Dental conference I

Dental plaque biofilm

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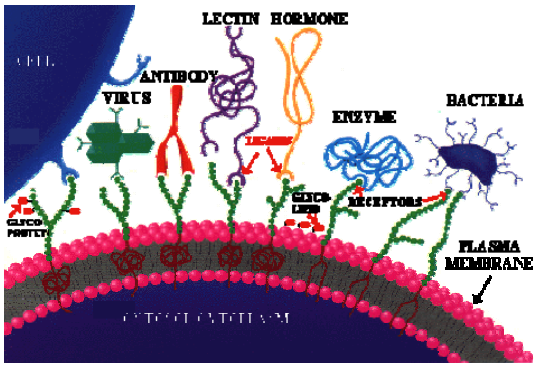
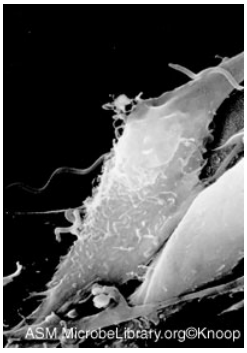
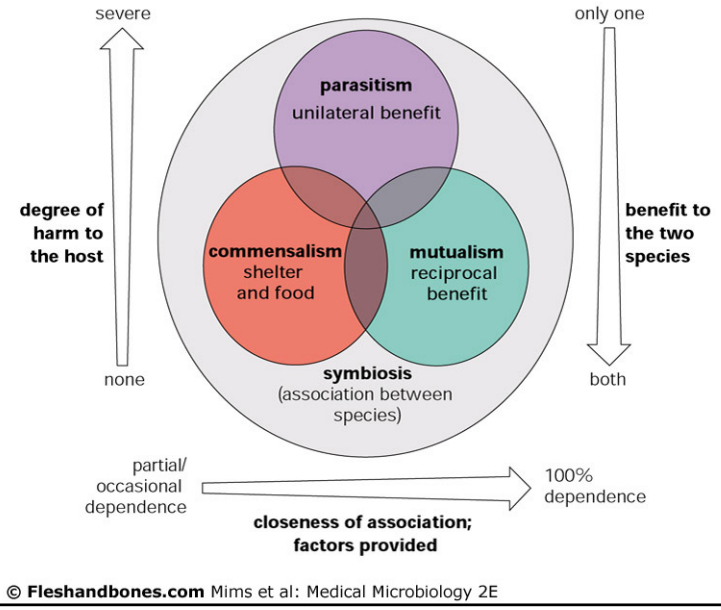
Infection as an interaction between organisms

- **Specific** interaction of **molecules**
  - Ligand
  - Receptor





Symbiosis (association between species)



Dental (oral) disease as an infectious disease

- Understanding microbial etiology
  - Pathogenicity (virulence) of pathogens
    - Virulence factors
  - Understanding microbial pathogenesis
    - Genetic and Molecular Basis for Virulence
- Understanding host response
  - Outcome of disease
- Applying to diagnosis, treatment, prevention

Nature of periodontal disease

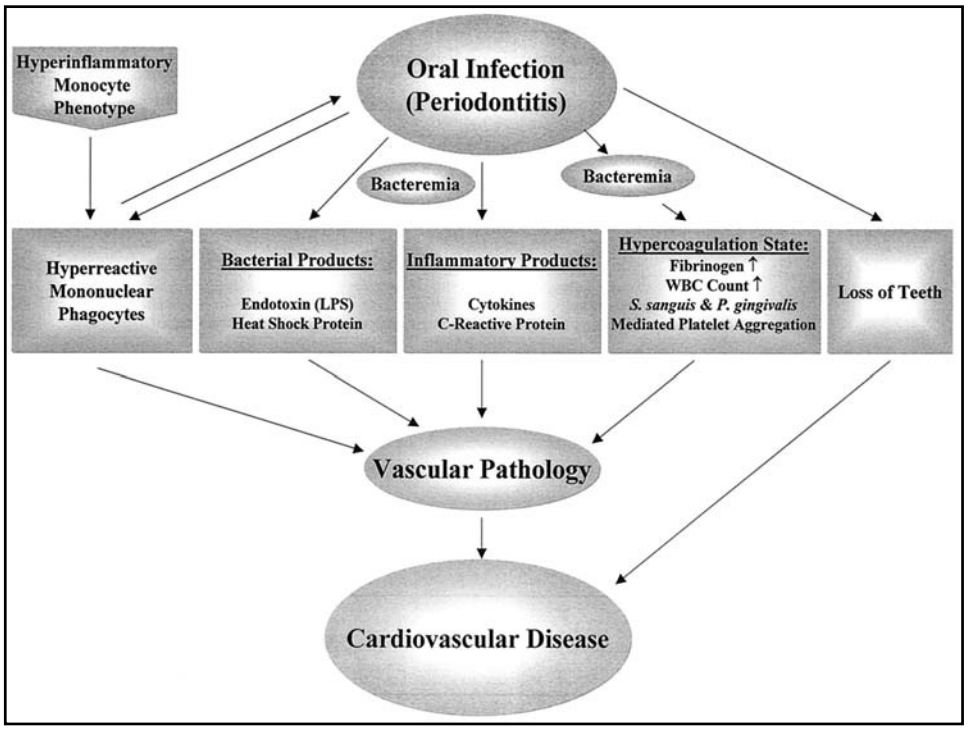
- Chronic infection
- Endogenous infection
  - vs. exogenous
- Opportunistic infection
  - indigenous bacteria
- Polymicrobial mixed infection
- Localized infection

