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MICROBIAL BIOFILM – I

Lecture for the dentistry students
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Two forms of microbial growth

- **Growth in planktonic form**

  Isolated microbial cells float freely in a fluid environment

- **Growth in biofilm form**

  Result of the natural tendency of microbial cells to stick to one another and to a solid surface and to form a community connected by an extracellular matter

Which form is more frequent?

- **Planktonic form**
- fairly common in the laboratory (e.g. in nutrient broth)
- relatively scarce in a natural environment

- **Biofilm form**
- standard and crucial in the natural environment
- more advantageous for microbes

Definition of biofilm

Microbial biofilm is a community of microorganisms that

- forms at the boundary of phases (usually of the solid and fluid phase)
- sticks to inert as well as to live surfaces
- is surrounded by an extracellular matter, in which a complex system of channels forms

Three examples of biofilm

- **Have you ever slipped on a wet stone in a creek?**

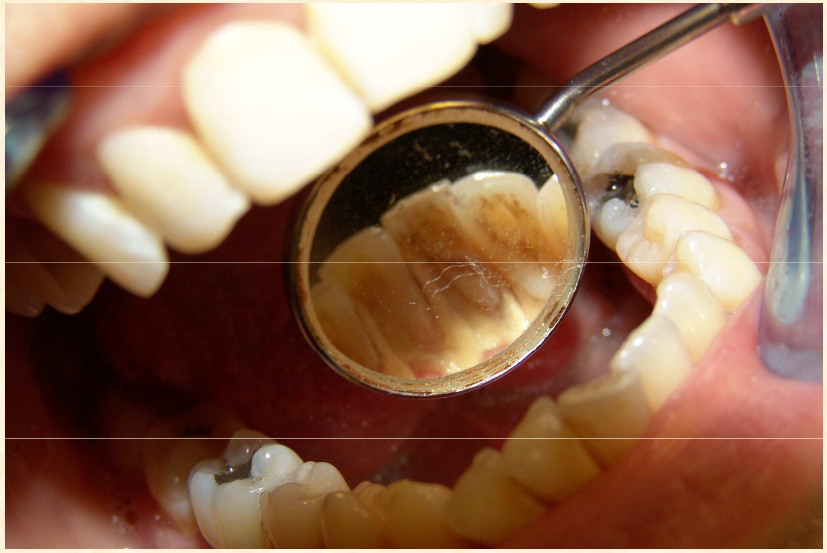
  Certainly – and in was biofilm that you slipped on

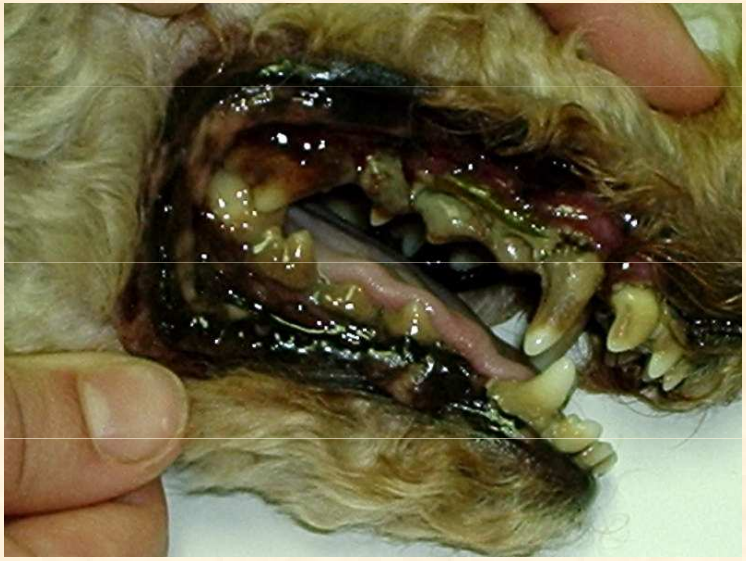
- **Have you an aquarium and do you clean its walls?**

