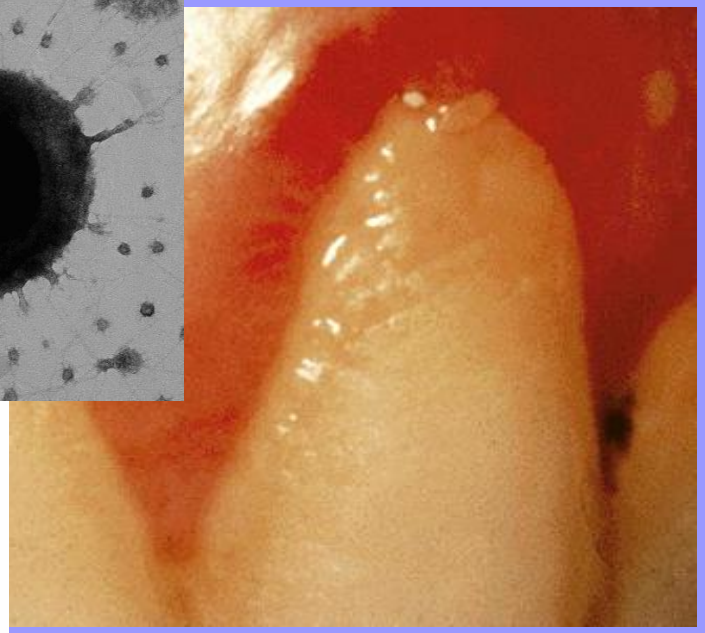
  change of diet (low-carbohydrate diet), application of fluorides and proper dental care

- Ozone – low efficiency, Műller, Eur J Oral Sci, 2007

Review:
Azarpazhooh A, Limeback H. The application of ozone in dentistry: A systematic review of literature.
J Dent. 2008 Feb;36(2):104-16.

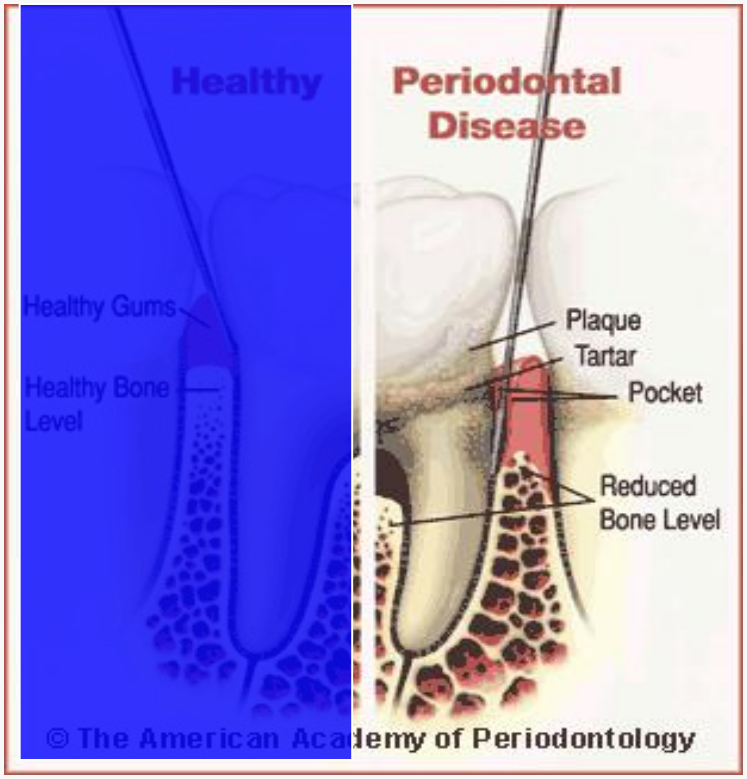
IV. Periodontitis

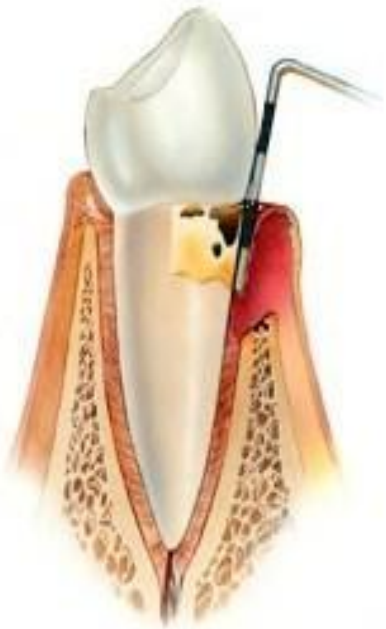


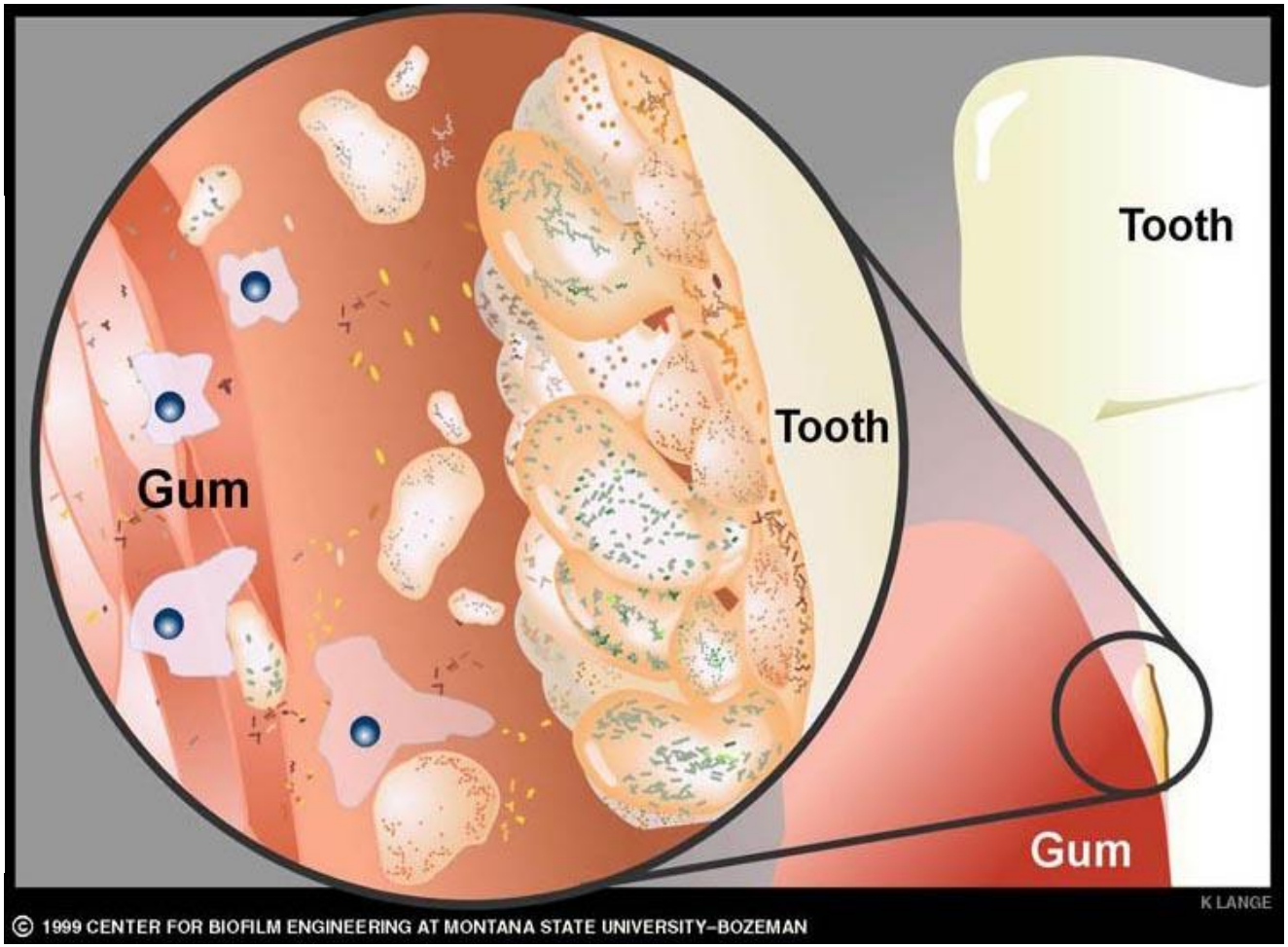


Periodontitis

- Almost 80 % adults
- Inflammation of gums, **scarcement of dentogingival junction**
- **Resorption of alveolar bone tissue**
- A periodontal pocket develops in the place of gingival sulci**, there is bleeding on probing,** purulent content
- Dental plaqe and calculus sediment on the cervical **surface**
- A teeth starts to move