Chronic oral infections and systemic disease

- Oral infections may affect the course and pathogenesis of a number of systemic diseases including:
  - Diabetes
  - Heart disease
  - Premature birth (low-birth-weight)
  - Lung disease

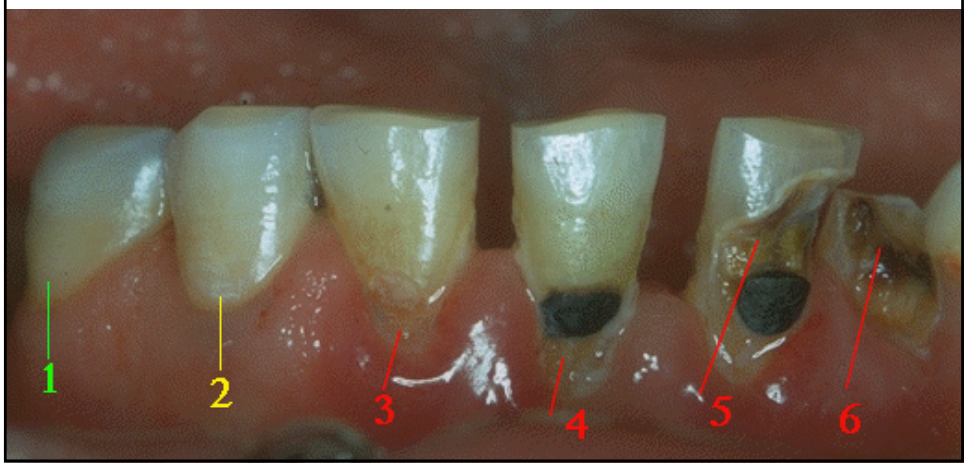
Possible mechanisms

- Spread of infection from the oral cavity as a result of transient bacteremia
- Injury from the circulating oral microbial toxins
- Inflammation caused by bacterial component-virulence determinants
- Subgingival dental plaque as reservoirs of gram-negative bacteria, resulting in inflammatory mediators