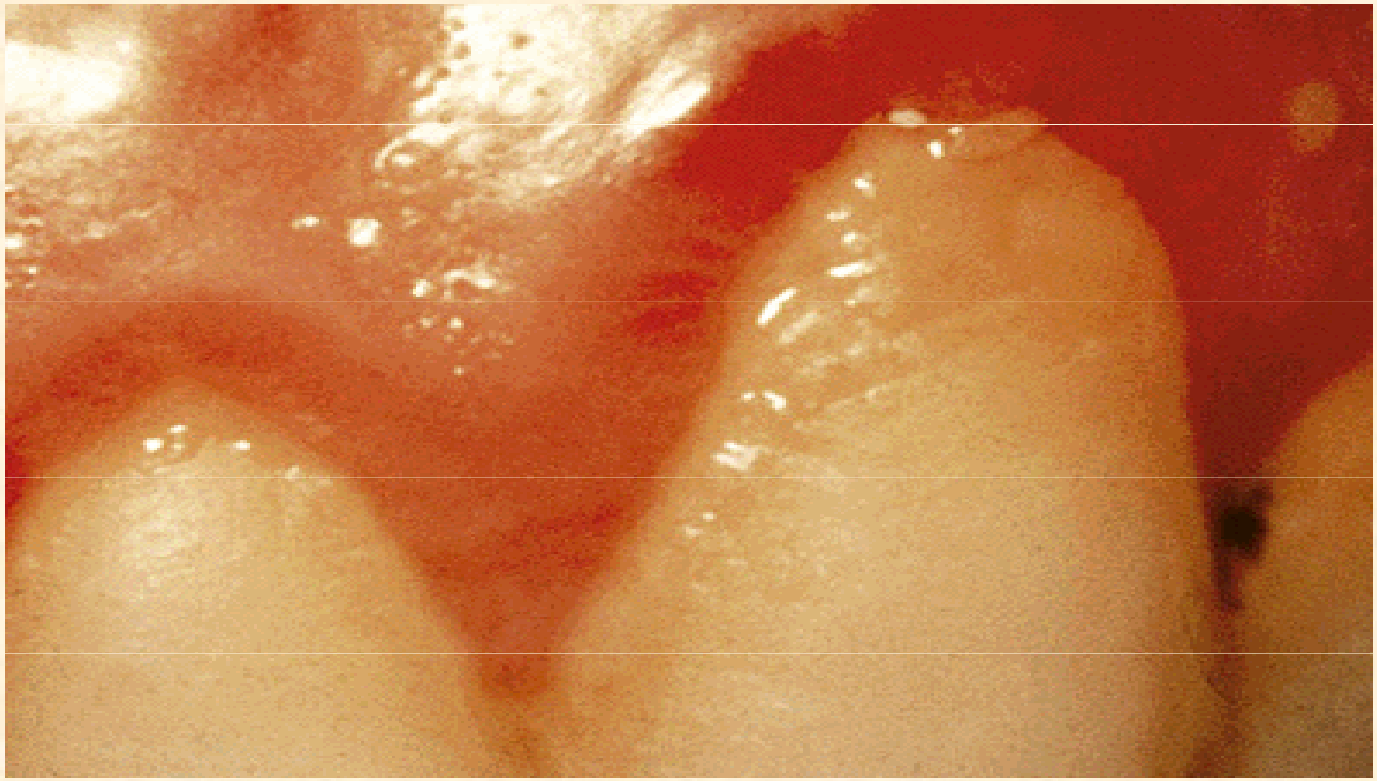
  If you do, what you wipe from them is the biofilm formed by algae

- **Do you clean your teeth regularly?**

  I hope so and by doing this you remove the biofilm called dental plaque







History of biofilm

- **1676 Antony van Leeuwehoek**

  bacteria in dental plaque
- **1935 C. E. Zobel**

  the first description of biofilm in marine bacteria
- **1950 – 1960**

  first information about problems with the biofilm
- **1978 J. W. Costerton**

  drawing attention to the ubiquity of biofilm
- **1999 Costerton, Stewart & Greenberg**

  biofilm involvement in persistent infections

Microbiology lead astray – 1

- **For 100 years since Pasteur and Koch times**

  it never occurred to anybody that in nature bacteria grow in other ways than as a freely floating plankton in seas or as colonies on agar

- **From the half of the 19th to the half of the 20th century,**

  throughout the whole „golden age of bacteriology“, the only subject of study were planktonic forms

  If signs of the biofilm growth appeared the experiment was quickly „sewered“

Microbiology lead astray – 2

The whole microbiology has been mislead

by efforts to examine and investigate pure cultures of planktonically growing cells only, whereas the natural microbial growth is in the form of biofilm

The last area of microbiology

that started to be concerned with the biofilm is regrettably the medical microbiology, proud of its achievements with planktonic forms

How does the biofilm develop?

