Institute of Microbiology shows

TRACING THE CRIMINAL

Part Eleven:

Criminal in a Organized Group

Bacterial biofilm

Survey of topics

Clinical cases related to biofilm

Characterisation of biofilm

Diagnostic and experimental method for biofilm

Pictures of biofilm

Clinical cases related to biofilm

Story one (today a real one)

- Male, 58 let, 2001 cardiostimulator, 2002 repeatedly hospitalized on an internal department with fever of unknown origin, elavation of inflamatory markers
- In blood cultures, *S. epidermidis*, very good susceptibility
- Several times treated by high doses of antibiotics in combinations (oxacilin, gentamicin, rifampicin, cafazolin, cefalotin, clindamycin)

Story – contiuing

- In the beginning, a good response, later attacks of fever again
- At transoesofageal examination, vegetation on a chamber electrode sized 1,5 × 1,5 cm.
- Cardiologists repeatedly refuse cardiostimulator removal. A combination oxacilin + gentamicine + rifampicine, pacient in a good state.
- Nevertheless, again temperature and CRP rises. Vancomycin and rifampicin starts to be used, after improval, patient‘s trombus is removed and the electrode changed (under antibiotics), so the patient starts to be better.

Who is guilty? The biofilm!!!

- The therapy could not be succesfull, because **high resistance of bacteria growing in form of a biofilm** was not taken into account.
- The therapy was not strong enough from the beginning and **the biofilm was not erradicated**.
- Only **electrod removal** (under antibiotic therapy) enabled **pacient status improval**.

Catheter biofilm

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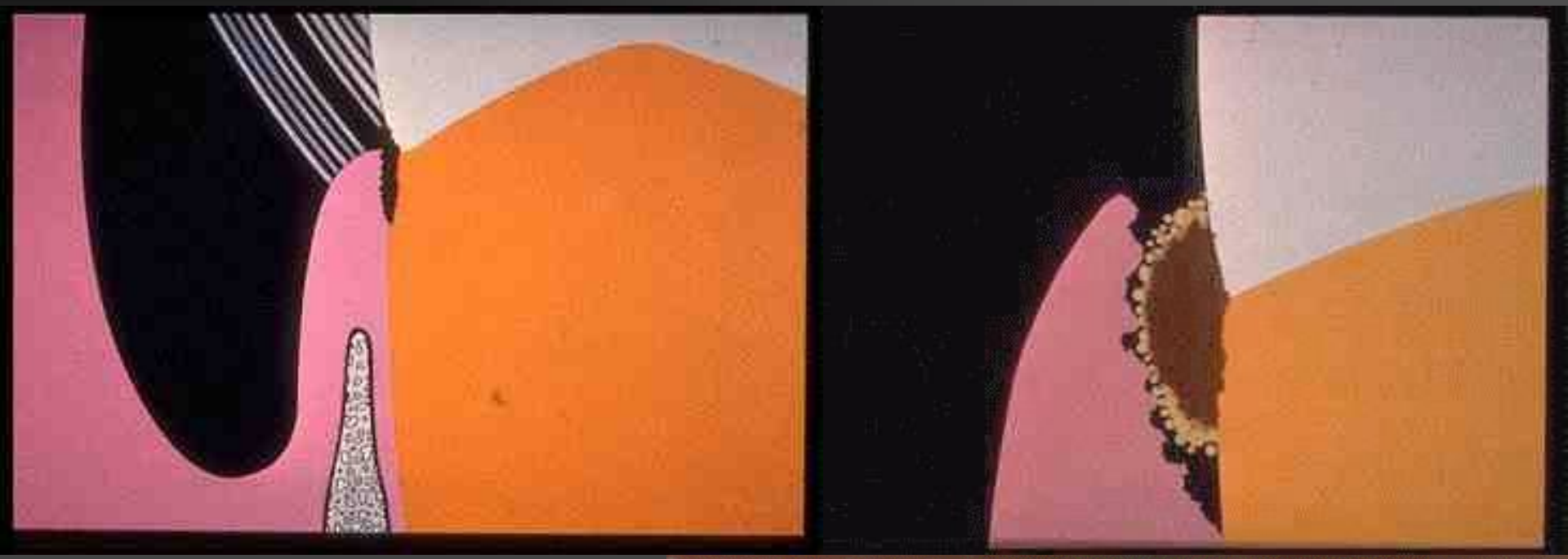
Story two

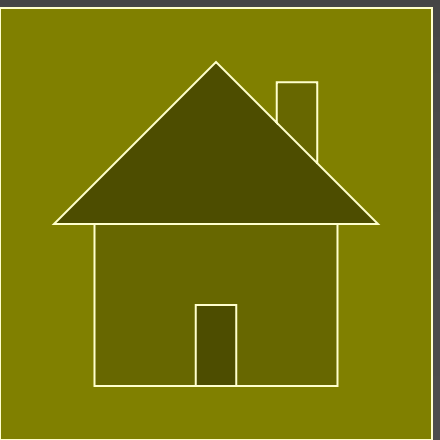
- Michal was a **13-year old boy.** He hated his parents and he decided to do anything against them.
- He decided that one of methods to fight them could be to resist everything his parents insist on him to do.
- So he decided to **stop cleaning his teeth**, keeping order in his room and some more activities like that.
- But very soon, a **toothache** started. He had to visit a dentist. The dentist said he has a **severe dental caries**. She repaired his teeth, but also wanted him to clean his teath again, otherwise he would have problems again – not only with her (and other „come-back-to-your-grave generation), but mostly with his own teeth.

The criminal agent was

- …an overgrown biofilm again.
- **In oral cavity, a biofilm is normal**. It is even useful: the normal oral microflora is organised in it, and so it is more resistant to outer influences, including pathogenic agents
- Nevertheless, too **overgrown biofilm** (as a result of too many sugars eaten and too little teeth cleaning) makes oral biofilm to be an **enemy instead to be a friend** of a patient.

Biofilm missed by a toothbrash may lead to a caries formation





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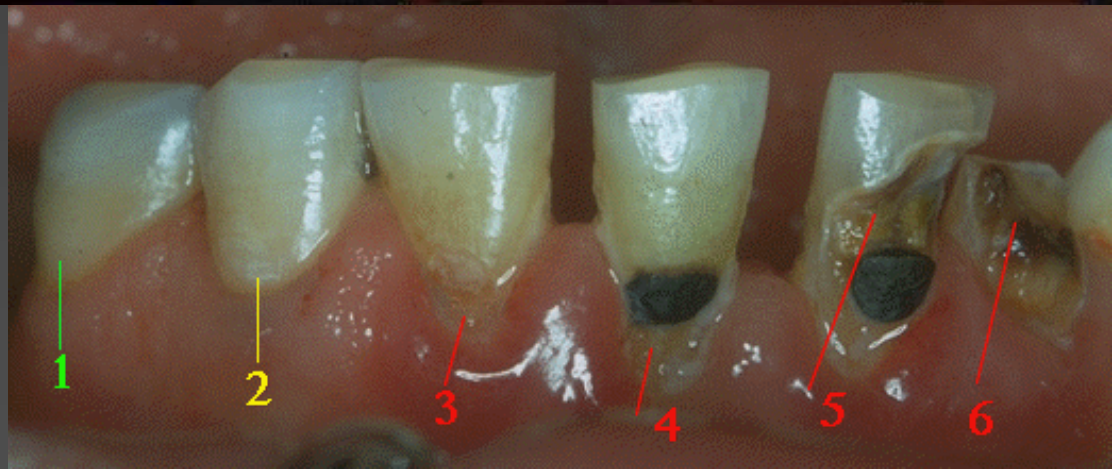