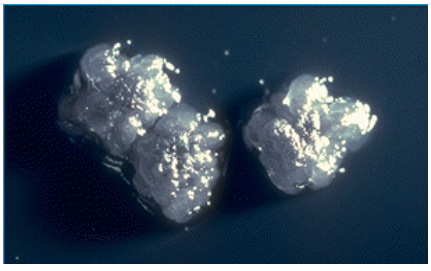


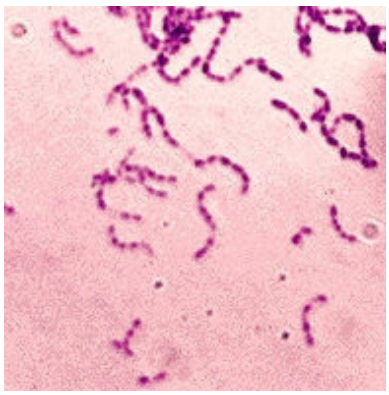
Dental Caries

Demineralization of the tooth surface caused by bacteria

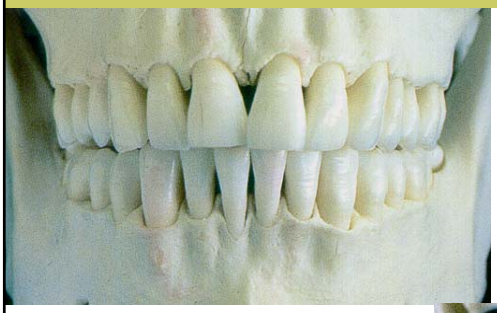


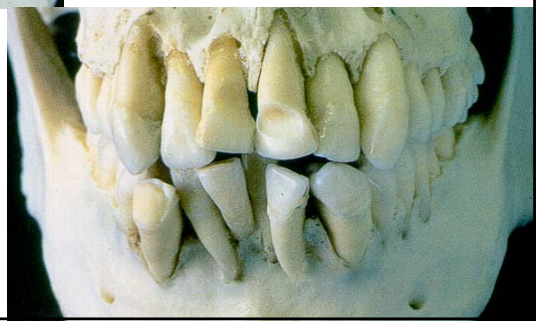
Streptococcus mutans





Periodontal Disease



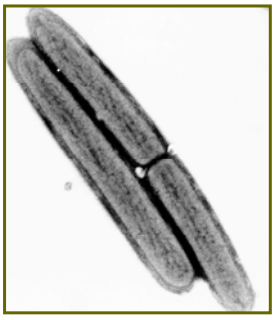


Tannerella forsythia

Gram Negative Anaerobic Rod

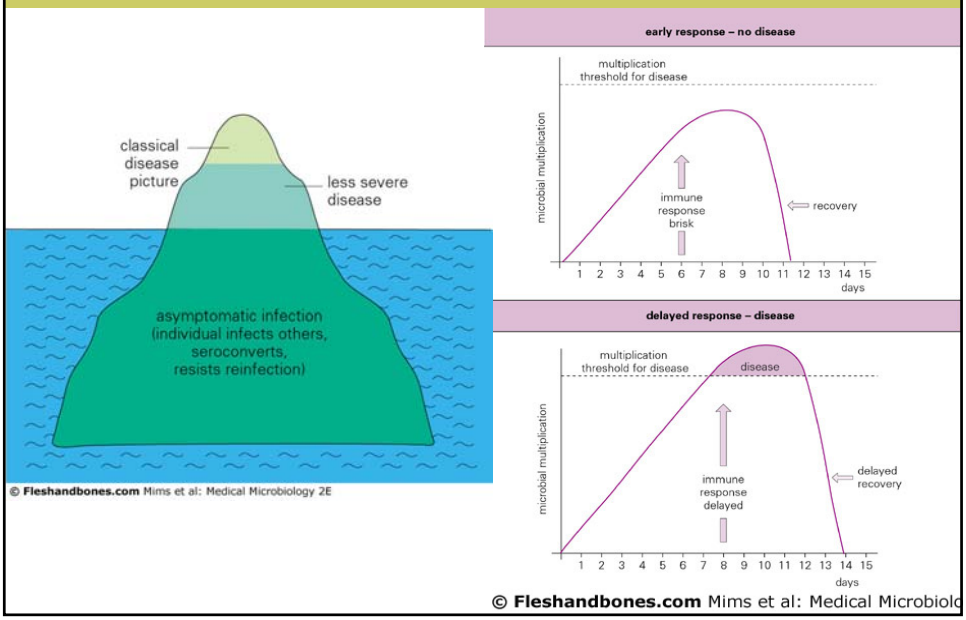
Colony

Gram stain



EM Negative staining

Infection and disease



Virulence factors

- Gene products that enhance a microorganism's potential to cause disease
- Involved in all steps of pathogenicity
  - Attach to or enter host tissue
  - Evade host responses
  - Proliferate
  - Damage the host
  - Transmit itself to new hosts
- Encoded by virulence genes

Identifying virulence factors

- Microbiological and biochemical studies
  - *In vitro* isolation and characterization
  - *In vivo* systems
- Genetic studies
  - Study of genes involved in virulence
  - Genetic transmission system
  - Recombinant DNA technology
    - Isogenic mutants
    - A molecular form of Koch's postulates (Falkow)

Specific virulence factors

- Adherence and Colonization Factors
- Invasion Factors
- Capsules and Other Surface Components
- Endotoxins
- Exotoxins
- Siderophores

