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Biofilms in Medicine

The 5th lecture for the students of General Medicine

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Water shortage – revision

Water = 80 % live weight of the bacterial cell

(only 15 % live weight of the bacterial spore)

Hygrophile organisms (most of bacteria) need freely accessible water

For xerophiles (actinomycetes, nocardiae, moulds) water bound to the surface of environmental particles (e.g. in soil) suffices

Resistance to drying up – revision

Very sensitive: agents of STD – gonococci,
treponemes

Less sensitive: all Gram-negative bacteria

A bit more resistant: skin flora – staphylococci,
corynebacteria
acidoresistant rods –
mycobacteria

Rather resistant: xerophiles – actinomycetes,
nocardiae, moulds
parasite cysts, helminth eggs

Highly resistant: bacterial spores

Practical application of water shortage – revision

Lowering water activity stops action of most microbes → we use it for food preservation

- drying – meat, mushroom, fruit (prunes)
- concentration – plum jam
- salting – meat, fish, butter
- sugaring – sirups, jams, candied fruit

Nutrient deficiency – revision

Microorganisms do not multiply in clean water

The problem lies in keeping water pure

After some time, even in distilled water e.g. *Pseudomonas aeruginosa* or *Pseudomonas fluorescens* start to multiply

In shower sprinklers: *Legionella pneumophila* grows (and can cause pneumonia)

However, *Salmonella* Typhi lives longer in well water than in waste water – why?

Temperature – revision

Cardinal growth temperatures:

Optimum – psychrophiles: 0 – 20 °C

mesophiles: 20 – 45 °C (medically important microbes)

thermophiles: 45 – 80 C

hyperthermophiles: >80 C

Growth temperature range:

narrow (gonococci 30 – 38.5 °C)

wide (salmonellae 8 – 42 °C)

The influence of cold – revision

Cold shock: gonococci will die if inoculated at cold agar media freshly taken out of the fridge

Growth temperature minimum:

at 5 °C: salmonellae & campylobacters survive, yersiniae & listeriae even multiply!

Tissue cysts of *Toxoplasma gondii* in meat do not survive common freezing

The influence of heat – revision

The temperature higher than optimum → heat shock and gradual dying of cells

The number of killed cells depends on the duration of the exposure to higher temperature

The relation between the number of surviving cells and the duration of heating is logarithmic one

The time needed for killing the whole population depends on the initial number of microbes

Thermal death time – revision

Thermal death time (TDT) = the shortest time needed to kill all microbes in a suspension. For most bacteria it averages 10-15 minutes at 60-65 °C (important exception: spores)

Osmotic pressure – revision

Hypotony – the damage is prevented by the cell wall

Hypertony mostly hinders microbes in multiplying (therefore fruit is candied, meat salted)

Higher osmotic pressure is endured by:

halophiles – halotolerant: enterococci (6.5% NaCl)

staphyloccoci (10% NaCl)

– obligate: halophilic vibria (in sea water)

moulds – tolerate higher content of saccharose (in jams)

pH – revision

Neutrophiles: growth optimum at pH 6 až 8 – most microbes

Alkalophiles: e.g. *Vibrio cholerae* (pH 7.4-9.6)
alkalotolerant: *Proteus* (it splits urea), *Enterococcus* (broad range of pH 4.8-11)

Microbes sensitive to extremes of pH: e.g. gonococci

Acidophiles: facultative: yeasts, moulds, lactobacilli (>3), coxiellae (tolerate low pH of phagosome)

Microbes sensitive to low pH: mainly vibrios, streptococci, putrefactive bacteria; low pH hinders most bacteria

Low pH keeps spores from germinating → botulism can be obtained from oil-preserved mushrooms or preserved strawberries, not from pickled gherkins or mixed pickles

Redox potential (rH) – revision

Level of rH depends both on the composition of the environment and of the atmosphere

Aerobes – need high rH levels (>200 mV)

Anaerobes – need low rH levels (≤0 mV)

Anaerobes are killed by O_2, aerobes without O_2 will live

Even so, anaerobes prosper both in nature and in our bodies – thanks to the cooperation with aerobes and facultative anaerobes (e.g. in biofilms)

Anaerobes in the body:

- large intestine (99 % of bowel microorganisms)
- vagina
- oral cavity (sulci gingivales)

Radiation – revision

UV radiation (maximum effect around 260 nm)

In nature airborne bacteria protect themselves by pigments → they have coloured colonies

Artificially: UV radiation is used for disinfection of surfaces, water, air

Ionizing radiation (X and gamma radiation)

For sterilizing disposable syringes, infusion sets, materials for dressing and sewing, tissue grafts, some drugs, waste and food (this not in EU)

Record holders for radiation resistance: bacterial spores

Toxic substances – revision

In general (and contrary to drying): G– bacteria are more resistant to toxic substances than G+ bacteria (because of different structure of bacterial cell wall → presence of enzymes in periplasmatic space of G– bacteria)

For application it is vital to know the effects of the particular substances used for disinfection