Development of biofilm = cyclic process

1. Attraction of planktonic cells to a surface
2. Adhesion of planktonic cells to the surface
3. Aggregation of cells and the development of colonies – quorum-sensing phenomenon
4. Accumulation of exopolysaccharide matrix (slime) – development of typical architecture
5. Dispersal of cells from the surface of biofilm

Development of biofilm – attraction

Attraction does not concern solid surfaces only but in general the boundaries of phases

Prominent in mobile bacteria with flagella

How does the bacterium know the proximity of a surface?

It sends out chemical signals that diffuse more quickly into free areas while they concentrate in the vicinity of boundaries of phases

Development of biofilm – adhesion

Bacterial adhesins

fimbriae (pilli)

colonization factors of enteropathogenic *E. coli*

proteins and lipopolysaccharides of outer membrane

generally in most of Gram-negative bacteria

slime

both coagulase-negative and golden staphylococci

curli

E. coli

Development of biofilm – aggregation I

1. Movement

by means of flagella (*E. coli, Vibrio cholerae*)

by means of fimbriae (type IV pilli of *Pseudomonas aeruginosa*)

divergent – continuous layer of cells forms

convergent – aggregates develop, even of different species (e.g. coaggregation of *Streptococcus gordonii* + *Fusobacterium nucleatum* in dental plaque)

2. Multiplication

both aggregation and cell division in aggregates lead to the development of microcolonies

Development of biofilm – aggregation II

3. Quorum sensing

During division individual cells emit chemical signals (homoserinlactones in *P. aeruginosa*)

After reaching a particular number of cells (quorum) the elevated concentration of signals causes the change of cellular properties:

- switching off some so far functioning genes (e.g. a gene for the production of flagellin)
- expression of other genes, and from this ensuing
- production of new molecules (in particular exopolysaccharides)

Development of biofilm – accumulation

Production of exopolysaccharides

- colanic acid (*E. coli*)
- alginate (*P. aeruginosa*)
- polysaccharide intercellular adhesin *(Staph. epidermidis)*

leads to the development of typical biofilm architecture

- Its appearance depends mainly on the nature of the environment

Development of biofilm – dispersal

After reaching the critical amount of biomass or after the reduction of the amount of nutrients in the environment the character of cells at the surface of biofilm changes

- e.g. in *P. aeruginosa* the superficial cells
  - cease producing alginate
  - begin producing lyase and flagellin
- superficial layer of biofilm starts to disintegrate
- cells grow flagella and get loose of biofilm