Etiology of dental disease

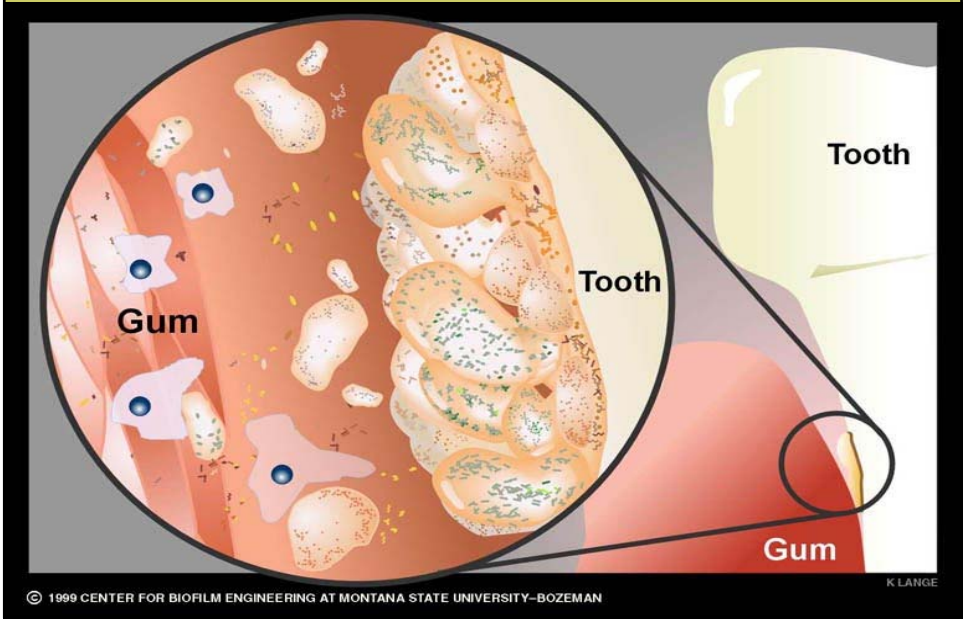
is

Dental Plaque

Dental plaque and calculus

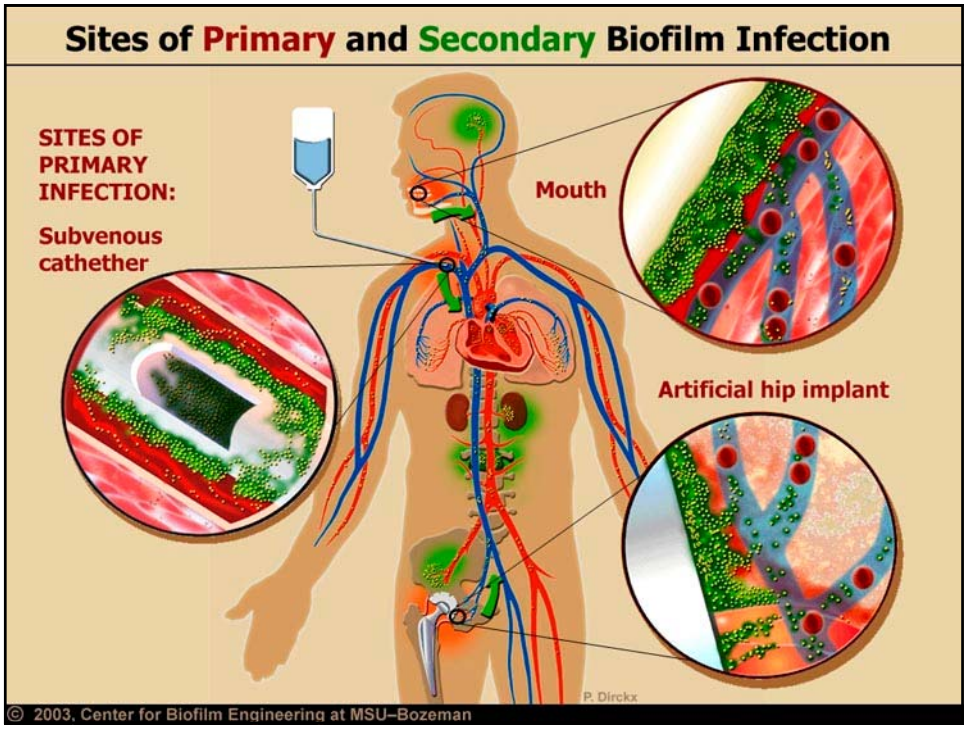
After removal

Dental plaque as a biofilm



Definition of biofilm

- Matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces
- May form on a wide variety of surfaces, living tissues, indwelling medical devices, water system piping, natural aquatic systems
- Prevailing microbial lifestyle (vs. planktonic)
- Like a complex, highly differentiated, multicultured community
- Of single or multi-species