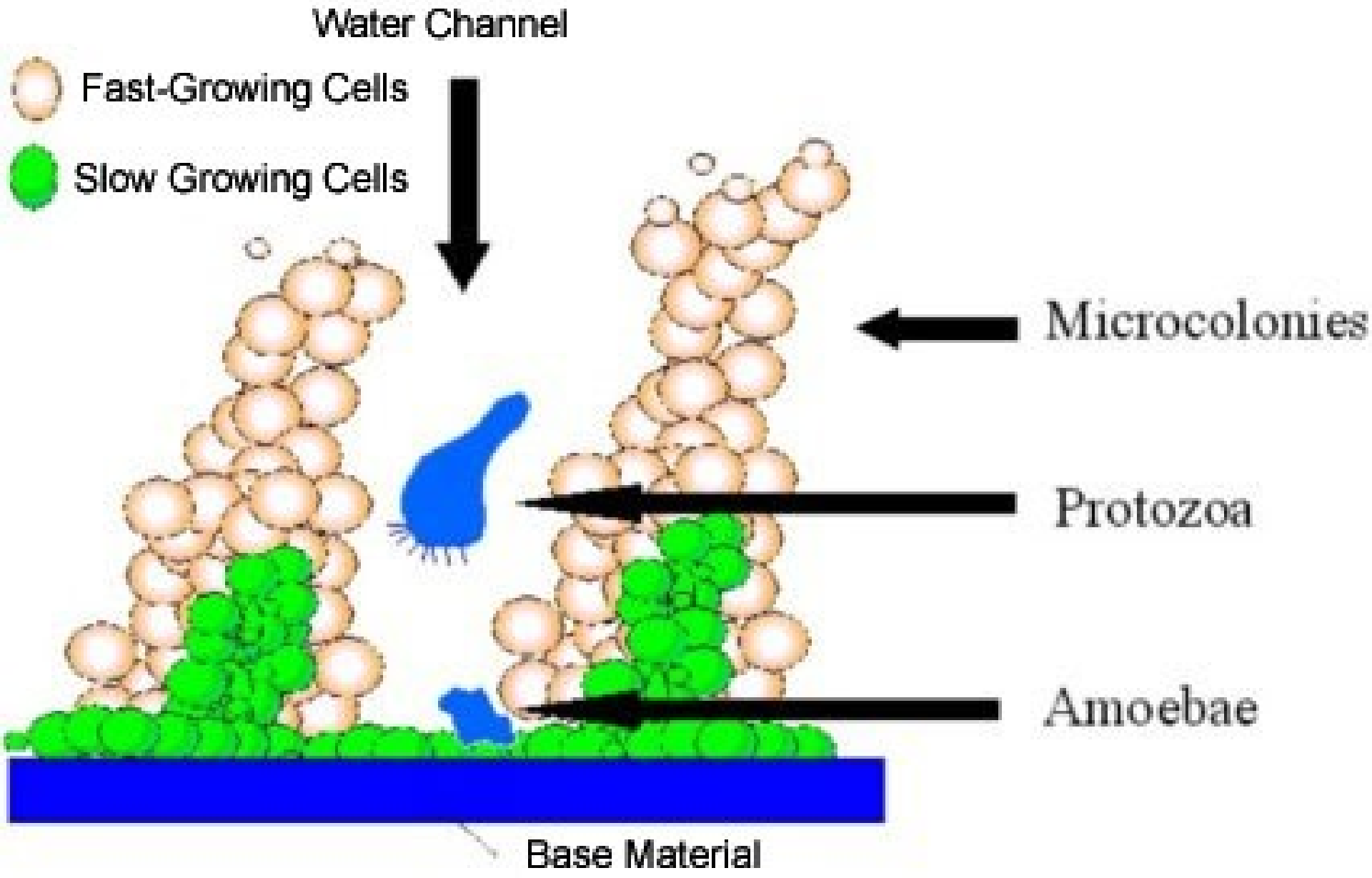
Characterisation of biofilm

Biofilm: what is it?

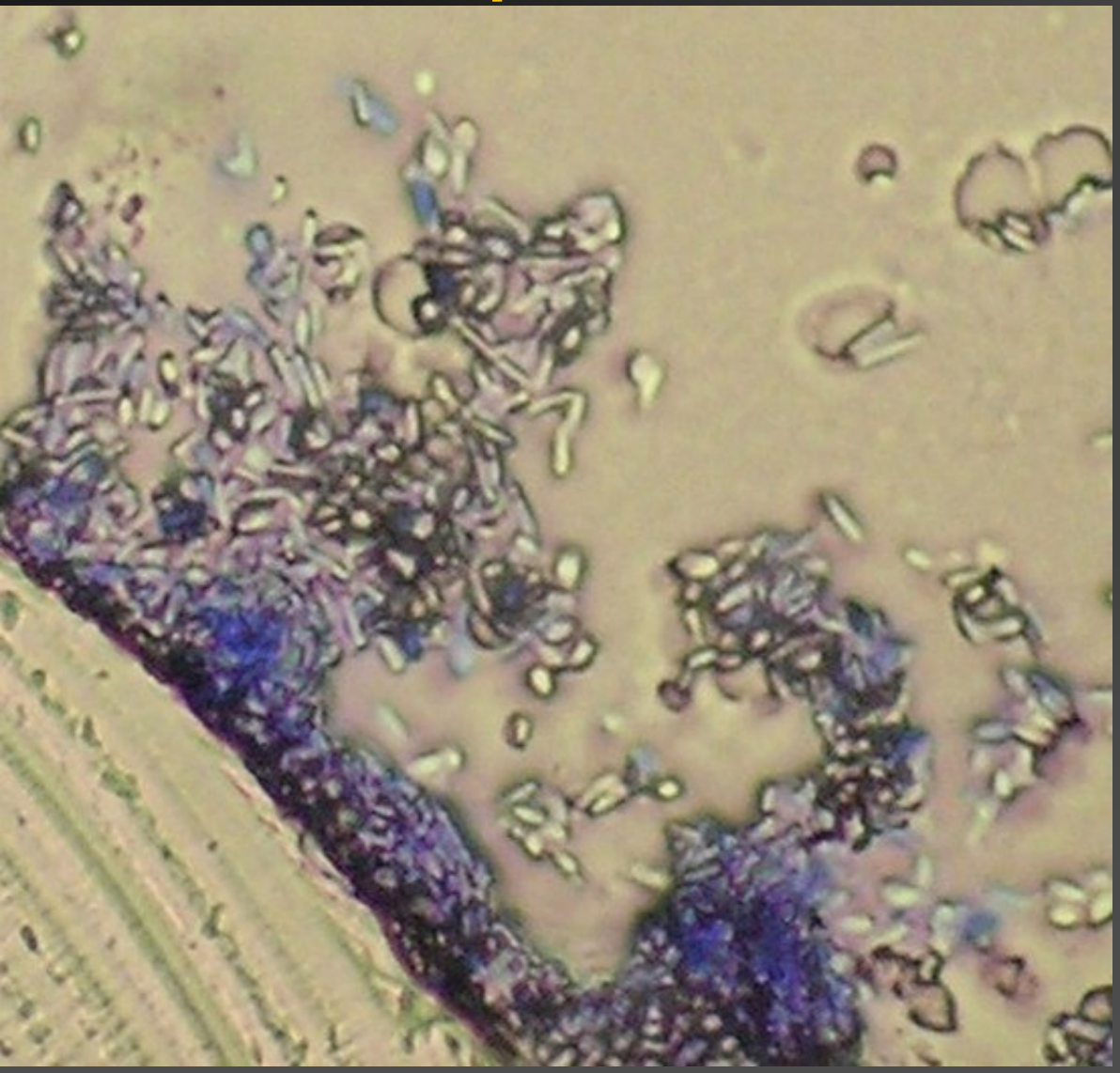
- A biofilm is a **complex, organised structure**
- It consists of **living cells** (mostly bacteria), **masses** produced by them (mostly polysaccarides) and **channels**
- It is present not only inside living body, but also **in the environment**. For example stones in ponds and rivers are often covered by a biofilm that makes them smooth.