Source: www.zahnarzt-hilpoltstein.de

Source: Center for biofilm engineering at MSU-Bozeman

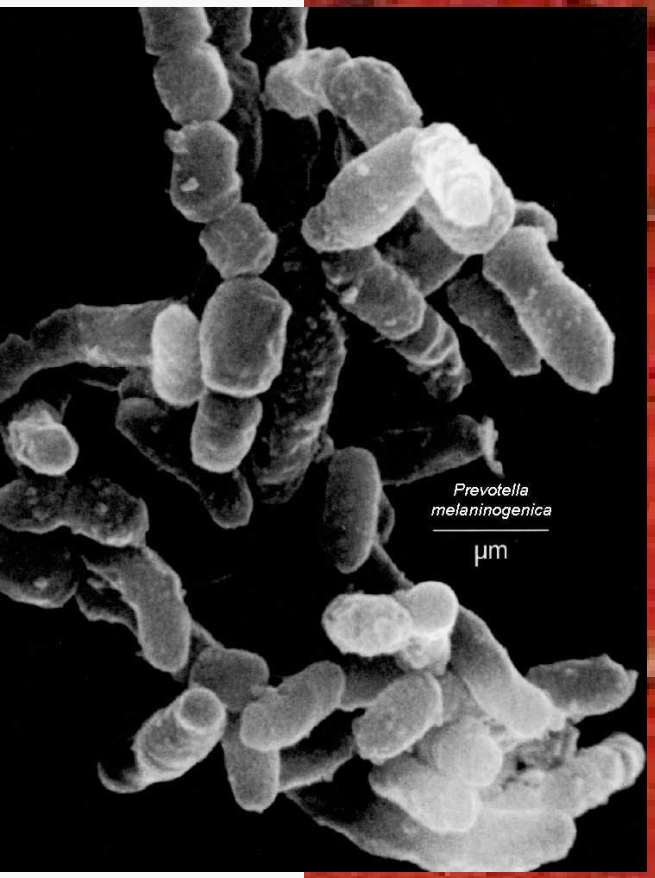
Gum reaction

- Dental plaque in the gum margine - chronic inflammation of the tissue around sulcus gingivalis = **marginal gingivitis**
- Exsudation – chemotaxis of anaerobic and proteolytic bacteria
- Increasing migration of leucocytes
- Inflammation breaks function of the junctional epithel, **plaque spreads apically to subgingival area**
- Symptoms much more intensive with older and thicker plaque

Microbiology of chronic marginal gingivitis

- Clinical symptoms - ocasional gum bleeding - inflamed, hurtfullness is minimal
- **Early stage** – after a one week course - **number of capnofile and strictly anaerobic microbes is growing** (especially Actinomyces sp. and anaerobic G- rods)
- **Late stage** – more microbes, **anaerobes are prevalent** (in black colonies growing e.g. *Porphyromonas gingivalis* and *Prevotella intermedia*, oral spirochetes)
- **Bleeding from gums** lead to multiplication of black-pigmented anaerobic rods, blood is a source of **haemin**

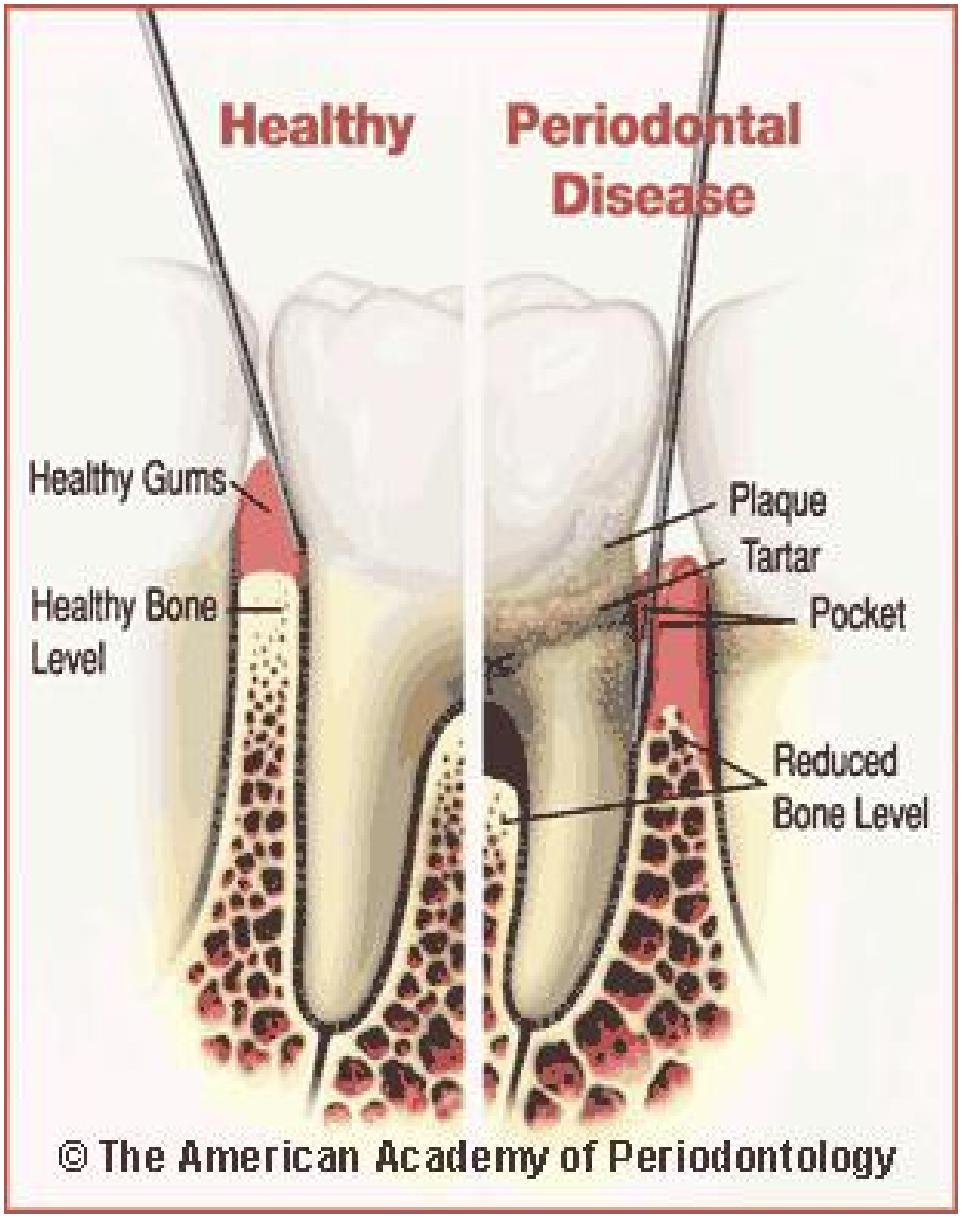
Prevotella melaninogenica (black pigment)



http://pharmacie.univ-lille2.fr

Changes in the periodontal pocket

Redox potential	**DECREASE**
Pockets / liquid	**INCREASE** = nutrient medium for the growth of anaerobes releasing proteolytic enzymes, proteins are cleaved by proteolytic bacteria
pH	**INCREASE** from normal neutral values to 7,4 – 7,8 - it enhances bacterial growth (e.g. *Porphyromonas gingivalis*)
Microflora	**INCREASE** G- anaerobic rods = *P. gingivalis, P. intermedia, F. nucleatum, T. denticola*, *A. actinomycetemcomitans* , and *C. sputigena*


Healthy
Periodontal Disease
Healthy Gums
Healthy Bone Level
Plaque
Tartar
Pocket
Reduced Bone Level
© The American Academy of Periodontology

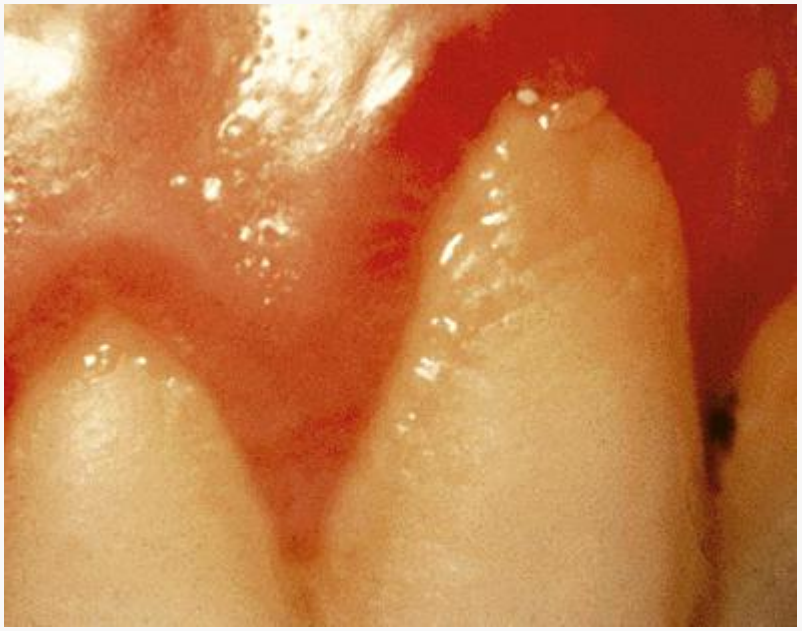
This may hurt, so I'm going to sedate your wallet.