The cells as a planktonic population drift away

to look for more suitable environment and to colonize new surfaces

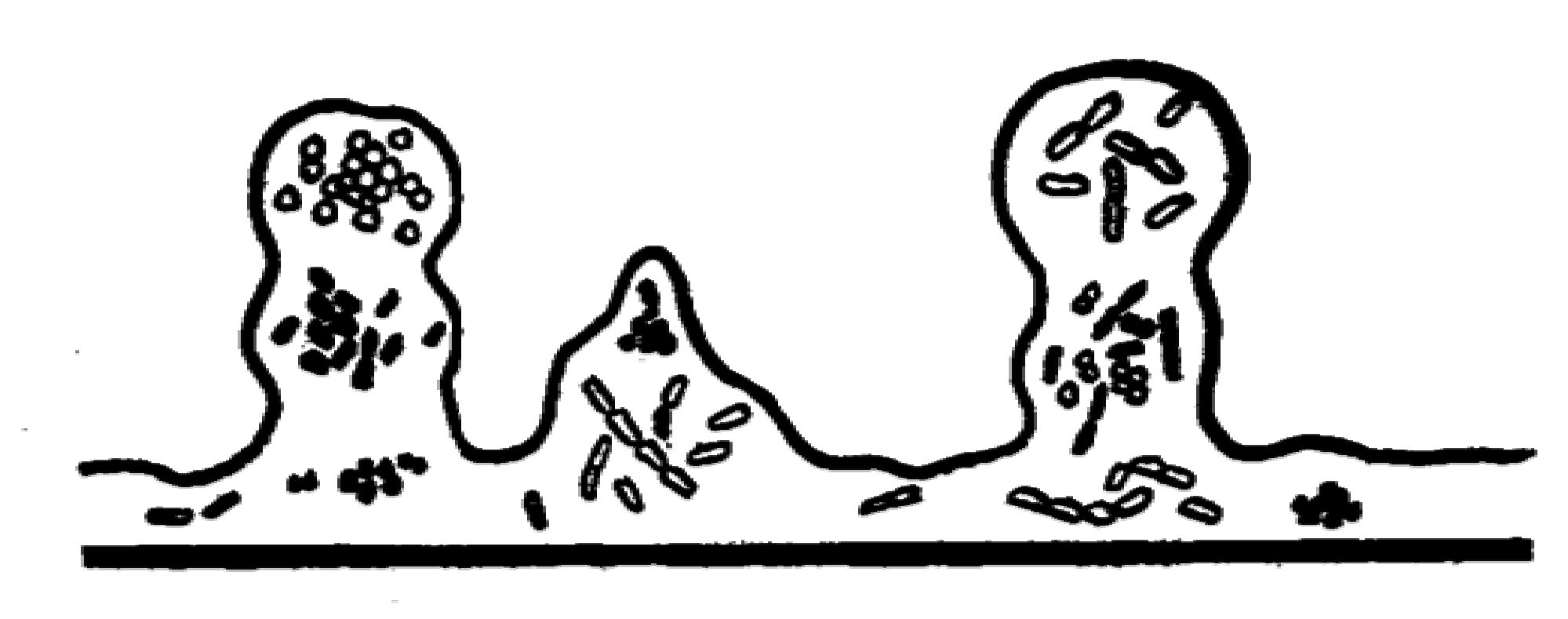
The cycle closes…

Architecture of biofilm – I

Depends above all on the concentration of nutrients

- **<10 mg/L** (mountain streams, lakes, open sea)
  heterogeneous mosaic (a thin layer + columns of microcolonies)
- **10-1000 mg/L** (majority of our rivers and ponds)
  complex system with channels (created by mushroom-like, partially merging microcolonies)
- **>1000 mg/L** (in the environment of macroorganism)
  compact biofilm (almost without traces of channels)

Architecture of biofilm – II



Low concentrations of nutrients (0.1 – 10 mg/L – mountain streams, lakes, open sea)

Heterogeneous mosaic = thin layer of individual cells above which columned microcolonies rise here and there