Biofilm in a river

www.sbs.soton.ac.uk



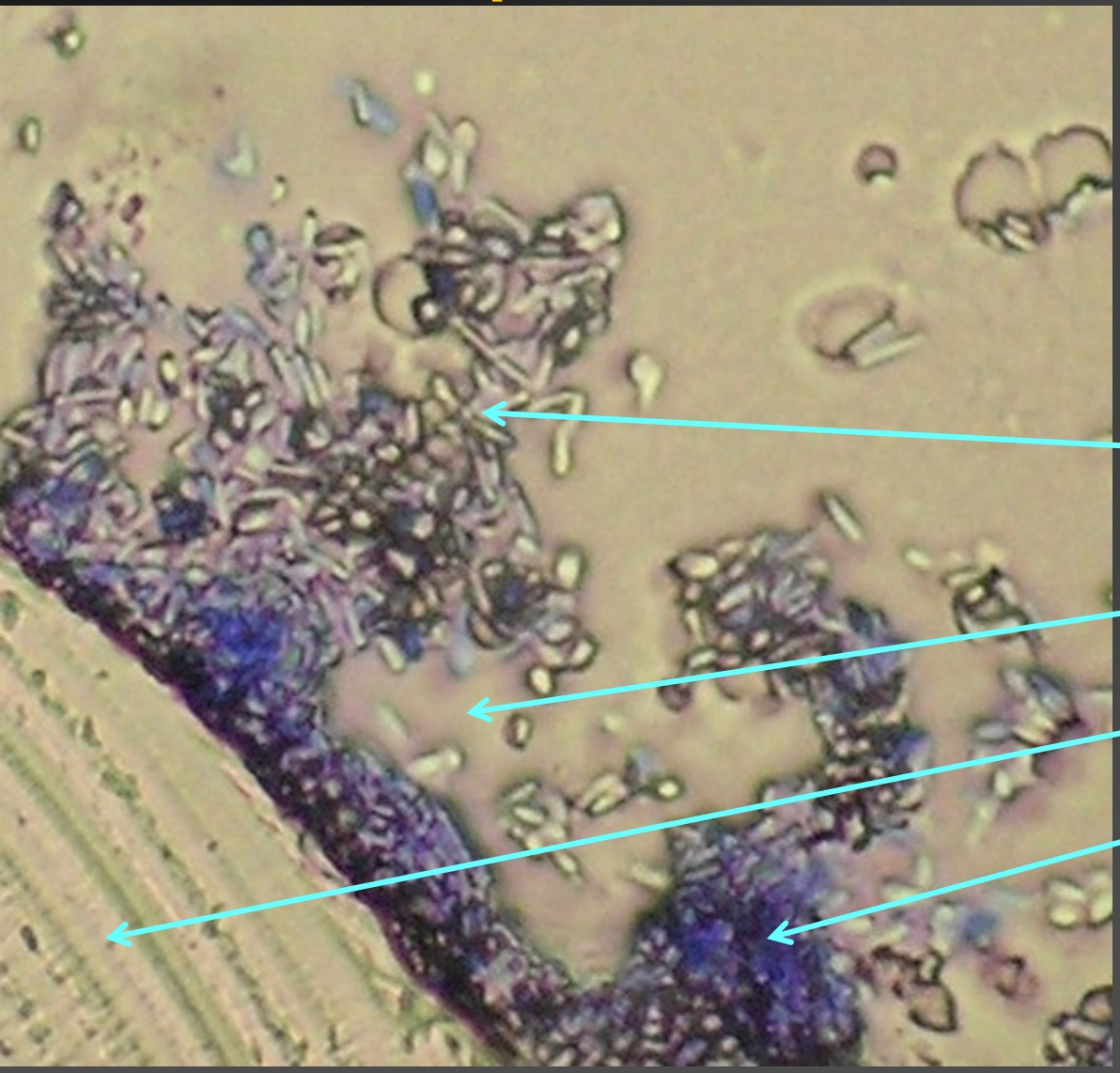
Various pictures of biofilm



Biofilm on a cathetre

Photo: Archive of Veronika Holá

Various pictures of biofilm



Biofilm on a cathetre

Bacteria

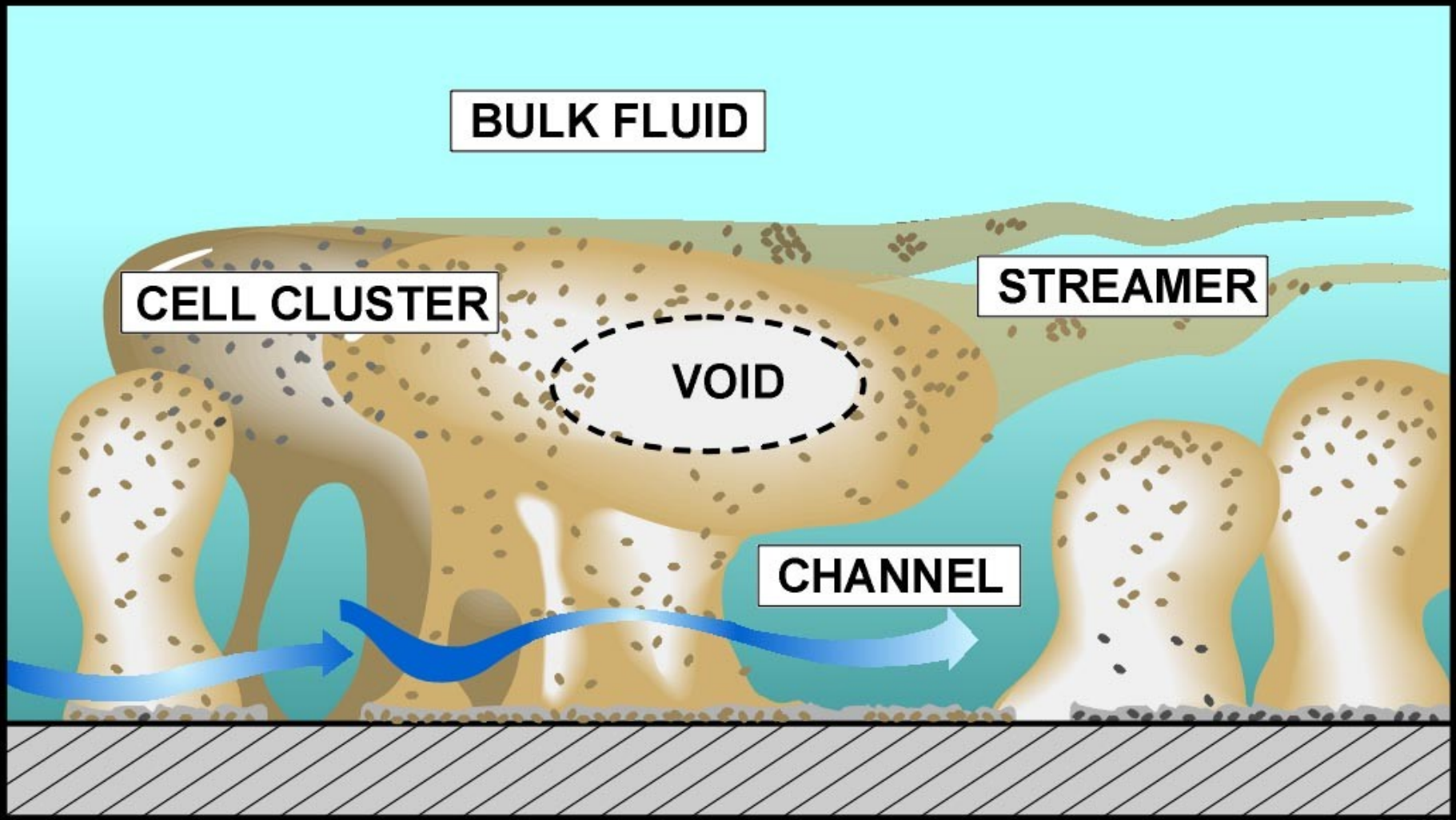
A channell

Catheter

Polysaccharides

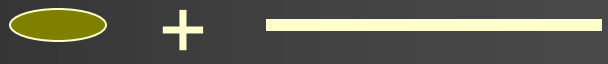
Photo: Archive of Veronika Holá

biology.fullerton.edu



Stages of biofilm development

- Direct contact of a planctonic bacteria with a surface

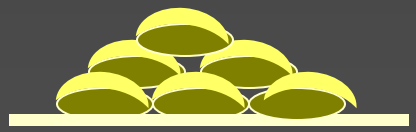