Infuence of subgingival plaque - studies

- Strong correlation between plaque volume and prevalence and severity of periodontal diseses
- **Volunteers studies** – poor dental hygiene = plaq growth and gingivitis – after plaq removal gingivitis heals
- Local application e.g. chlorhexidine diminish plaq and prevent gingivitis
- **Microbe-free animal models** - bacteria of „red complex“ from human plaq lead to parodontal infection and immunoinflammatory bone resorption (Kesavalu 2007)

Etiology of parodontitis

- Specific plaque hypothesis
- Non-specific plaque hypothesis
- Ecological plaque hypothesis



Specific plaque hypothesis

- **Etiology of parodontitis = specific microorganisms**
- Necrotizing ulcerative gingivitis – key agens fusobacteria and spirochetes
- Terapeutic success with antimicrobials inhibiting anaerobes – e.g. metronidazole
- Rapidly progreding juvenile parodontitis - *Aggregatibacter actinomycetemcomitans* – sensitive to tetracycline – treatment

Ecological plaque hypothesis

- **Endogenous infection is caused by opportunist species** = parodontitis caused by change in sulcar microflora based on changes of environment

- In the beginning, there is **plaque development and spreading to sulcus gingivalis** = macroorganism reacts by inflammation

- Increasing **production of sulcar fluid increases supply of proteins** - catabolised by proteolytic G- anaeroby easily

- **Změna in zastoupení bacterial species:**
  number of G- anaerobes is growing, whereas facultative G+ anaerobes not – the first ones produce sufficient amount of virulence factors and break host immunity – destruction is a result

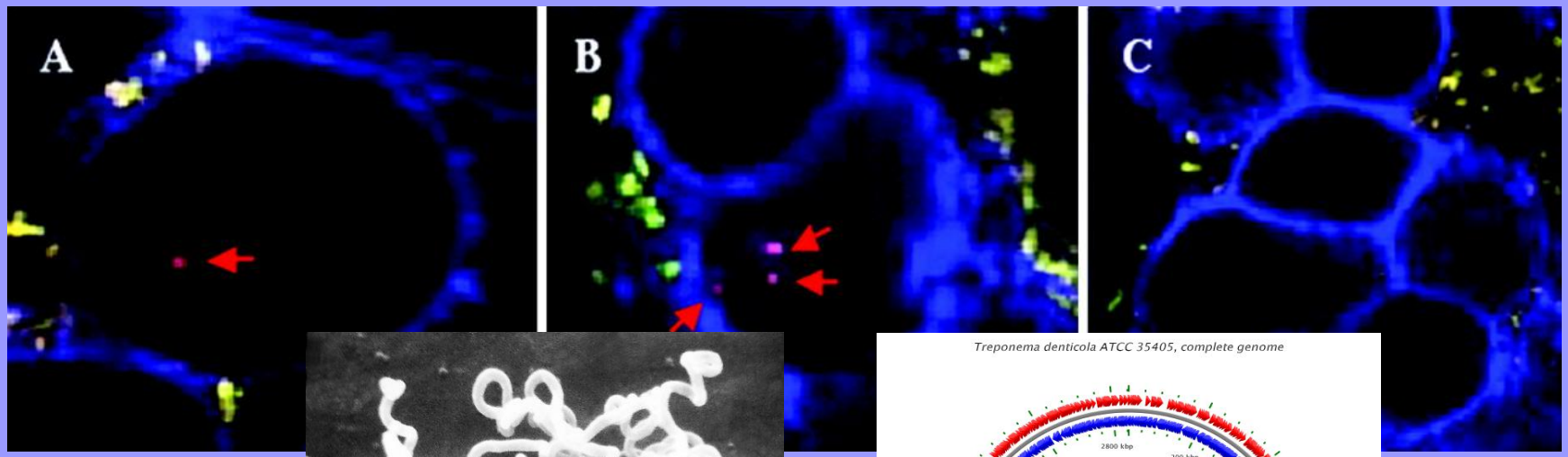
Therapeutic strategies

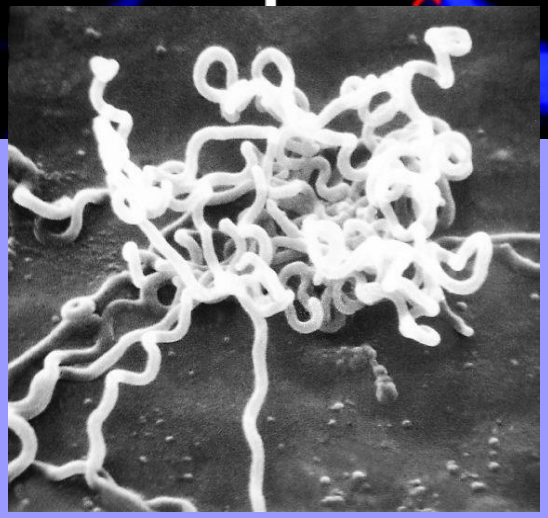
- **Specific plaque hypothesis** – therapy focused on specific pathogen removal, e.g. **antibiotics administration**

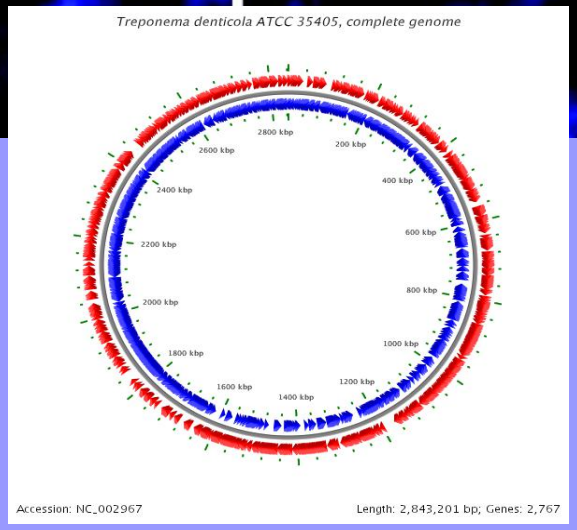
- **Non- specific and ecological hypotheses** - parodontal disease can be treated by measurments aimed at **reduction of plaque volume**

- **Regular removal of dental plaque** by proper cleaning of the teeth
- **Perfect removal of calculus**
- **Improvement of exogenous factors** (… impaired prothetic devices etc.)

Key pathogens

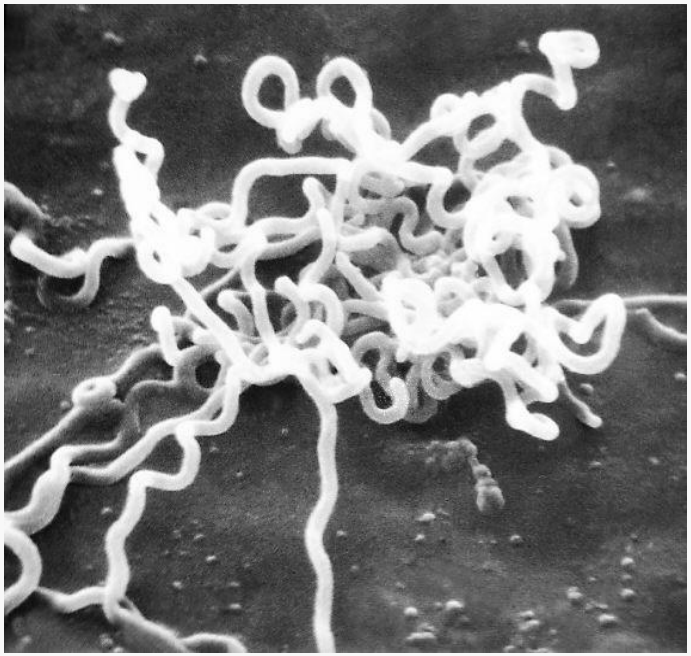






Treponema denticola

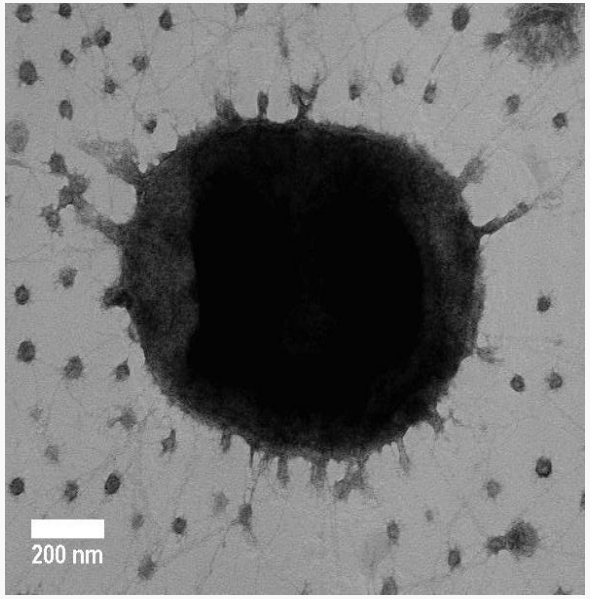
- Spirochete (a close cousin of *T.pallidum*)
- Proteolytic
- Colonizing older children (6 – 12 let 50 %, but 0,5 % microb. population) and adults
- Close relationship to *P. gingivalis* – growth factors