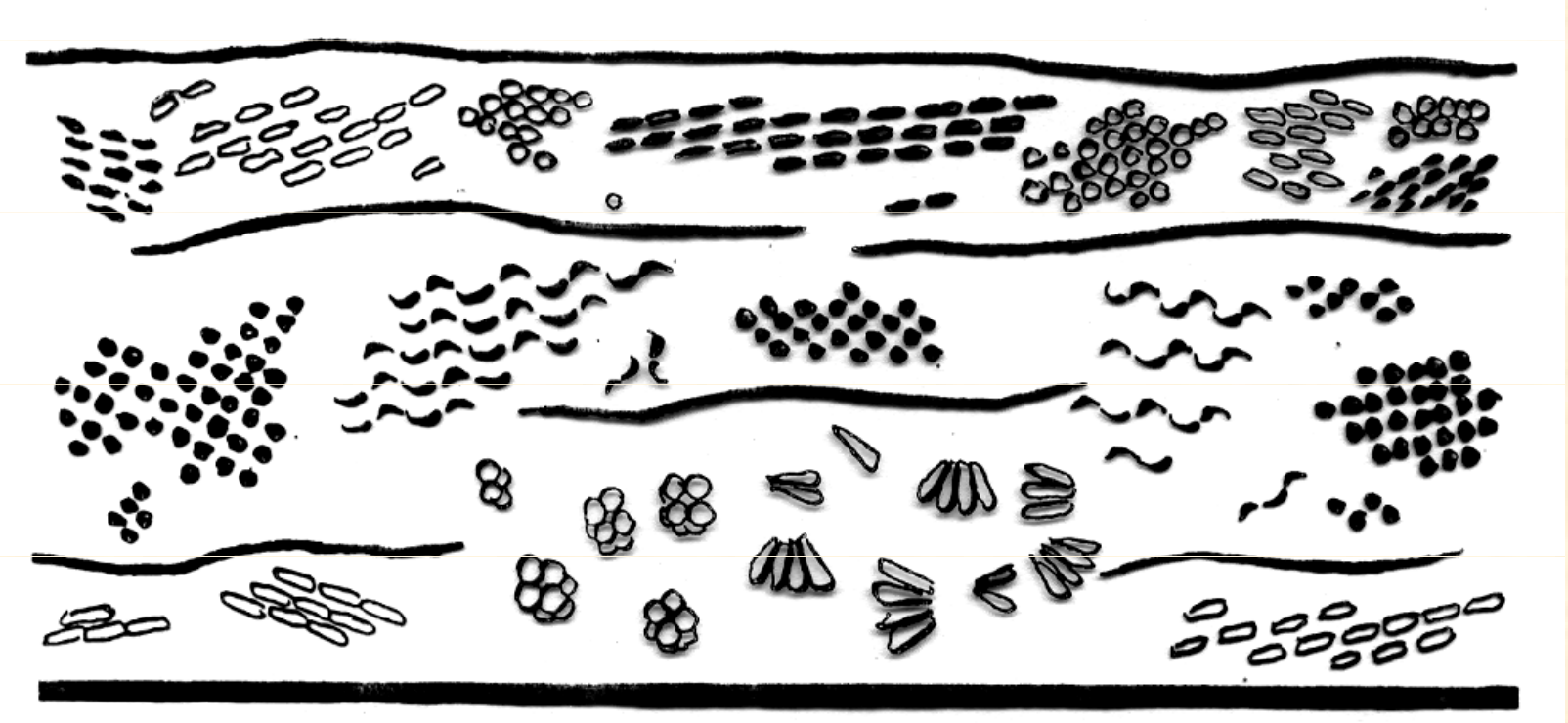
Architecture of biofilm – III

Medium concentration of nutrients (10 – 1000 mg/L – eutrophic water environment)

System with channels = mushroom-shaped microcolonies partially merging together, interwoven with water channels