- Attachment to this surface

- Adhesion, growth, and aggregation of cells into microcolonies

- Production of polymeric matrix

- Formation of three-dimensional structure known as biofilm

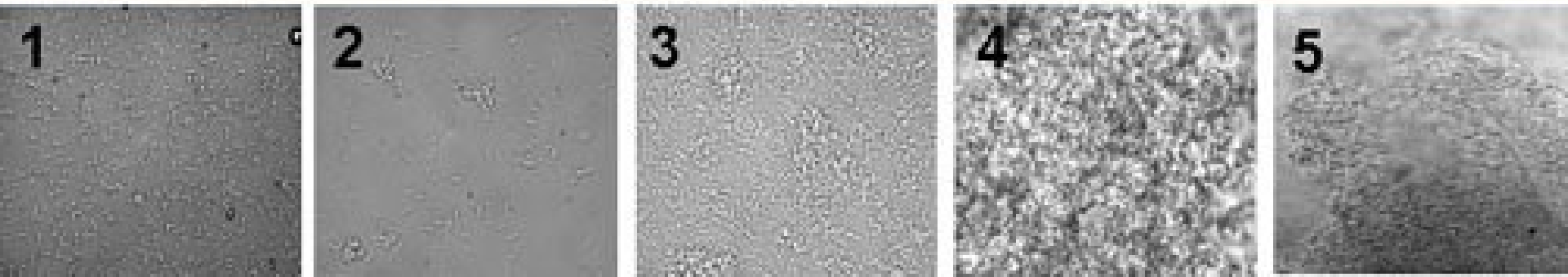


Development of biofilm – timing

0 h

Development of biofilm

biology.binghamton.edu



Biofilm development, another picture

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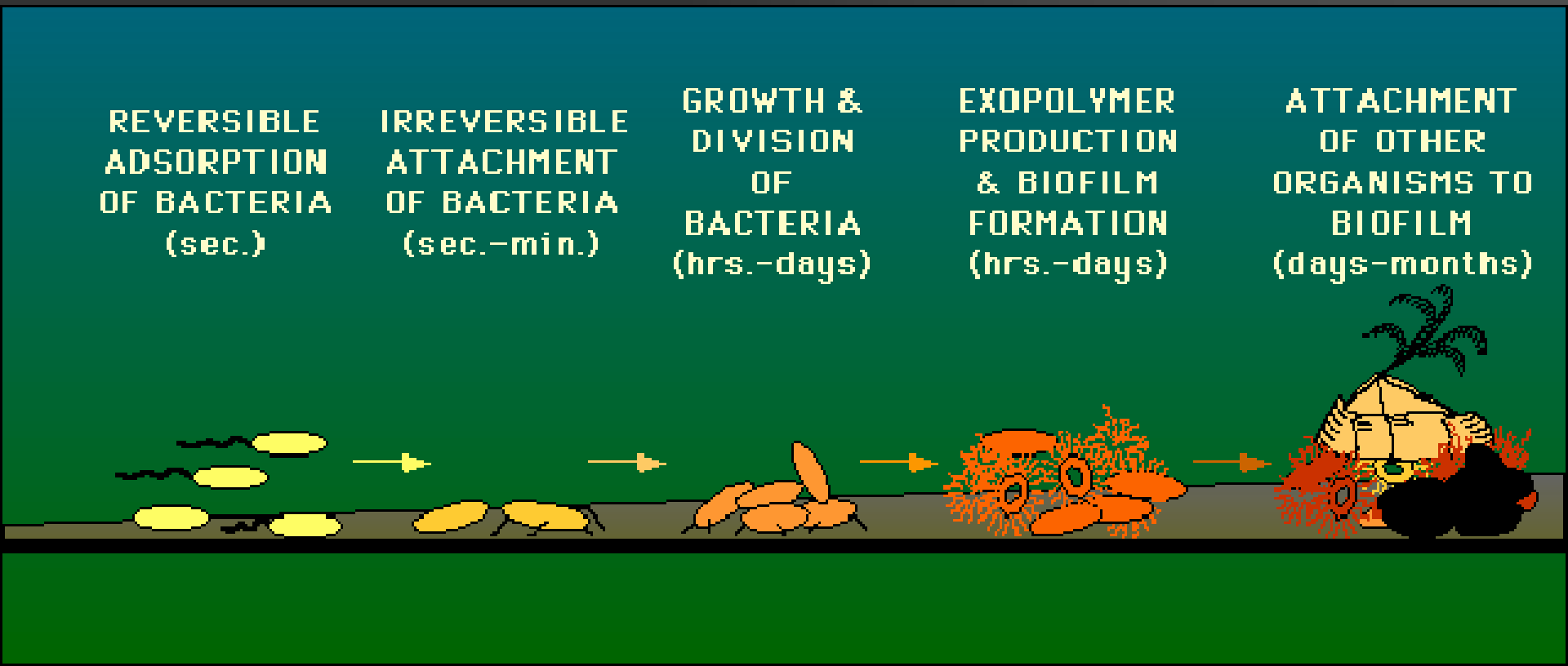
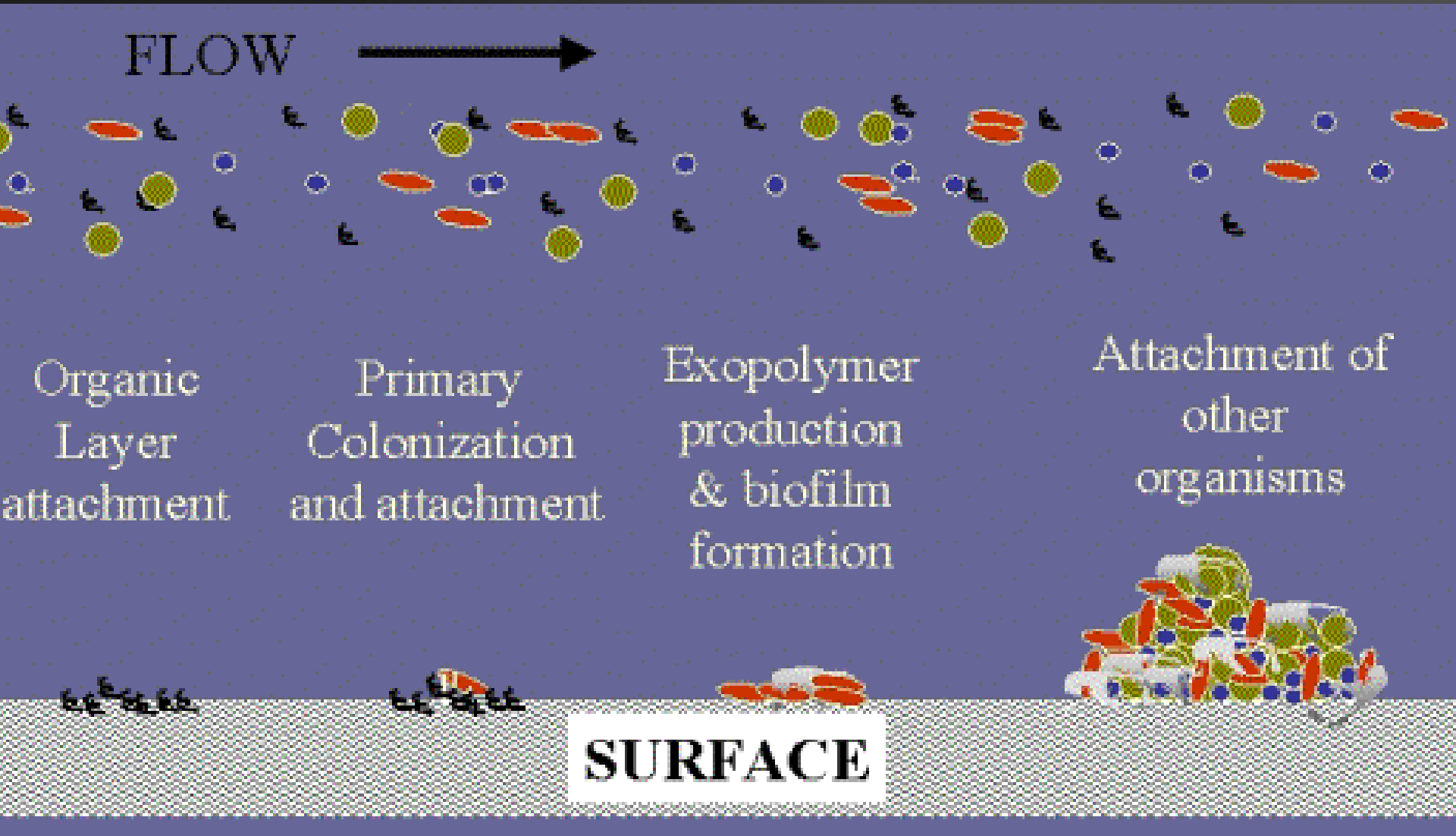
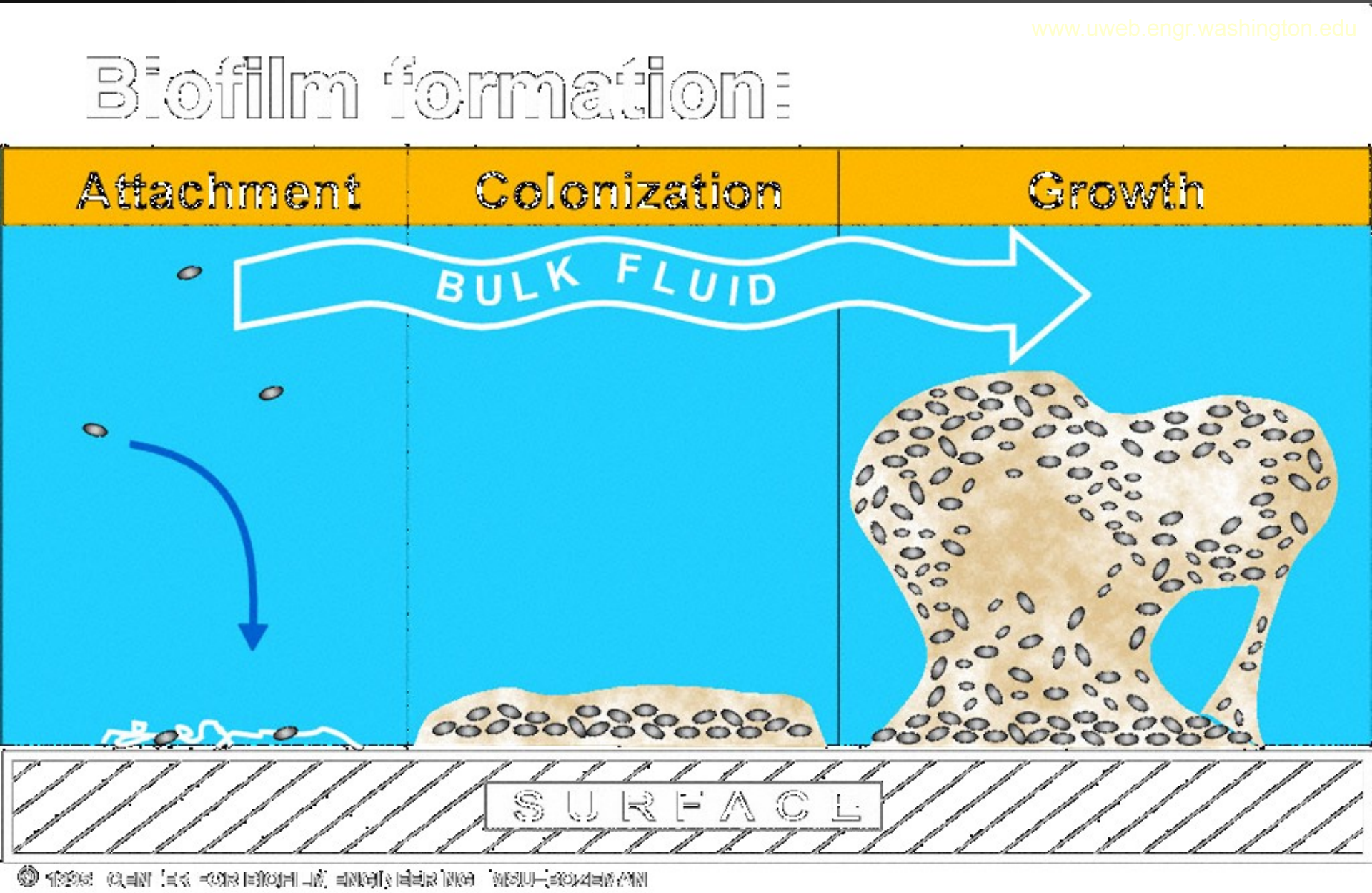