Zdroj: fr. wikipedia.org/wiki/Treponema

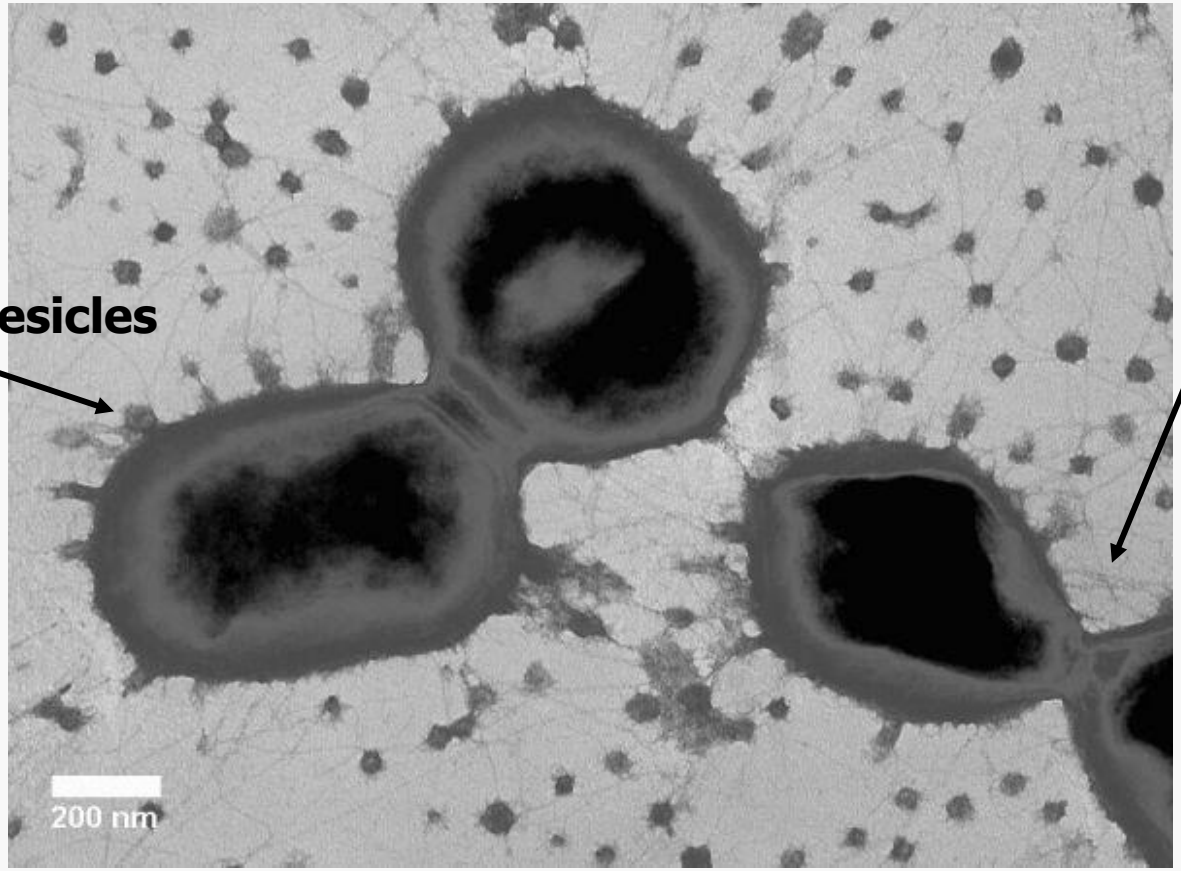
Porphyromonas gingivalis

- **Highly proteolytic**
- **Fimbriae** – adhesioin and colonisation
- Releases **vesicles** containing parts of outer membranes - proteins, LPS, capsule etc.
- Vesicles - transport of toxins and enzymes, bacterial adherention and aggregation, adherention of thrombcytes
- Black pigment = acummulated hemin – a source of iron (a growth factor)



Zdroj: www.pgingivalis.org

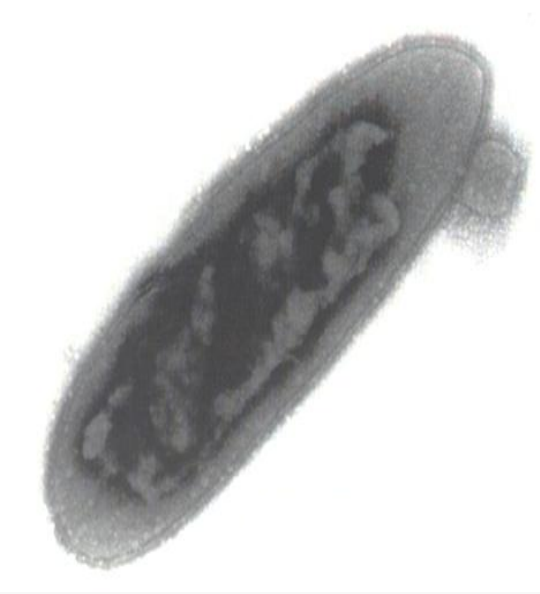
P.gingivalis



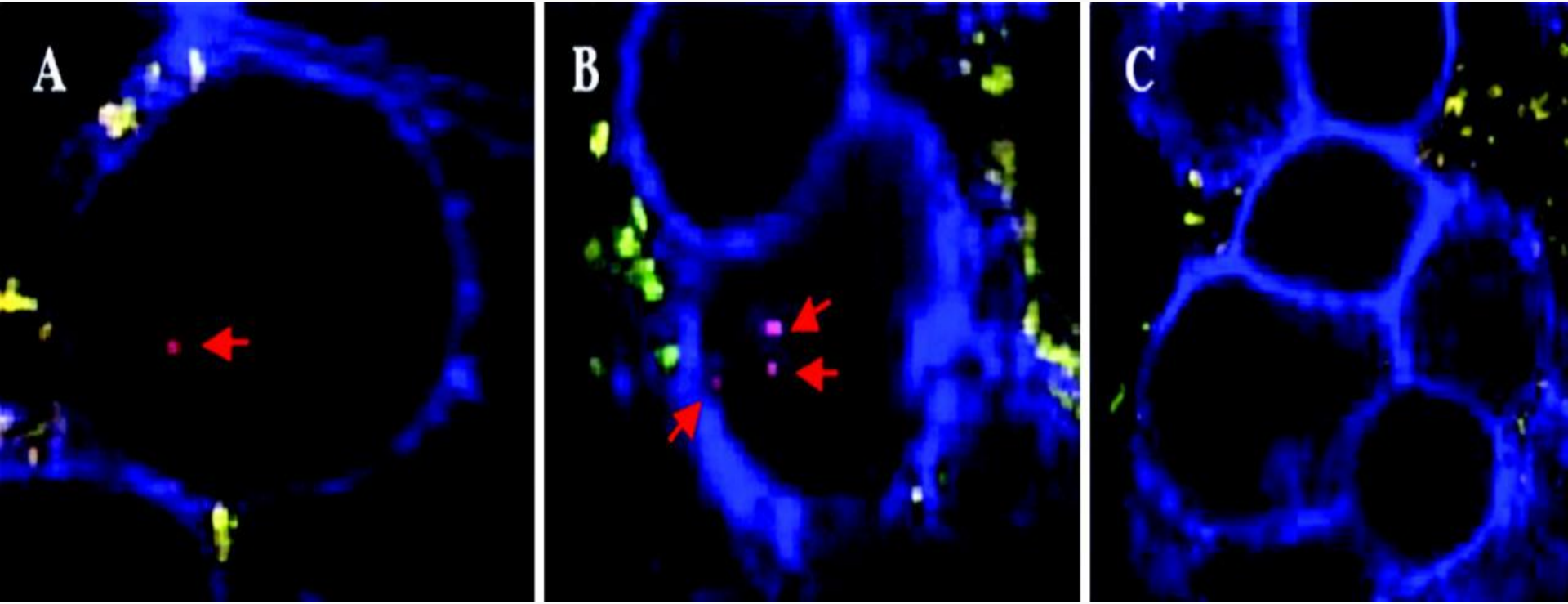
Zdroj: www.pgingivalis.org

Tannerella forsythia

- **Interaction between *T. forythia* a *P. gingivalis***
- *P. gingivalis* supports **adhesion to host cells and invasion**
- **Epitelia with** invading bacteria are th source of **recurrent infection**