Architecture of biofilm – IV

Architecture of biofilm – V

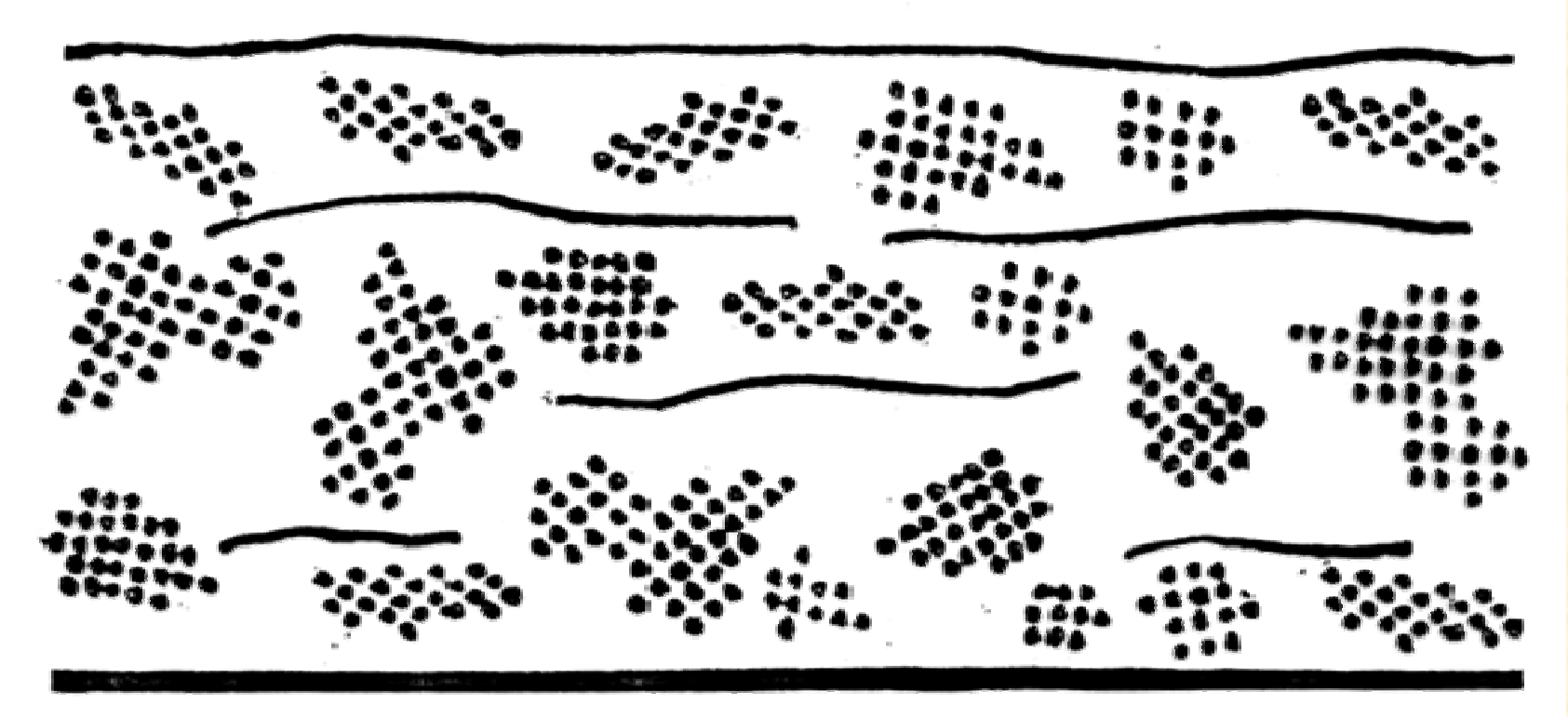


High concentrations of nutrients (>1000 mg/L – in the macroorganism)

compact biofilm = closely interconnected numerous microcolonies almost without traces of possible channels