Biofilm development

www.ul.ie



Biofilm formation, another picture



Importance of biofilm production in bacteria

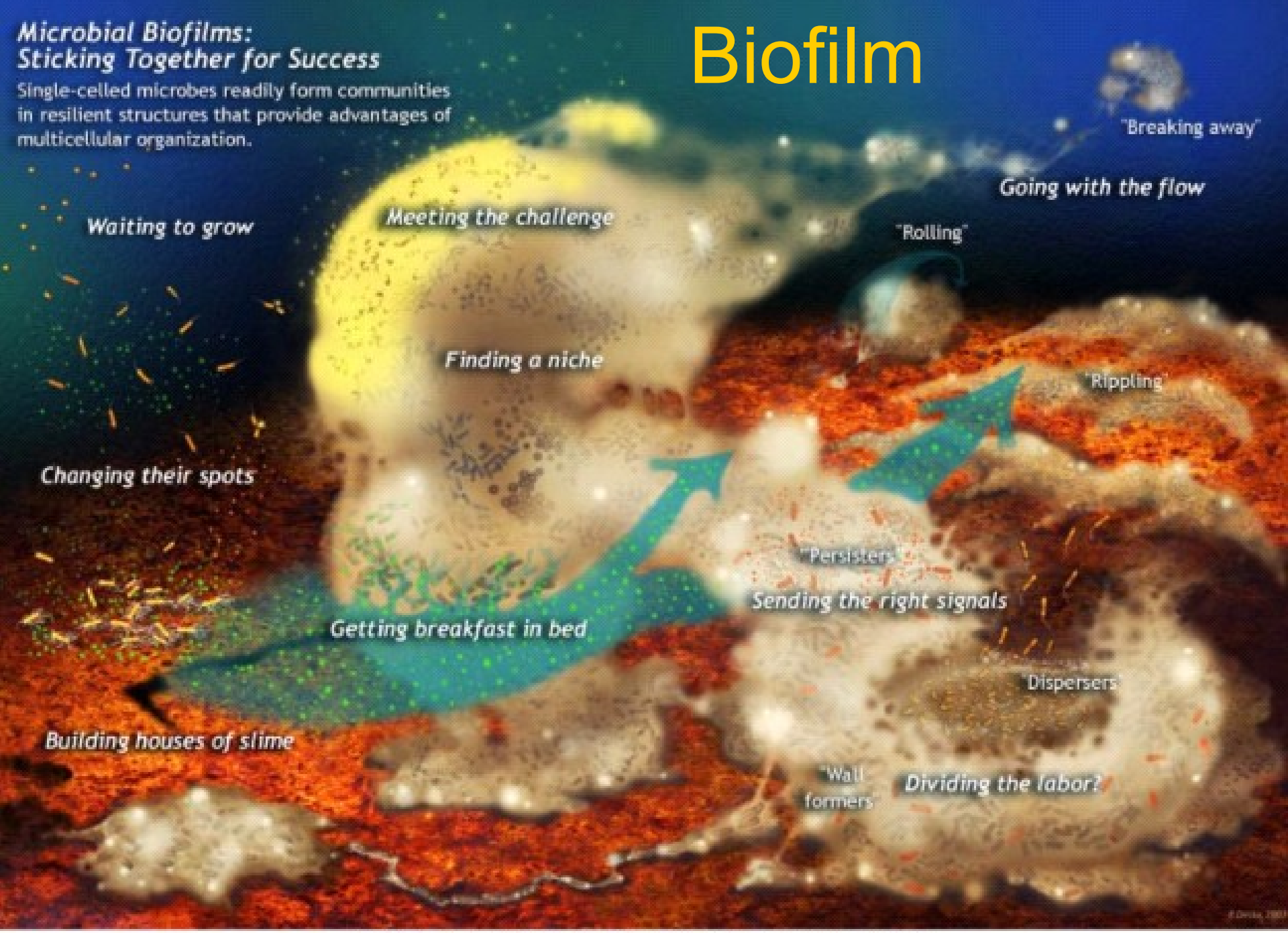
Bacteria may better regulate their quantity – in the biofilm they inform each other by production of various stuffs (quorum sensing)