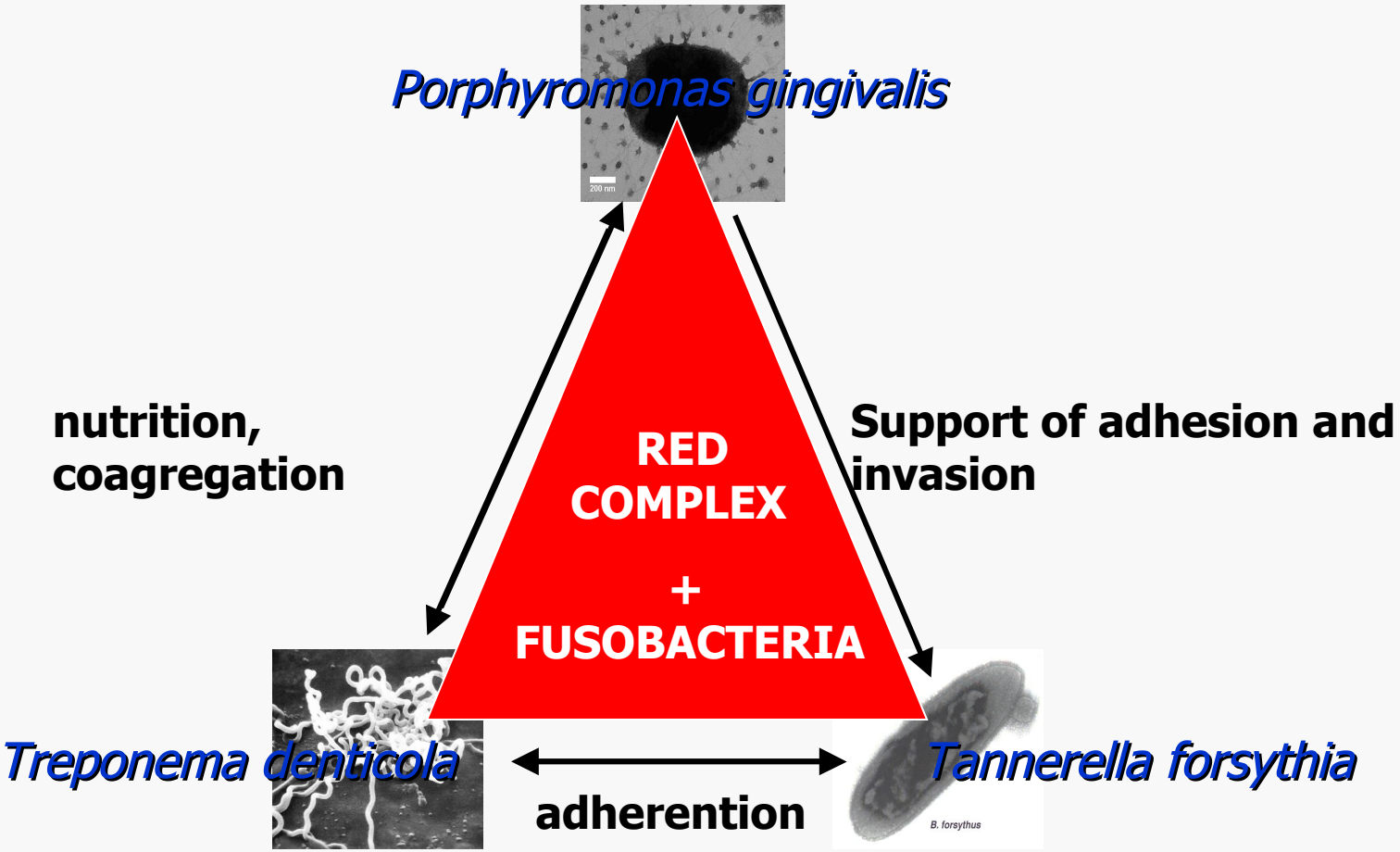


Zdroj: www.acsu.buffalo.edu



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

Mutual relationships in„the red complex"



Oral microflora in systemic diseases

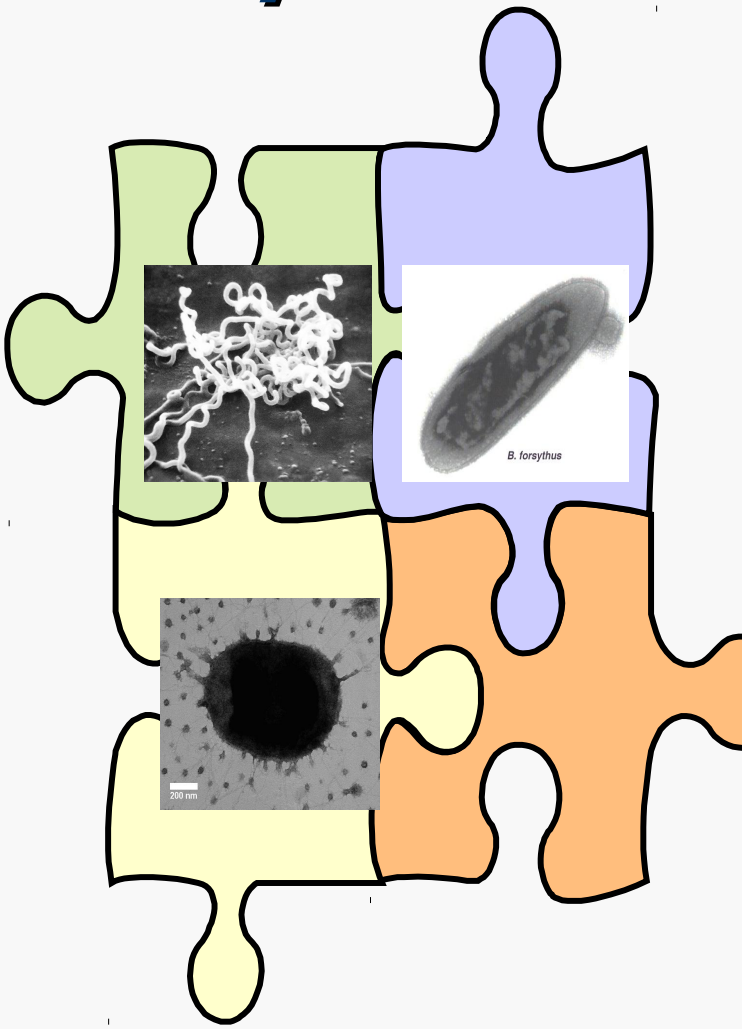
- **Cardiovascular diseases** - bacterial endocarditis, aterosclerosis - esp. coronary arterias (Gotsman et al. 2007)
- **Strokes** (Pussinen et al. 2004)
- **Pneumonias**
- **Diabetes mellitus** (Mealey, Rethman 2003)
- **Preterm births and low birth weight** (Lin et al. 2007)
- **Oesophagal carcinoma** (Narikiyo et al. 2004)

Mechanisms

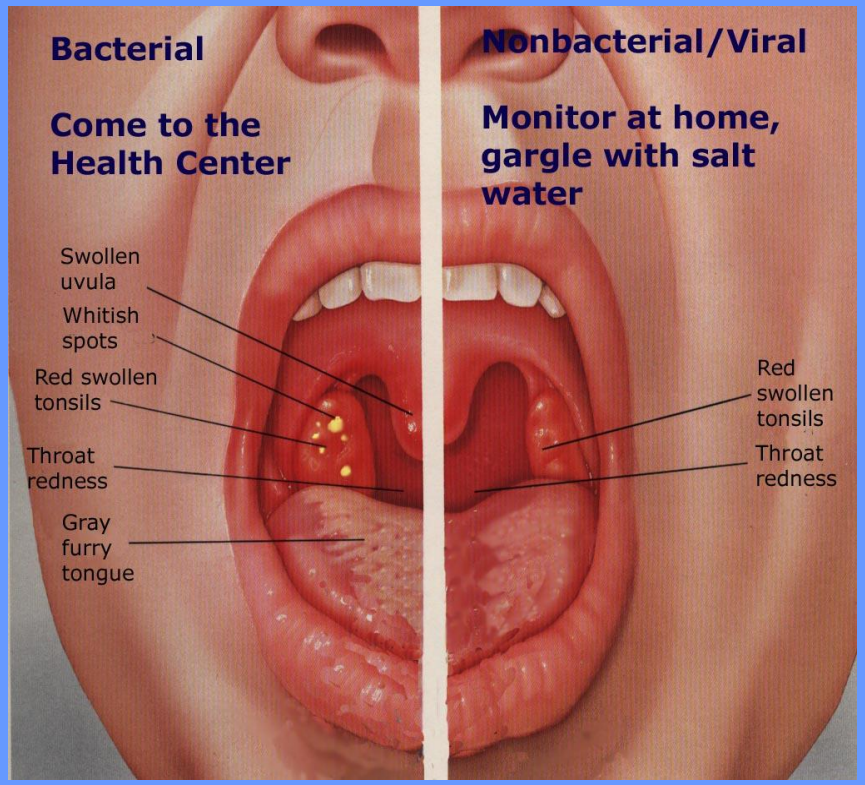
- **Microbes from the mouth** = metastatic infections (bacteremia after tooth extraction - bacterial endocarditis)
- **Bacterial enzymes and toxines from** parodontal focuses = metastatic damage (e.g. endotoxin G- bacteria from subgingival biofilm)
- **Antigens of oral bacteria and pro-inflammatory cytokines** from inflamed parodont = metastatic inflammation (reaction Ag-Ab where immunocomplexes)