Biofilm: analogy to city

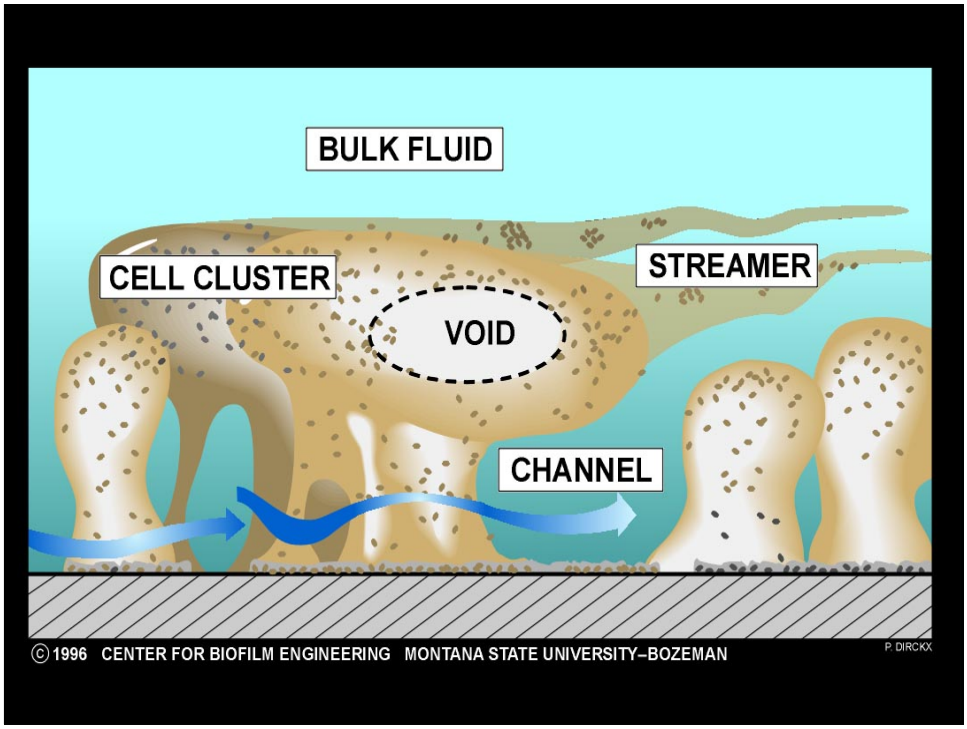
- Planktonic (nomad) vs biofilm (city)
- Initial colonization followed by lateral spread, vertical direction growth
- Shared resources and activities only possible through biofilm
- Protection from other species, host, and harsh environment
- Need communication – quorum sensing, exchange of genetic information

The nature of biofilms

- Natural method of growth for microorganisms
- Provides advantages for colonizing species
  - Protection from
    - Competing microorganisms
    - Environmental factors, host defense
    - Toxic substances, such as lethal chemicals, antibiotics
  - Facilitate processing and uptake of nutrients, cross-feeding, removal of harmful metabolic products
  - Development of an appropriate physico-chemical environment

Structure of biofilms

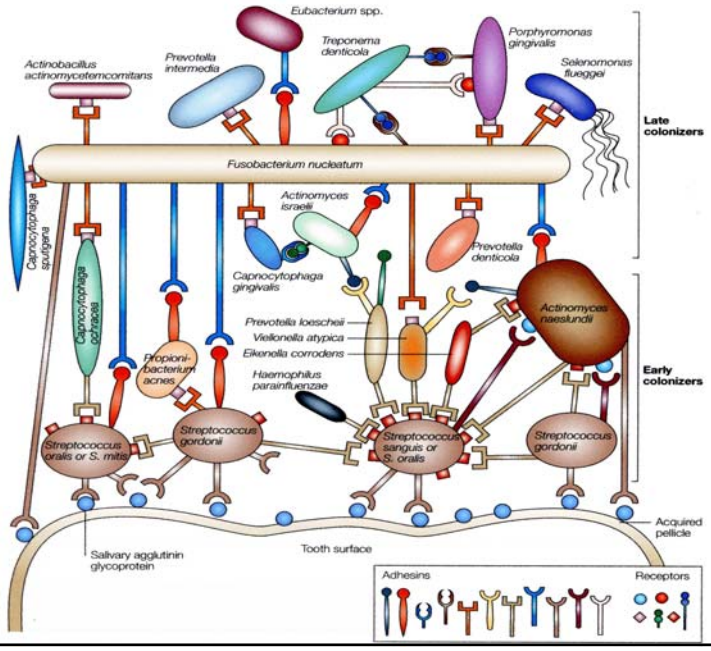
- Composed of microcolonies (15-20% by volume) disctributed in a shaped matrix or glycocalyx (75-80% volume)
- Presence of voids or water channels
  - Permit the passage of nutrients and other agents, acting as "circulatory" system
- Exopolysaccharides (EPS) – the backbone of the biofilm
  - 50-95% of the dry weight of the biofilm
  - Maintain the integrity of the biofilm
  - Act as a buffer and a substrate for resident bacteria
- Physiological heterogeneity within biofilms
  - pH can vary quite remarkably within a biofilm
  - Different chemical and physical microhabitats