Bacteria become more resistant to outer influences:

- desinfectants
- antibiotics
- host immunity response

Biofilm is formed both by common flora bacteria (rather positive for macrorganism and by pathogens

Biofilm



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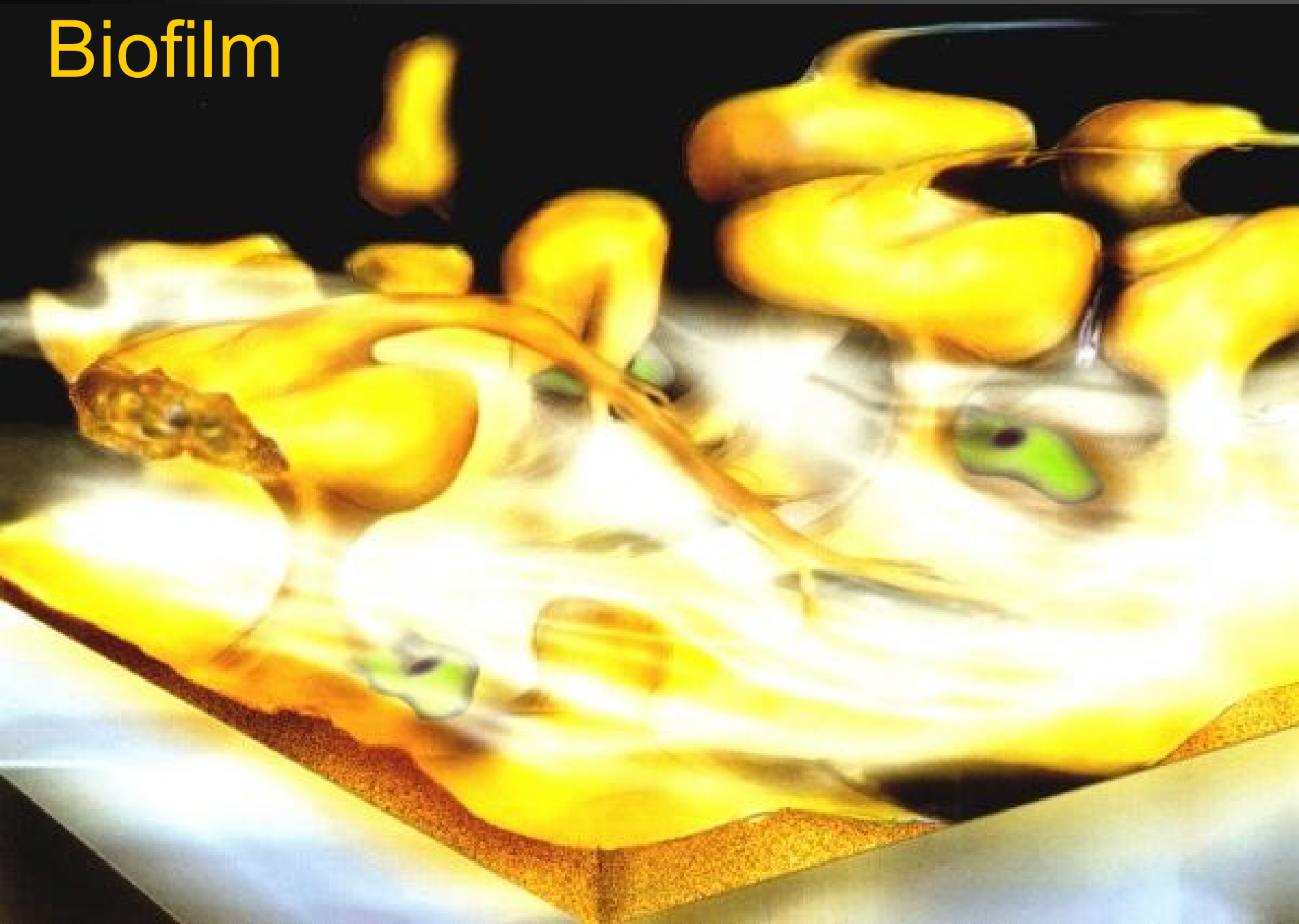
Mechanisms influencing bacterial resistance

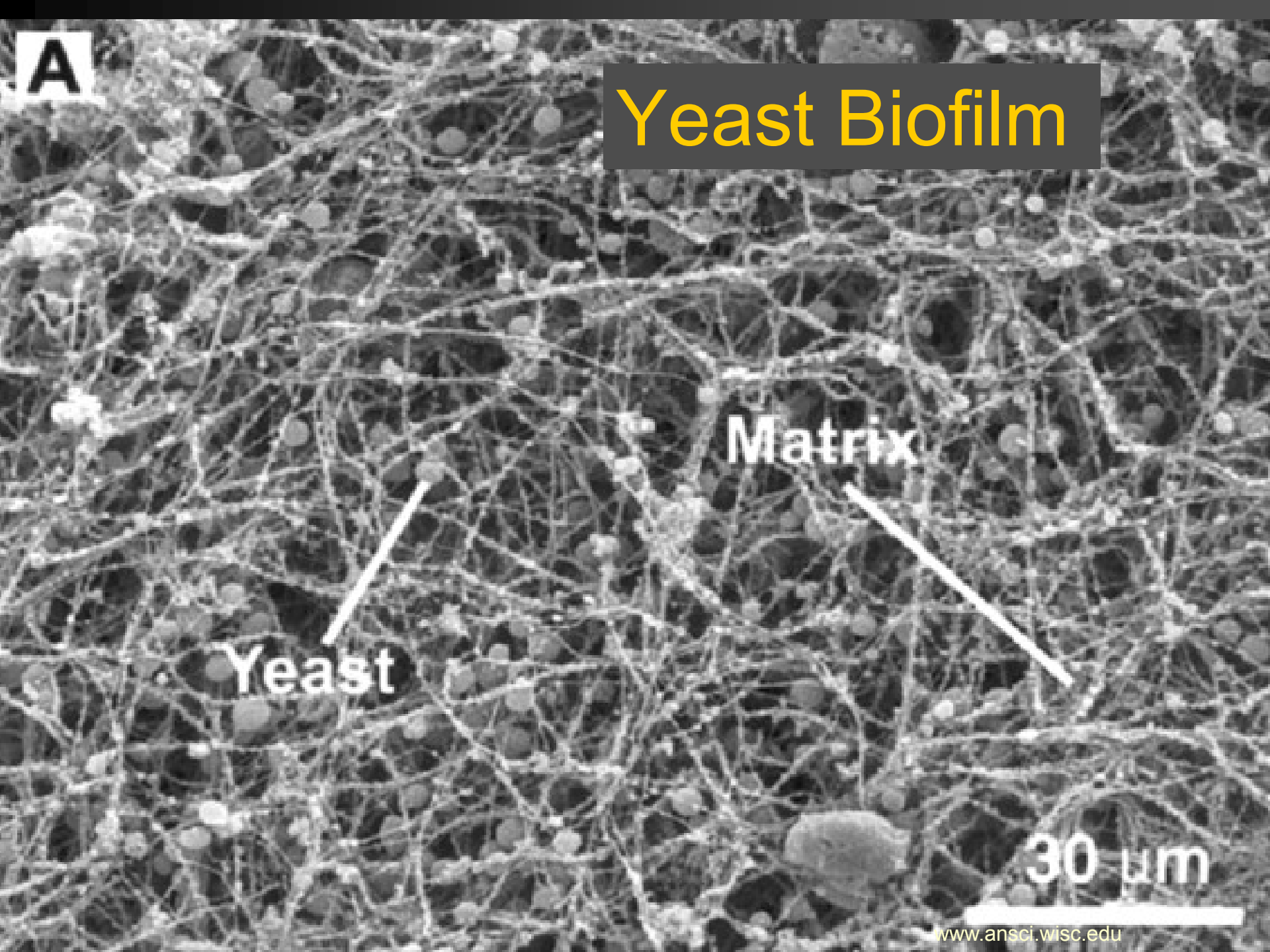
- Influence of **surface charge**
- Decrease of **growth rate**
- **Penetration bariere**
- Non-homogenous matrix
- **Fenotypic differences**
- **Intercelular signalisation**
- **Immunity mechanizms**…