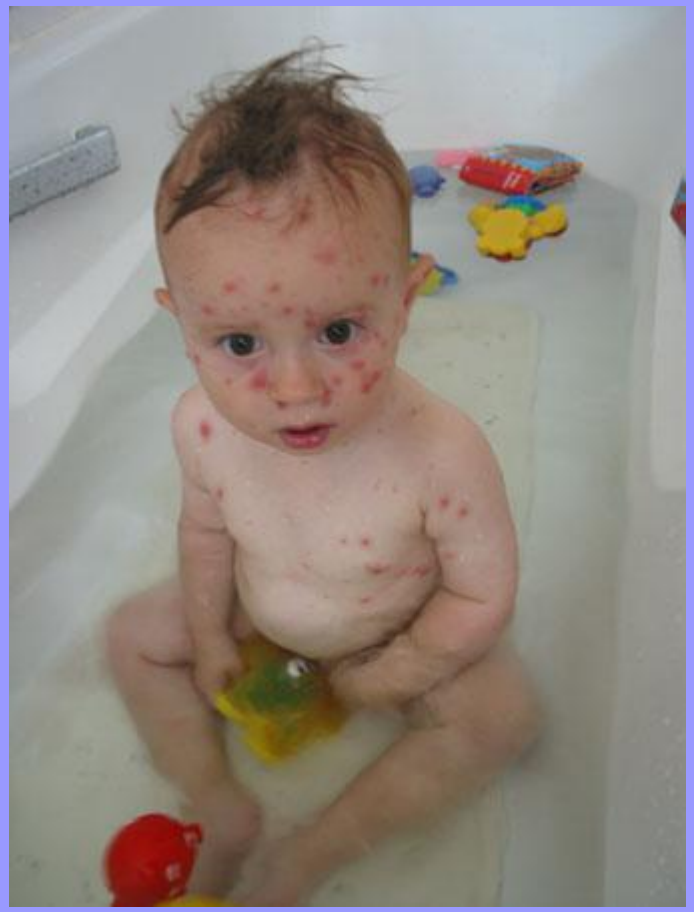
Periodontitis - summary

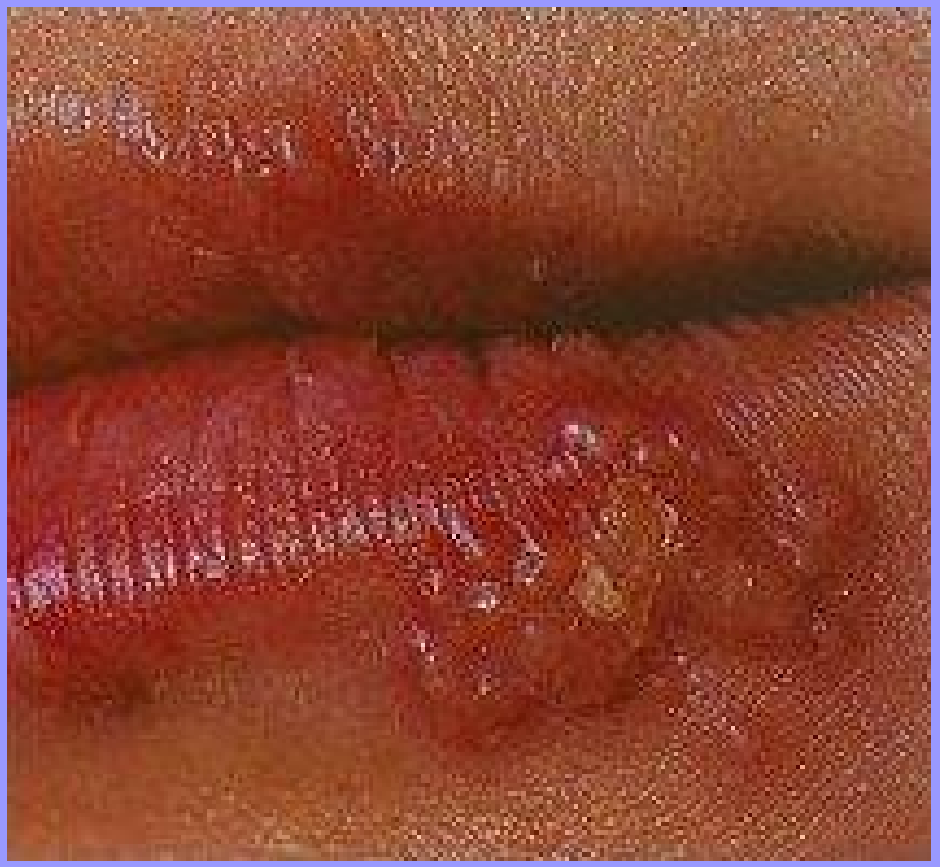
- Model polymicrobial disease
- Oral biofilm and bacterial interactions
- *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola*
- Influencing human health in a broader sense