Sterilization versus disinfection – revision

Sterilization = removal of all microorganisms from objects or environment

Disinfection = removal of infectious agents from objects and environment or from the body surface

Biocides = a new general term including also disinfectants

Types of disinfectants – revision

1. Oxidizing agents (peracetic acid, H_2O_2, O_3)
2. Halogens (hypochlorite, sol. iodi)
3. Alkylating agents (aldehydes)
4. Cyclic compounds (cresol, chlorophenols)
5. Biguanides (chlorhexidine)
6. Strong acids and alkali (e.g. slaked lime)
7. Heavy metal compounds (Hg, Ag, Cu, Sn)
8. Alcohols (ethanol, propanols)
9. Surface active agents (QAS; e.g. cetrimid)
10. Others (e.g. crystal violet & other dyes)

Relative resistance of different agents to biocides – revision

Enveloped viruses	very susceptible	herpesviruses
Some protozoa		*Trichomonas*
Gram-positive bacteria		*Streptococcus*
Gram-negative bacteria	**susceptible**	*Salmonella*
Yeasts		*Candida*
Moulds		*Trichophyton*
Naked viruses	**relatively resistant**	enteroviruses
Protozoal cysts		*Giardia*
Acidoresistant rods		*Mycobacterium*
Helminth eggs	very resistant	*Ascaris*
Bacterial spores		*Clostridium*
Coccidia		*Cryptosporidium*
Prions	**extremely resistant**	agent of CJD

Universally effective biocides

On small, naked viruses: oxidizing agents
halogens
aldehydes
strong acids and alkali

On mycobacteria: oxidizing agents
aldehydes
lysol
strong acids and alkali

On bacterial spores: (oxidizing agents)
aldehydes
strong acids and alkali
(not alcohols!)

Two forms of microbial growth

Growth in the planctonic form:

Isolated microbial cells freely float in the fluid environment

Growth in the biofilm form:

Microbial cells have a natural tendency to stick to each other and to solid surfaces and to form a community interconnected with an extracellular mass

Which form is more frequent?

Planctonic form

Common in the laboratory (e.g. in broth)

Biofilm form

Primary in the natural environment because it is more advantageous for the microbes

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
- They exhibit an altered phenotype with respect to the growth rate and gene transcription
- They are highly resistant to outer influences

Dental plaque as a biofilm

Nowadays, dental plaque is viewed as a microbial biofilm

It is composed of numerous bacteria that

– live in close juxtaposition
– communicate mutually
– influence each other

In disease, the balance of predominant bacterial populations typically shifts

Architecture of dental biofilm

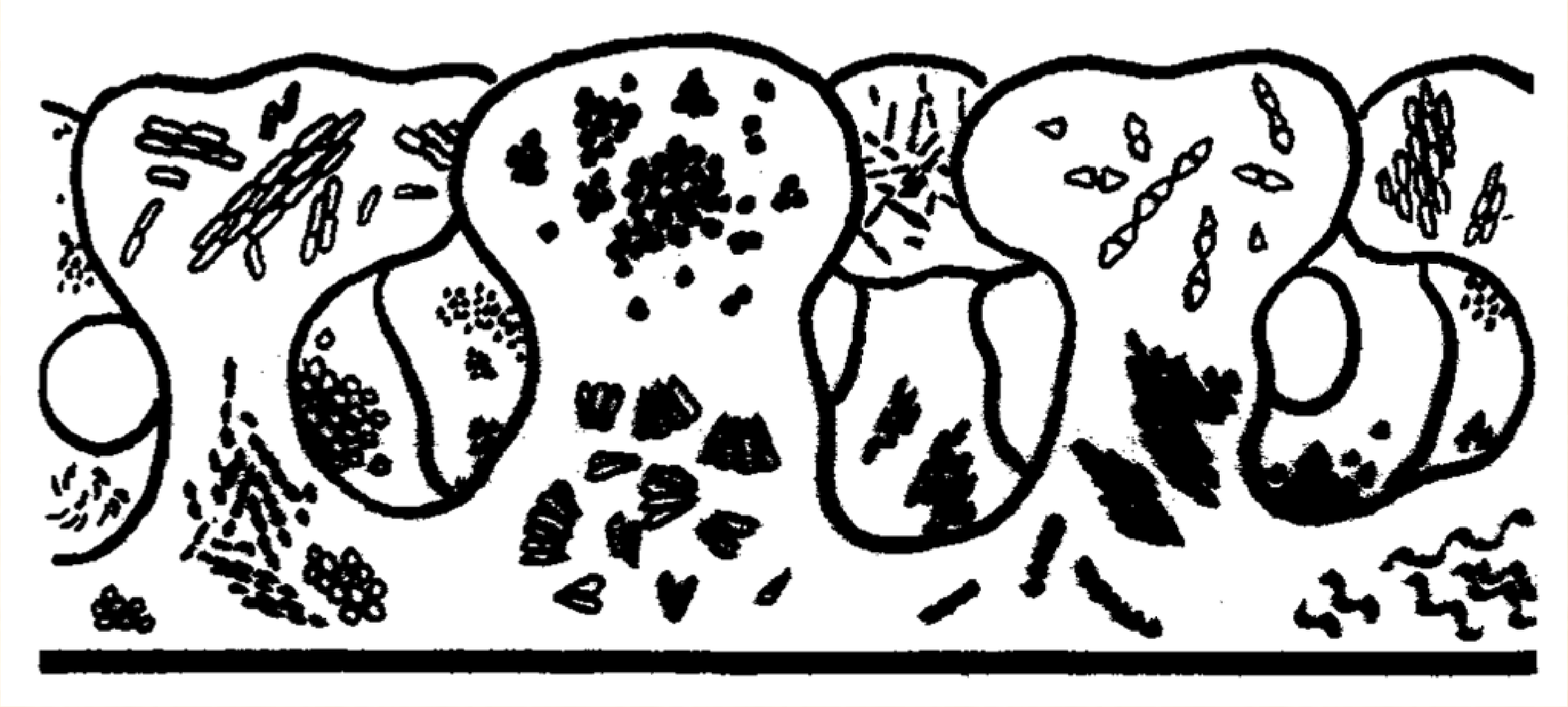
Original assumption:

Dental plaque has a compact structure

New technologies (confocal microscopy) say otherwise:

Dental plaque has an 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

Biofilm architecture

Mutual relations between biofilm bacteria

Bacteria in plaque communicate mutually

- **through coaggregation and coadhesion (physically)**
- **through conventional metabolic interactions (biochemically)**
- **via small diffusible signalling molecules (quorum sensing)**

Coaggregation in plaque

E.g. anaerobic *Fusobacterium nucleatum* rod literally forms bridges between

- **early colonizers of the tooth surface – streptococci, actinomycetes – and**
- **anaerobic late colonizers – *Treponema denticola, Porphyromonas gingivalis, Tannerella forsythia* (former *Bacteroides forsythus)* et al.**

Quorum sensing in plaque

Quorum sensing:

During multiplication the individual cells emit chemical signals (e.g. competence-stimulating peptide CSP in *Streptococcus mutans*)

After the cells reach certain number (quorum) the higher concentration of signals triggers the following change in cell properties:

- shutting off some so far operating genes
- switching on other genes, resulting in
- production of new products (chiefly exopolysacharides; in *S. mutans* it means – formation of biofilm – induction of acid tolerance and genetic competence)

Dental plaque – conclusion

Oral bacteria in plaque:

- **not independent entities**
- **function as a microbial community**
  - **coordinated**
  - **spatially organized**
  - **fully metabolically integrated**

The properties of the community:

greater than the sum of the component species

Biofilm in dental office – I

Dental unit waterlines = ideal environment for microbial colonization → biofilm

Source of microbes:

- public water supply
- occasionally aspirated patient saliva

Consequence of biofilm in dental waterlines: elevated concentrations of microorganisms in water emitting e.g. from high-speed handpiece

Biofilm in dental office – II

Microbes found in dental unit water in significant concentrations:

pseudomonads (chiefly *P. aeruginosa* potentially pathogenic for susceptible host)

legionellae (some species are pathogenic, dental personnel has higher rates of *Legionella* antibodies)

Evidence suggests exposure of patients and dental staff to potential bacterial pathogens via dental water

Biofilm in dental office – III

As yet, no efficient way of controlling dental water unit biofilm is known

Main interim recommendation:

Run water for several minutes at the beginning of each clinic day

Run high-speed handpieces for 30 seconds after use on each patient

Follow the instruction of the dental unit´s manufacturer

Chronic infections of natural bodily surfaces

dental caries (alpha streptococci)

periodontitis (gramnegative oral anaerobes)

otitis media (*Haemophilus influenzae*)

osteomyelitis (*Staphylococcus aureus*)

cholecystitis (enterobacteriae)

prostatitis (*Escherichia coli*)

subacute bacterial endocarditis (alpha streptococci)

pneumonia during cystic fibrosis (*Pseudom. aeruginosa*)

Chronic infections of artificial devices

central venous catheters (coag. neg. staphylococci, candidae)

artificial cardiac valves (*Staph*. *aureus*, *Staph*. *epidermidis*)

artificial joints (*Staphylococcus aureus*, *Staph*. *epidermidis*)

surgical sutures (*Staph*. *aureus*, *Staph*. *epidermidis*)

vascular grafts (Gram-positive cocci)

endotracheal cannulae (various bacteria and yeasts)

intrauterine devices (*Actinomyces israelii*)

urinary catheters (*E. coli* and others, mostly G-negative rods)

contact lenses (*Pseudomonas aeruginosa,* G-positive cocci)

Recommended reading material

Paul de Kruif: Microbe Hunters

Paul de Kruif: Men against Death

Axel Munthe: The Story of San Michele

Sinclair Lewis: Arrowsmith

André Maurois: La vie de Sir Alexander Fleming

Could you kindly supply me with another work in connection with microbes or at least medicine?

Please mail me your suggestions at:

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Thank you for your attention