Biofilm



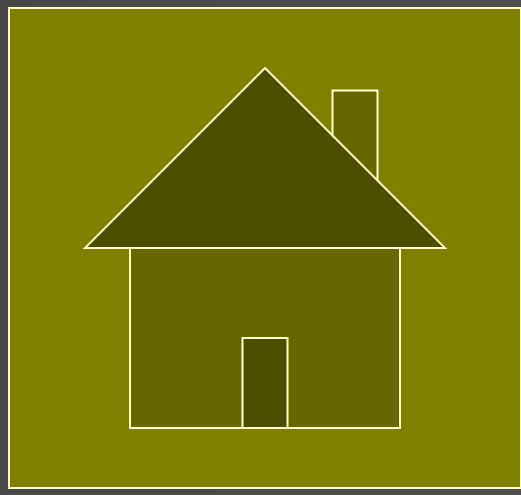
Biofilm eradication

- Antibiotic therapy often only supresses symptomas of infection caused by cells released from biofilm matrix and reacting with immunity system. Cells fixed in biofilm matrix cannot be destroyed by such therapy.
- To biofilm eradication we often to use high ATB concentrations (monotherapy or combinations), when treatment is not effective, the biofilm focus should be removed.
- In future we will possibly try to destroy the biofilm, e. g. by enzymotherapy


A
Yeast Biofilm
Matrix
Yeast
30 µm
www.ansci.wisc.edu

Prevention

- Catheters and bone cements
  - made of new generation plastic material (risk of adhesion and biofilm formation lower)
  - with coloid silver and similar surface-active compounds
  - with antimicrobial substances, e. g.
    - minocycline
    - rifampicine
- Catheter washing
- Correct asepsis, decontamination methods etc.



Diagnostic and experimental methods for biofilm

Biofilm and microbiologic diagnostics

a) Biofilm assessment

aa) by phenotypic methods (Christensen‘s method, Congo red agar cultivation)

Foto: Archiv Veroniky Holé

ab) by genotypic methods

b) Assesment of bacterial susceptibility in biofilm to individual antibiotics or combinations (mostly MBEC)

c) Regarding to biofilm formation at common bacteriological diagnostics, e. g. at venous catheter cultivation we choose specific methods (see later) instead of classic multiplication in broth

Microscopy of oral biofilm

Besides official methods for biofilm detection there are also other methods how to visualise biofilm.

For oral biofilm:

Gram stain may only visualise cell clusters (both G+ and G-) and eventually macrooranism cells (epitheliae etc.). Polysacharidic masses remain invisible.

Alciane blue stain enables visualisation of polysaccharicic material, i. e. the acellullar part of biofilm. Cells are visualized by negative staining.

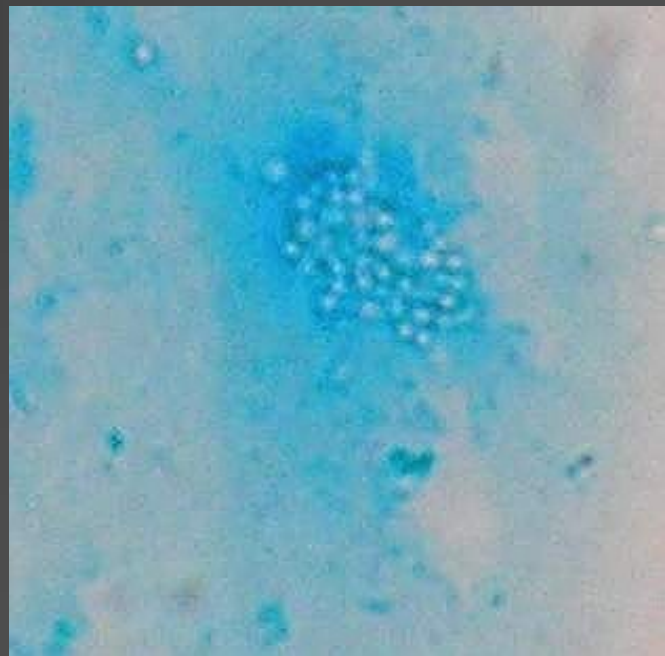


Photo: Archive of Veronika Holá

Proof of influence of tooth cleaning to oral biofilm

Photo: Archive of Veronika Holá

- A volunteer has a iodine solution or pills with a stain effecting to tooth plaque.

Photo: Archive of Veronika Holá

- The iodine is let to work in oral cavity during approx. 2 min.

Culture of biofilm producing bacteria

- In case of likelihood of biofilm formation, it is usually necessary to perform special methods for pre-processing the biological material, that precede the proper culture
- For central venous catheter culture, there exist two methods. Both of them are better than classical culture in broth without any pre-processing, sonification still remaining better than the Maki method