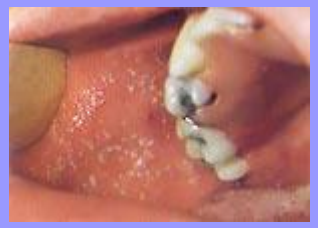
MICROBIAL DISEASES IN THE MOUTH

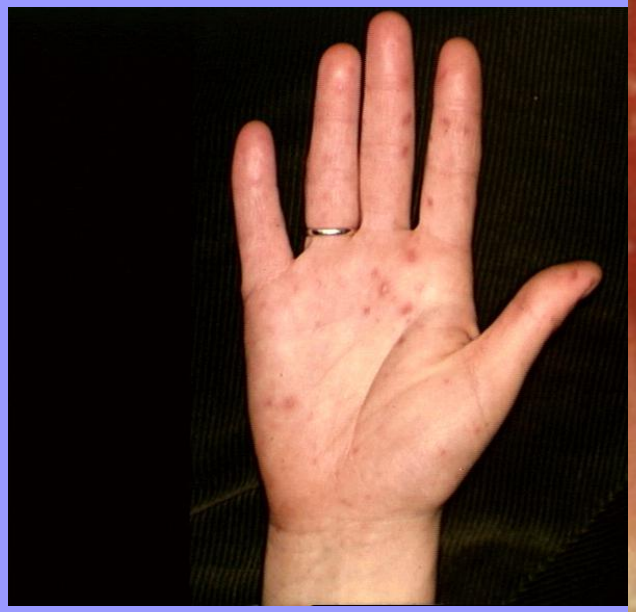




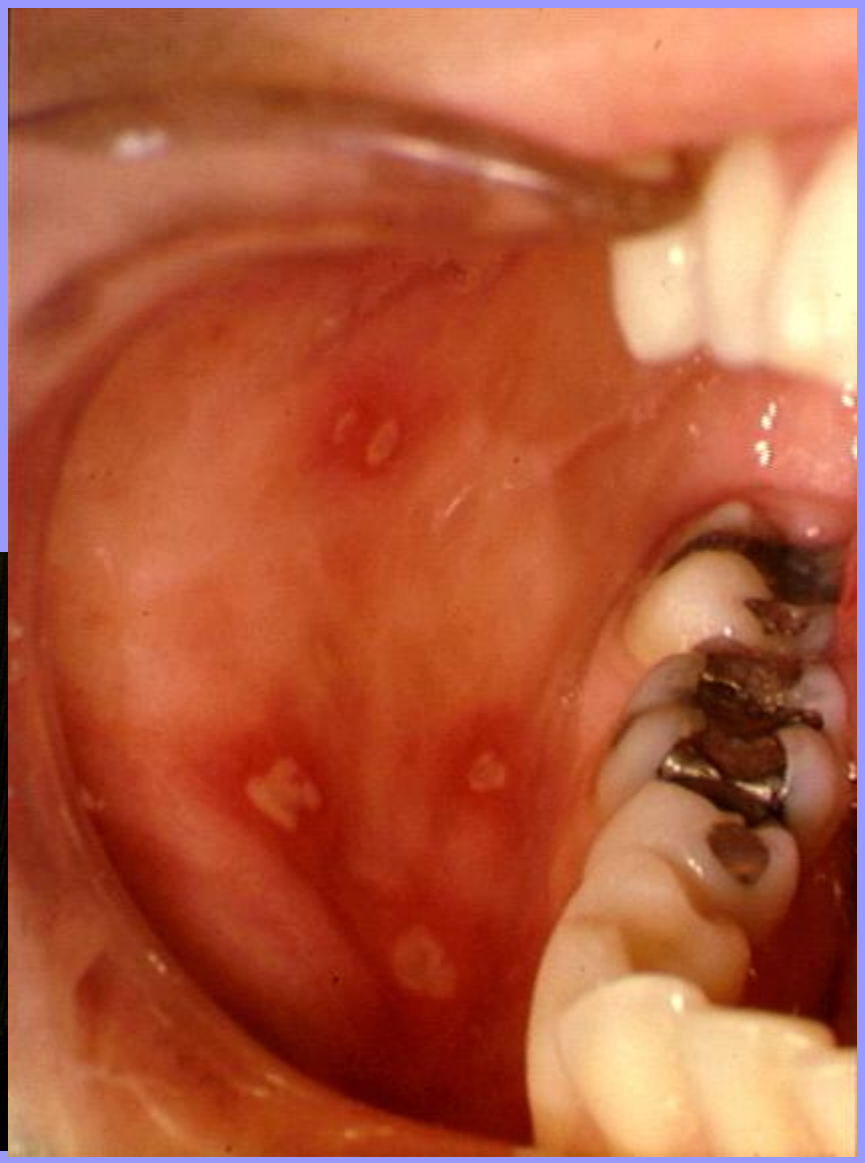


Koplik's spots / measles

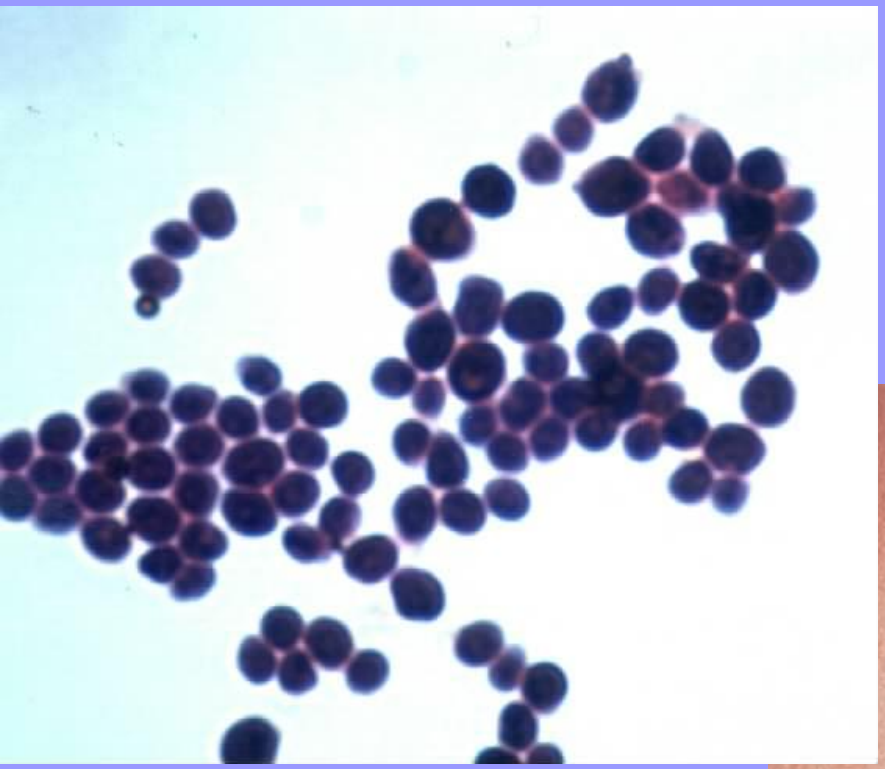


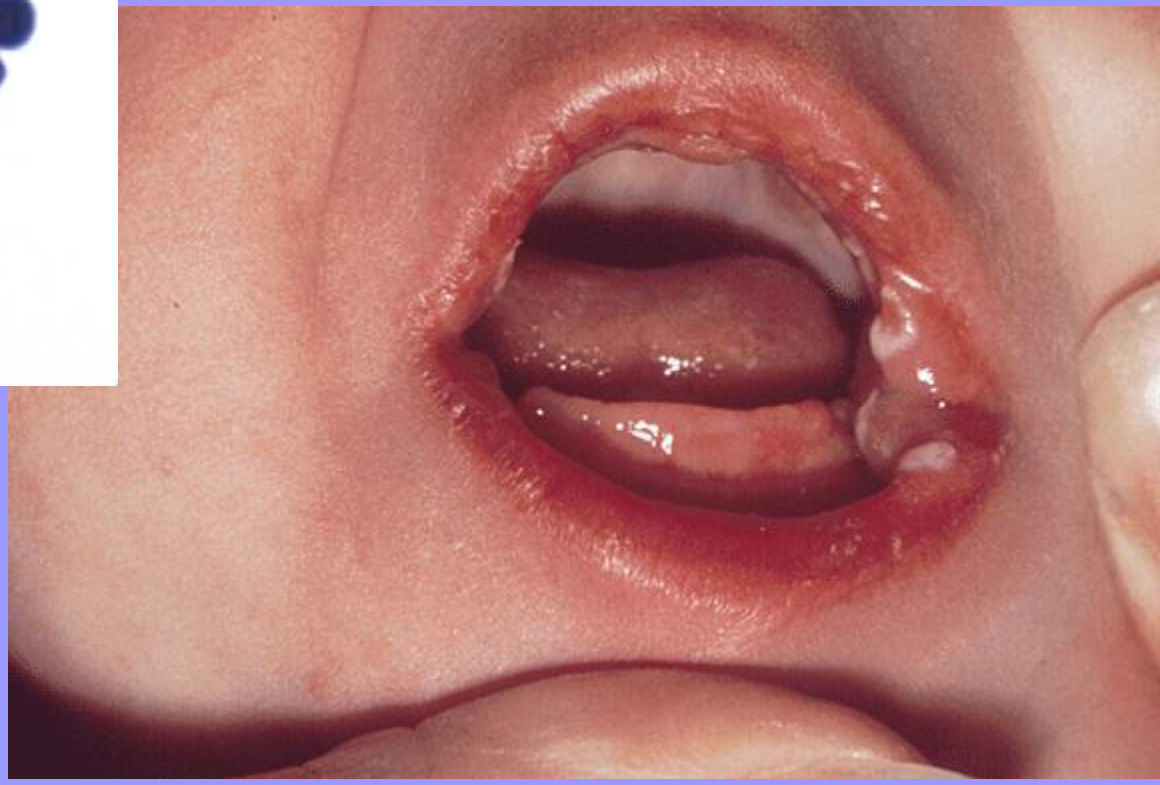


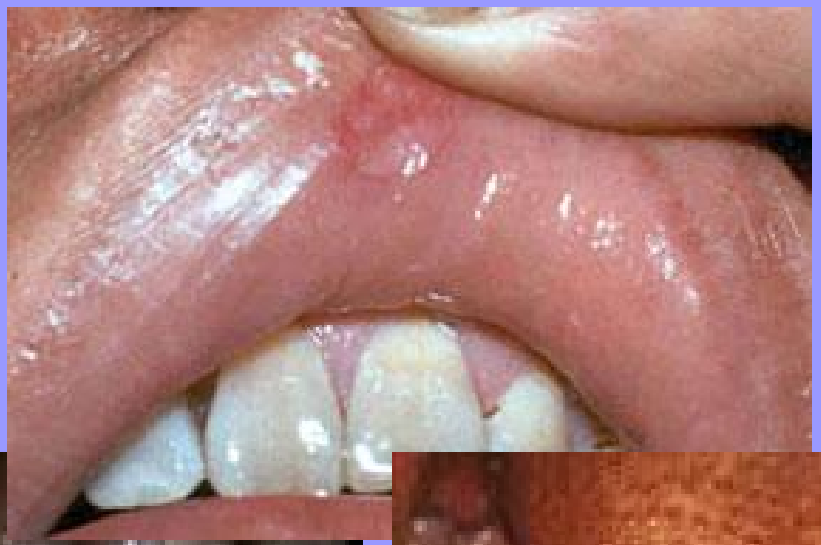
Hand-foot-mouth disease

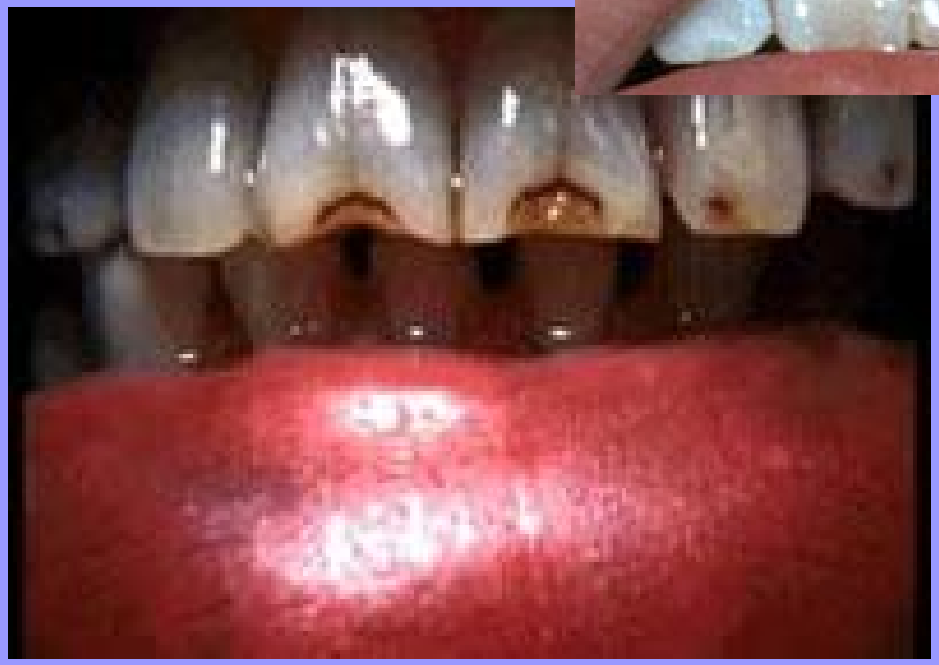


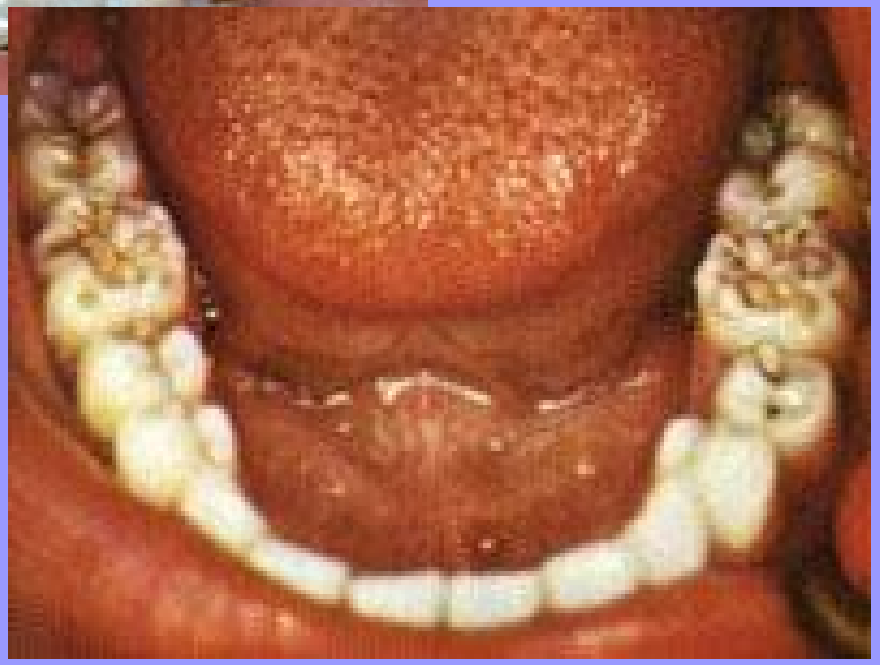
http://pathmicro.med.sc.edu/virol/picorna.htm