Diversity of dental plaque bacteria

- Study methods
  - Cultivation and biochemical characterization
    - Up to 300 species
  - Molecular methods by comparing 16s rRNA genes
    - PCR amplification using universal and specific primers
    - Cloning of the amplicons into *E. coli*
    - DNA sequencing of 16s rDNA
    - Comparison to database
- **~ 700 different 16S rRNA sequences identified**
  - Less than half are cultivated and characterized
  - 400 bacterial species identified in subgingival dental plaque

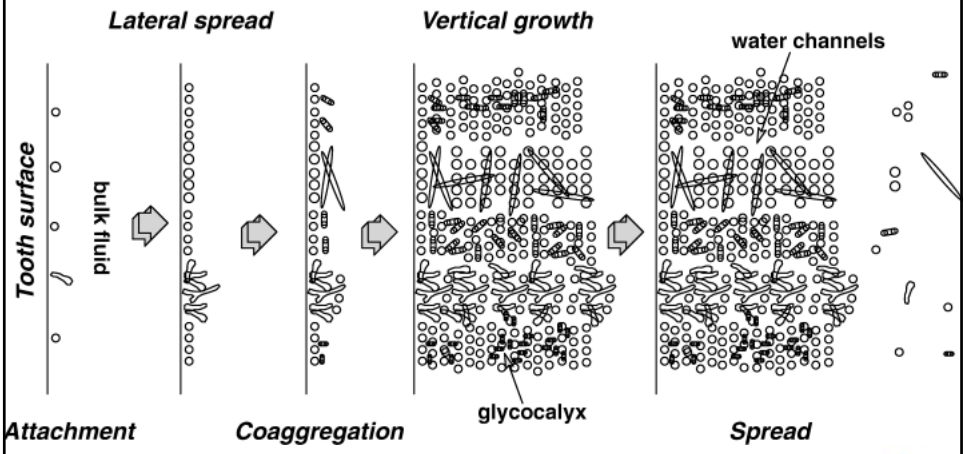
Interactions among dental plaque bacteria



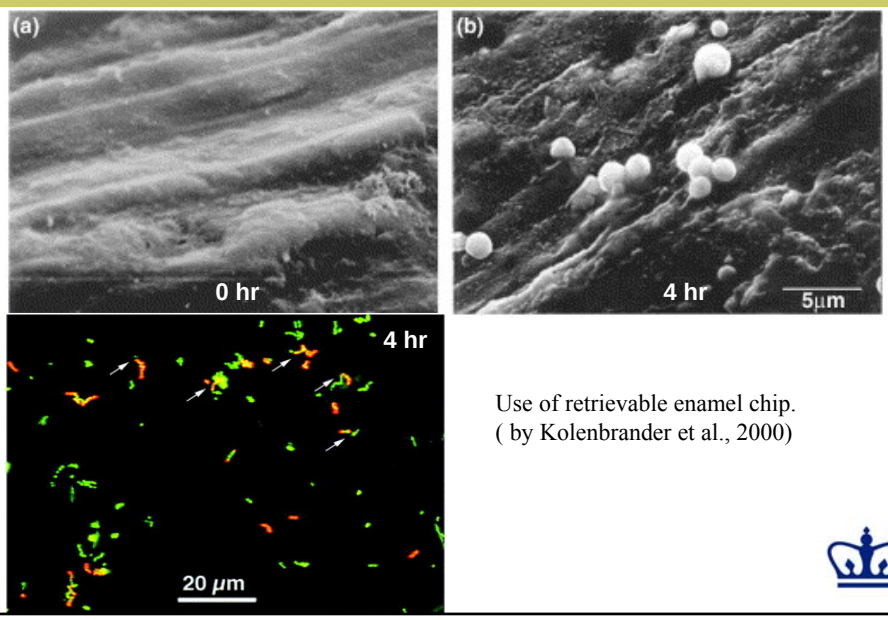
Formation of dental plaque

1. Acquired pellicle formation
   - Adherence of salivary glycoprotein on tooth surface
2. Rapid colonization by pioneer species (Gram (+) cocci and rods)
   - *S. sanguis*, *S. oralis*, *S. mitis*, *A. viscosus* can adhere to pellicle by specificity
   - Resisting shear force (saliva) and electrostatic repulsion
3. Predominance by Gram (-) filaments (in 5 days)
   - Microbial interaction, replacing Gram (+) cocci and rods
   - Emergence of Gram (-) filamentous bacteria
   - Matrix of microorganisms and a ground substance