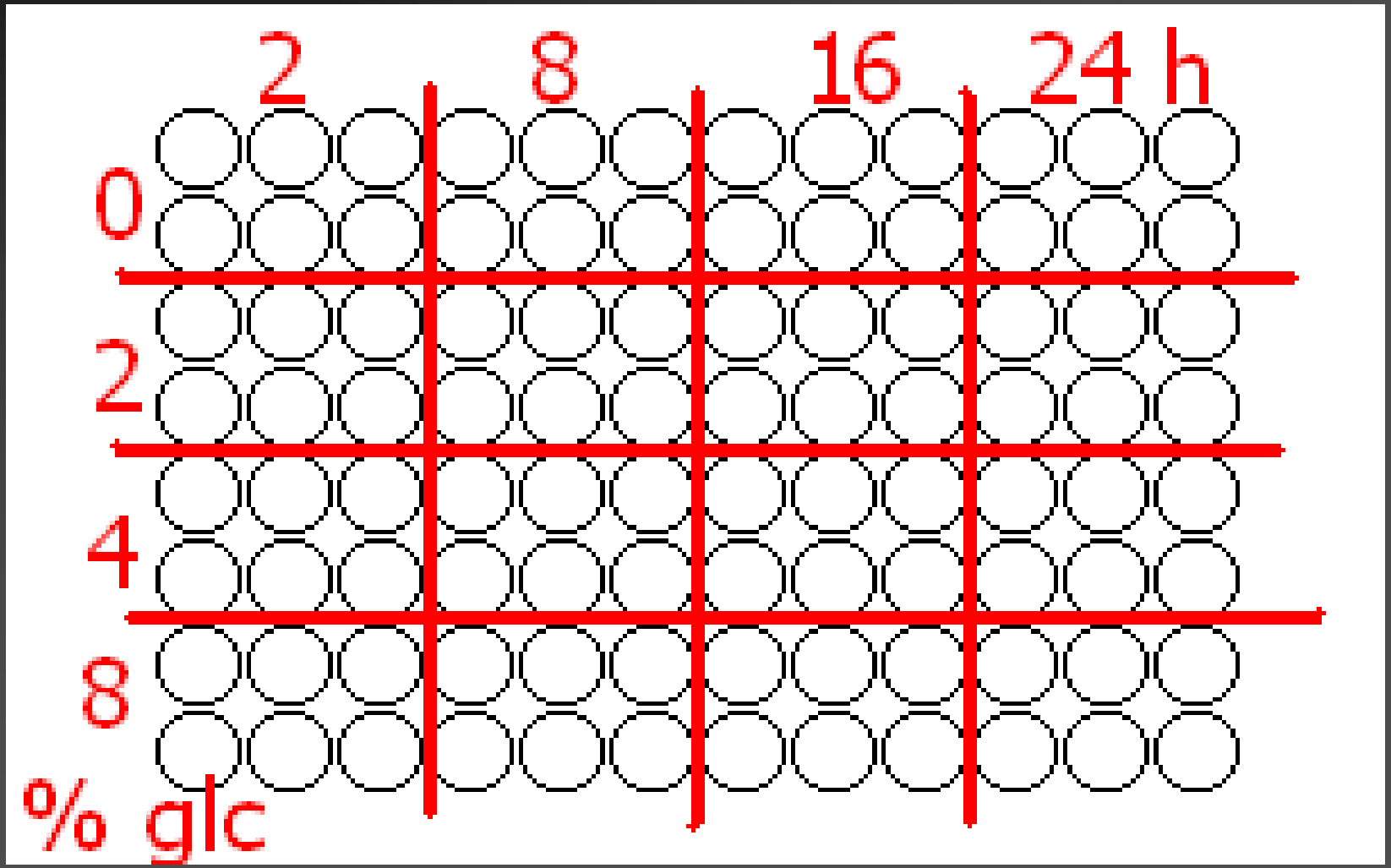
Methods

- *Classical broth culture: Bacteria in planctonic form are released. Bacteria in form of a biofilm are released. Bacteria in biofilm form are released less, or not at all. As broth is used as multiplying medium, we know nothing about its quantity (contamination × infection).*
- Semiquantitative (Maki) method: It enables us to assess catheter surface and semiquantitativelly assess the finding, but we have no information about intraluminal bacteria and bacteria are not necesarilly released from the biofilm.
- Sonification: destroys biofilm on the catheter surface and catheter lumen. Inoculation of a defined specimen volume is a quantitative method, that enables as to assess microbial amount.

Proof of influence of saccharides presence to dental plaque formation

- The experiment has a simple principle. One of oral bacteria is cultured on plastic surface (simulating tooth surface) with presence of various concetrations of glucose and for various time value
- After the incubation, biofilm is visualised using gentiane violet and its density quantified as absorbance using a spectrophotometre

To avoid accidental mistake, six adjacent wells have always the same values of both glc concentration and time



Old and new abbreviations in antibiotic effect measuring

MIC – minimal inhibition concentration is the growth limit of bacteria (the lowest concentration that disables bacterial growth)

MBC – minimal bactericidal concentration is the survival limit of bacteria (the lowest concentration that kills bacteria). In viruses, we would use „minimal virucidal“ etc.

MBIC – minimal biofilm inhibiting concentration

MBEC – minimal biofilm eradication concentration

Diagnostic methods MBEC assessment

MBEC … minimal biofilm eradicating concentration

(Another value exists: ***MBIC*** *… minimal biofilm inhibitory concentration – a value not approved by all scientists)*

Photo: Archive of Veronika Holá

Photo: Archive of Veronika Holá

MIC versus MBEC

- While MIC determinates minimal

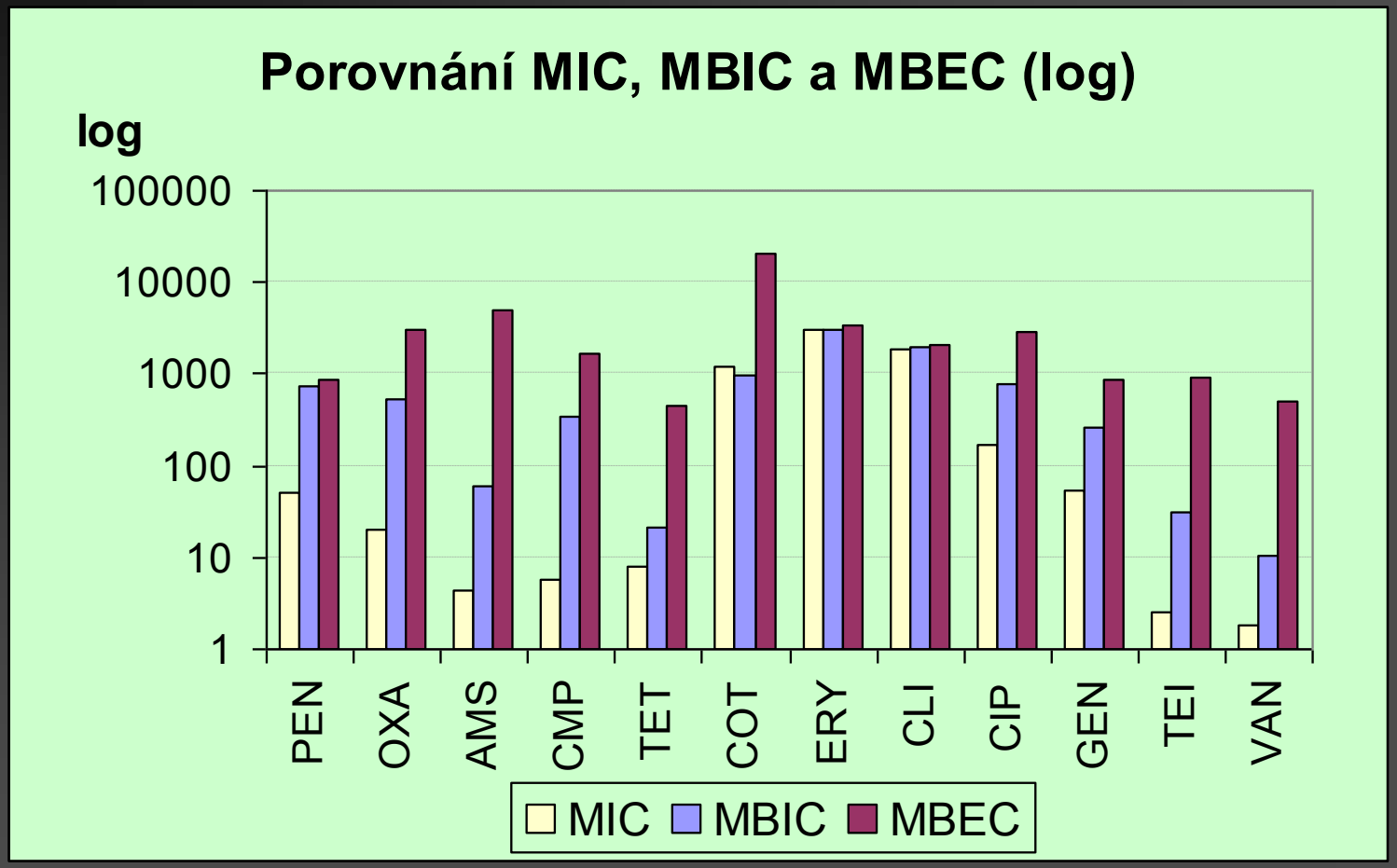
inhibitory concentration of atb in planctonic form,

MBEC shows us if eradication of bacterial biofilm is present.

So it tells us more about effect of antibiotic on normally living bacteria

- MBEC corresponts the lowest concentration of antibiotic, where biofilm eradication is proven (absence of living cell, no pH medium change, the well remains red)

Differences in MIC, MBIC, MBEC



Abbreviations: pen – penicilin, oxa – oxacilin, ams – ampicilin/sulbactam, cmp - chloramphenicol, tet – tetracycline, cot – co-trimoxazole, ery – erythromycine, cli – clindamycine, cip – ciprofloxacine, gen – gentamicine, tei – teicoplanine, van – vankomycine

Diagnostic methods II.

- Values of **MBEC** are often over break point for given antibiotics (bacterie are resistant to them)
- Values of **MBEC** use to be several times higher than MIC
- Microbes in biofilm are usually resistant even to antibiotic combinations, the only possibility is then biofilm focus removal (a catheter, joint implants, tooth implants etc.)

The End

Photo: Archive of O. Z.

This slideshow was prepared in cooperation of ing. Veronika Holá, MUDr. Lenka Černohorská, PhD., and MUDr. Ondřej Zahradníček

(Student K. C. four years ago forgot to bring her index, so she got the credit in the evening in a pub ☺)