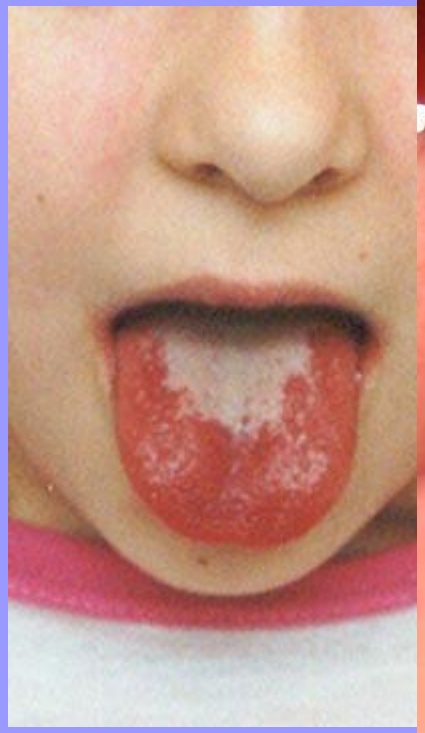


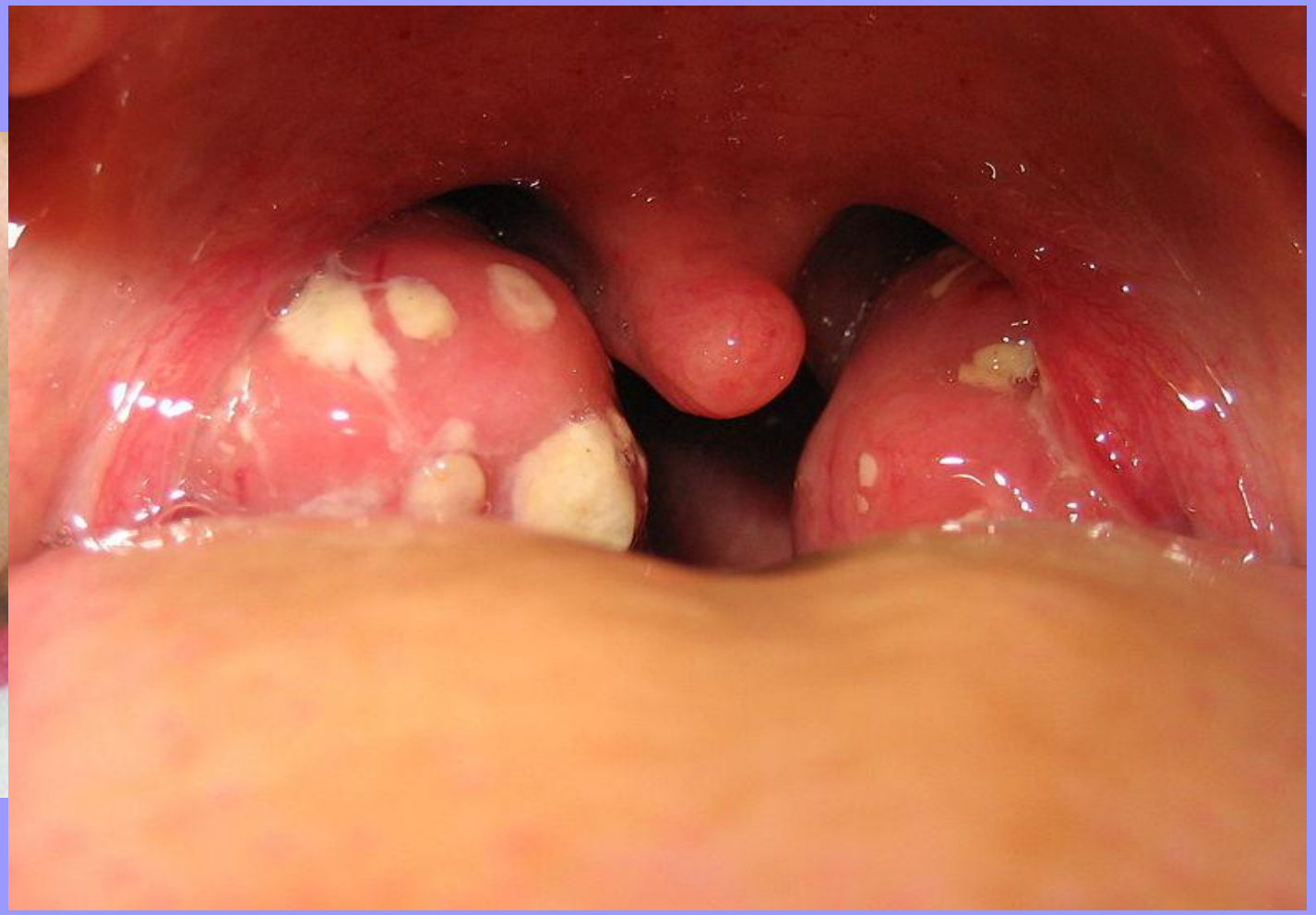


Hutchinson's teeth

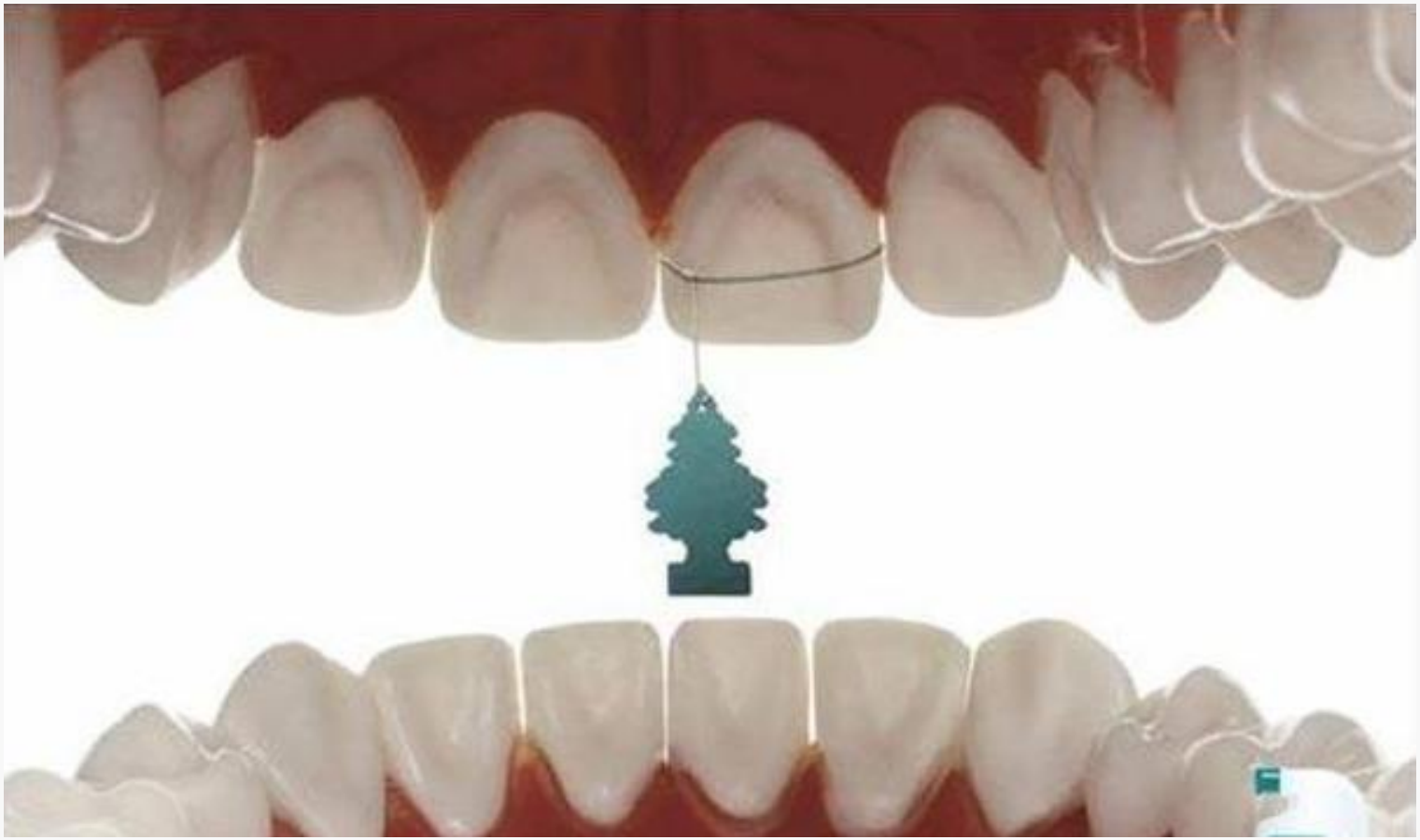
Moon's molar

kmil.trios.cz/ObrLues/hutchin1.JPG





Zdroj: Wikipedia



Thank you