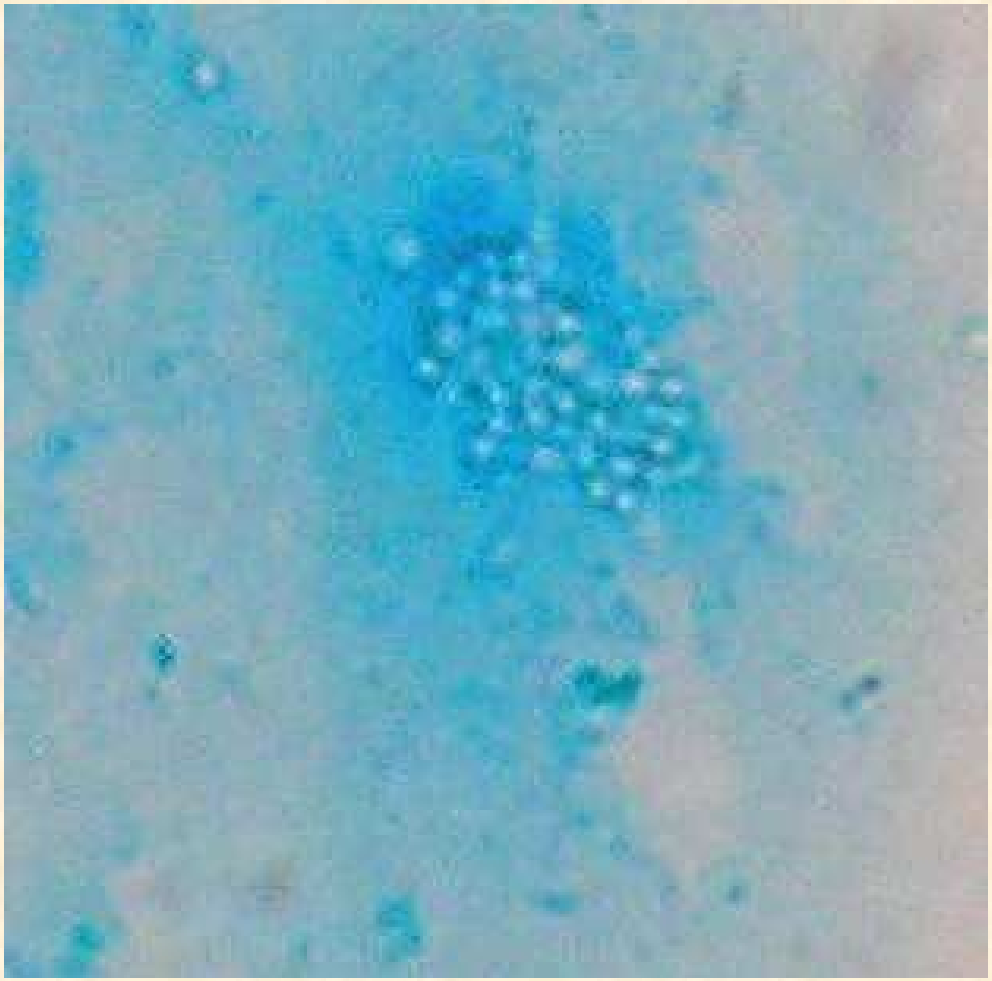
a) polymicrobial = e.g. dental plaque, normal microflora of mucous membranes

Architecture of biofilm – VI



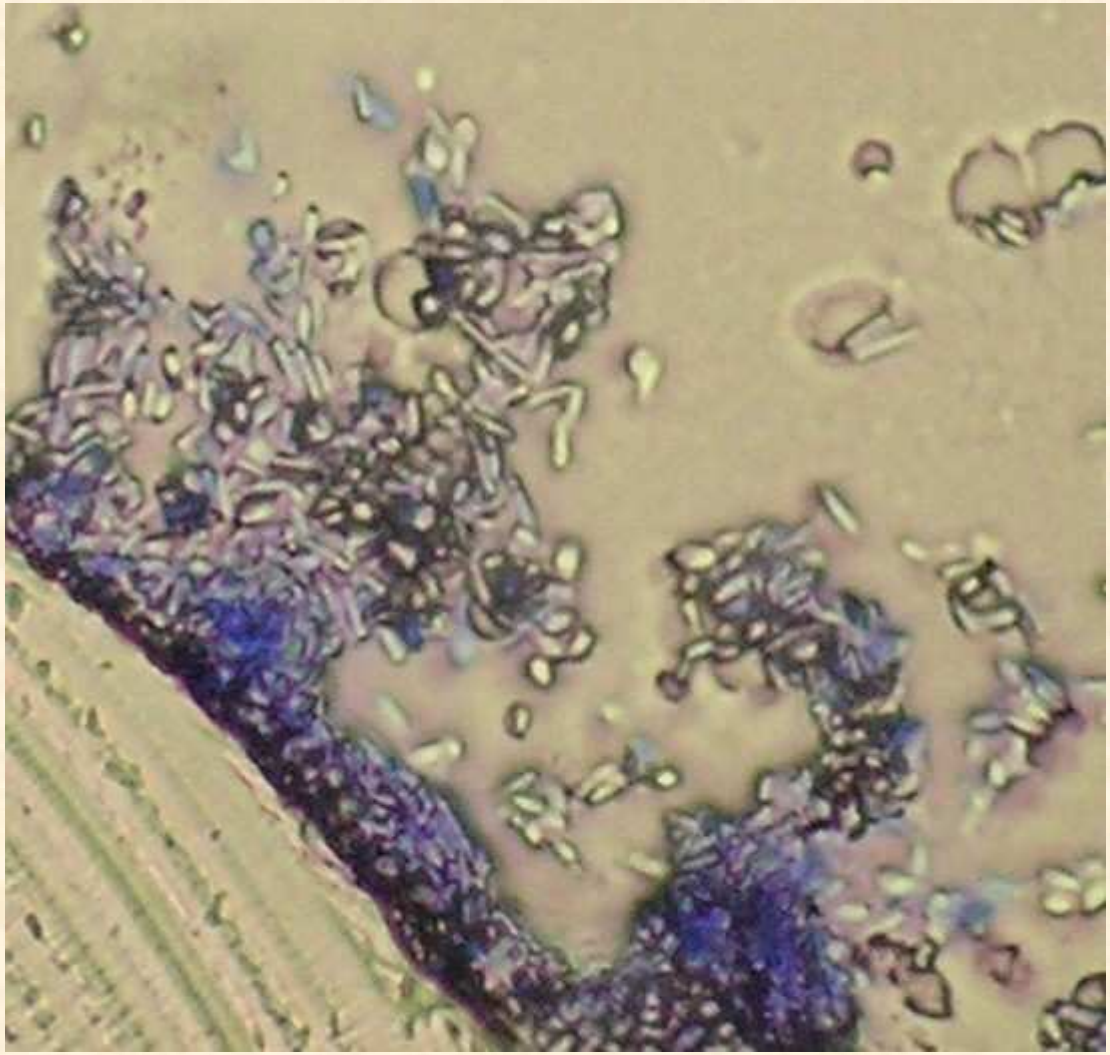
High concentrations of nutrients (>1000 mg/L – in the macroorganism)
compact biofilm = closely interconnected numerous microcolonies almost without traces of possible channels
b) monomicrobial = e.g. chronic osteomyelitis
biofilm on inert surfaces of medical devices