Development of dental plaque biofilm

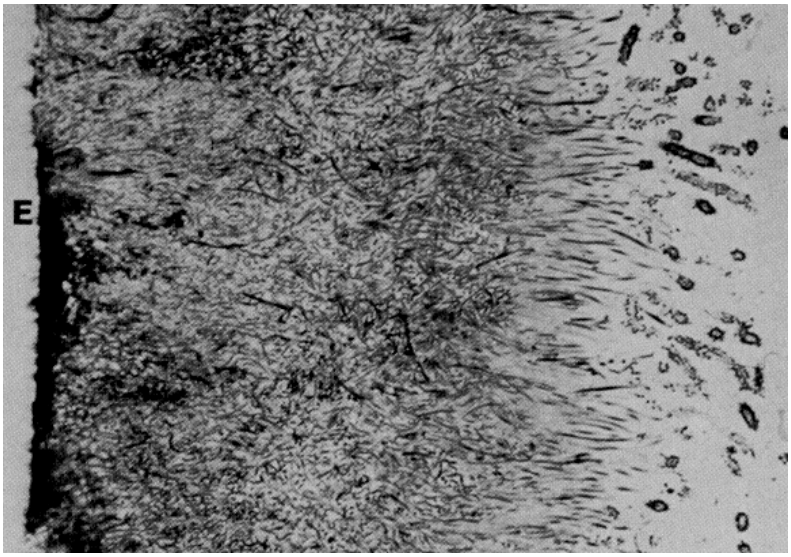


Early colonizers of human mouth



Use of retrievable enamel chip.
(by Kolenbrander et al., 2000)

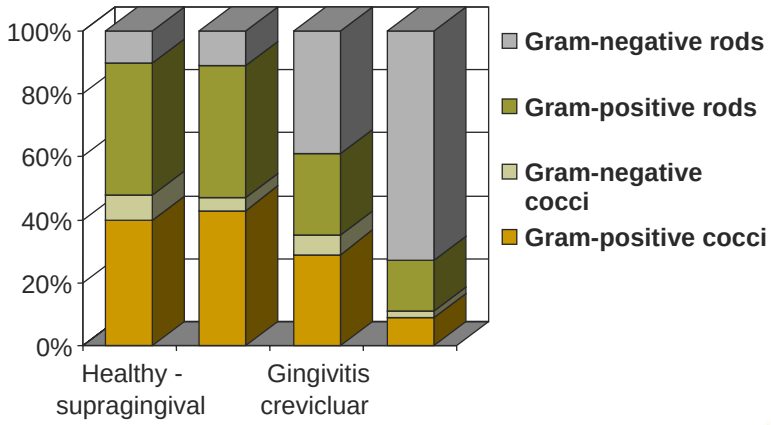
Supragingival dental plaque biofilm



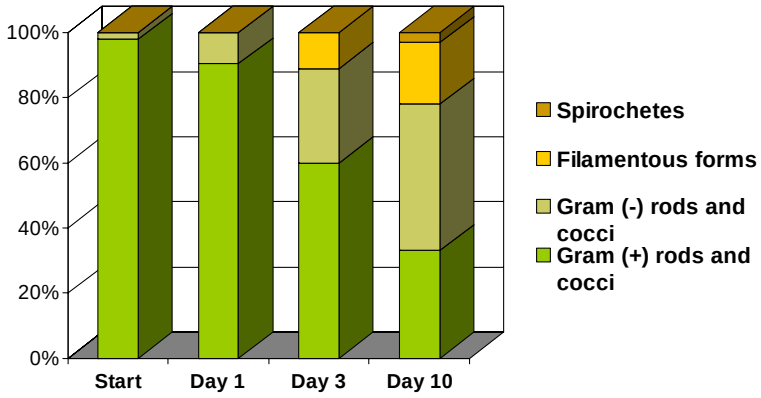
Dental plaque in health and disease

- Pattern of colonization (microbial succession) in dental plaque formation
- Difference in predominant species
  - Health-associated dental plaque
  - Disease-associated dental plaque
- Dental plaque hypotheses
  - Specific plaque hypothesis
  - Non-specific plaque hypothesis

Microbiota associated with periodontal health, gingivitis, and advanced periodontal disease