Architecture of biofilm – VII



Candida albicans biofilm. Alcian blue has coloured extracellular polysaccharides.
Photo: Veronika Holá

Architecture of biofilm – VIII



Candida albicans biofilm. Toluidin blue. At the photo mushroom-like structure of the biofilm is obvious.

Photo: Veronika Holá

Properties of biofilm

- Biofilm is a higher and more complex form of microbial growth
- It utilizes the opportunity of mutual cooperation of cells
- It enables the easier transfer of genes
- It is characterized by an effective homeostasis
- It shows features of a primitive circulation system
- It provides a high protection against antimicrobial factors
- It plays an important part in many significant occasions including medically important conditions

Properties of biofilm

Summary:

The properties of microbes growing in the biofilm form are fundamentally different from the properties of microbes growing in the planktonic form; the microbes

- express different genes
- produce different products (extracellular matrix × flagella)
- enjoy a higher degree of protection

Importance of biofilm for the life of microorganisms – I

More favourable environment for the life of microorganisms

Possibility of effective cooperation and specialization of cells

Stable mutual position of cells of different species in the intercellular matrix and corresponding presence of various enzymes

Considerably easier transfer of genes

Effective homeostasis

Inside the aerobically established biofilm anaerobic places may occur

Primitive circulation system

brings in and carries away nutrients, waste products as well as signal molecules

Importance of biofilm for the life of microorganisms – II

Protection against harmful influences

in environment: against amoebae, phages, dessication, washing away, toxic substances

in macroorganism: against phagocytes, washing away, complement, antibodies, antibiotics

Resistance of biofilm towards toxic substances

MICROBES IN THE BIOFILM FORM ARE ALWAYS MORE RESISTANT THAN IN THE PLANKTONIC FORM

- Higher resistance applies also to disinfectants and antibiotics
- Differences in sensitivity sometimes amount up to 3 orders
- General mechanism of the higher resistance is not known
- In each microbe-antimicrobial combination the mechanism can be different

Possible causes of higher resistance of biofilm

1. More difficult penetration of toxic matter through the biofilm
2. Altered character of environment in the biofilm
3. Altered microbial population in the biofilm

Recommended reading material

Paul de Kruif: Microbe Hunters

Paul de Kruif: Men against Death

Axel Munthe: The Story of San Michele

Sinclair Lewis: Arrowsmith

Thank you for your attention