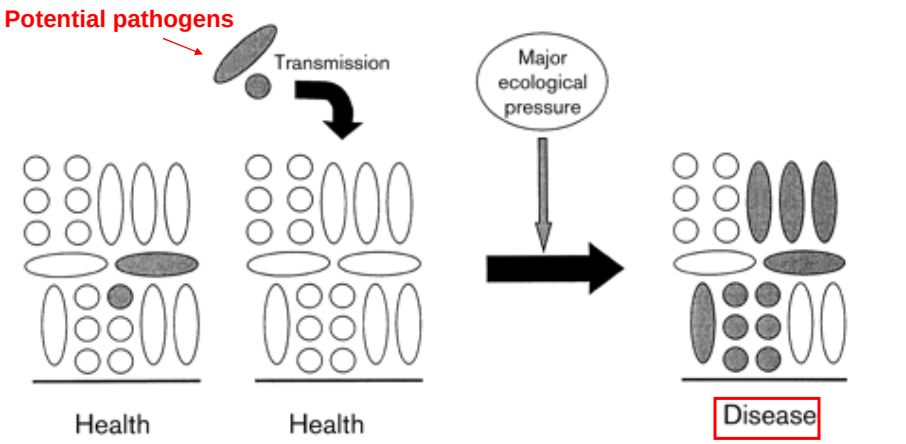
Plaque reformation after cleaning



Dental plaque hypothesis

- **Specific** plaque hypothesis
  - A single or limited numbers of specific pathogen within dental plaque
  - Specific forms of periodontal disease have specific bacterial etiologies, i.e. LJP
- **Non-specific** plaque hypothesis
  - Overgrowth of dental plaque will lead to disease
  - Plaque as a relatively homogeneous bacterial mass
  - Gingivitis
- **Intermediate**
  - Qualitatively distinct bacterial composition: healthy vs. disease (subjects, sites)
  - Pathogenic shift; disturbed equilibrium
  - A small group of bacteria: Gram (-), anaerobic

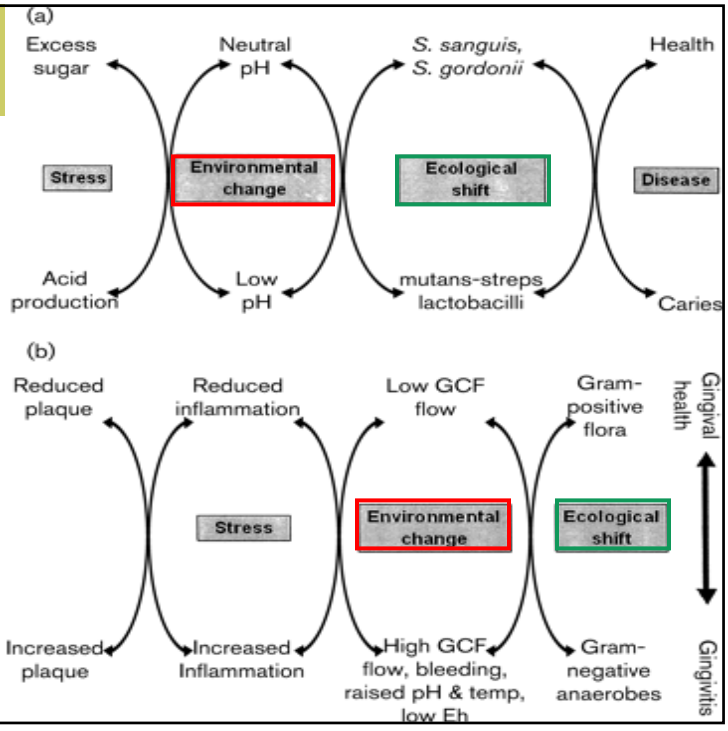
Health vs. disease microflora in dental plaque



Understanding dental diseases from ecological perspective

- The mouth - a unique microhabitat
- Multi-species biofilm
- "Selection" of "pathogenic" bacteria among microbial community
  - Selection pressure coupled to environmental changes
- Therapeutic and preventive measures - by interfering with the selection pressures responsible for their enrichment

Ecological plaque hypothesis



Dental plaque biofilm infection

- Ecological point of view
  - Ecological community evolved for survival as a whole
  - Complex community
    - Over 500 bacterial species
    - Adherence, coaggregation
- Dynamic equilibrium between bacteria and a host defense
  - Adopted survival strategies favoring growth in plaque
  - Disturbed equilibrium leading to pathology
- The dental plaque bacterial composition may result in a destructive inflammatory response