ETH zürich

Biomaterials II

Materials and Mechanics in Medicine HS 2019

Exercise 2 – 01.10.2019

Jack Kendall

Today

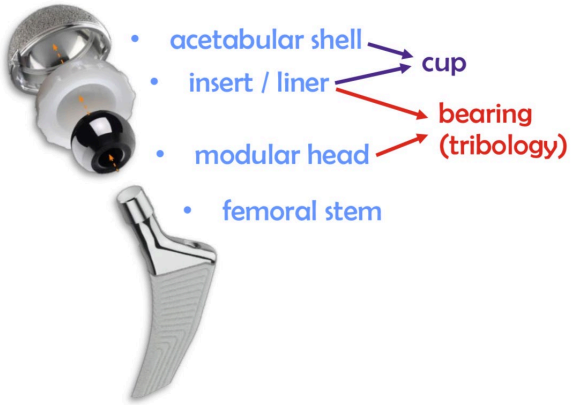
- Short run-through of last Exercise (5’)
- Biomaterials II Lecture Recap (20’)
- Exercise 2 (20’)

Today

- Short run-through of Exercise 1 (5’)
- Biomaterials II Lecture Recap (20’)
- Exercise 2 (20’)

Considerations for Biomaterial-Bone Interface

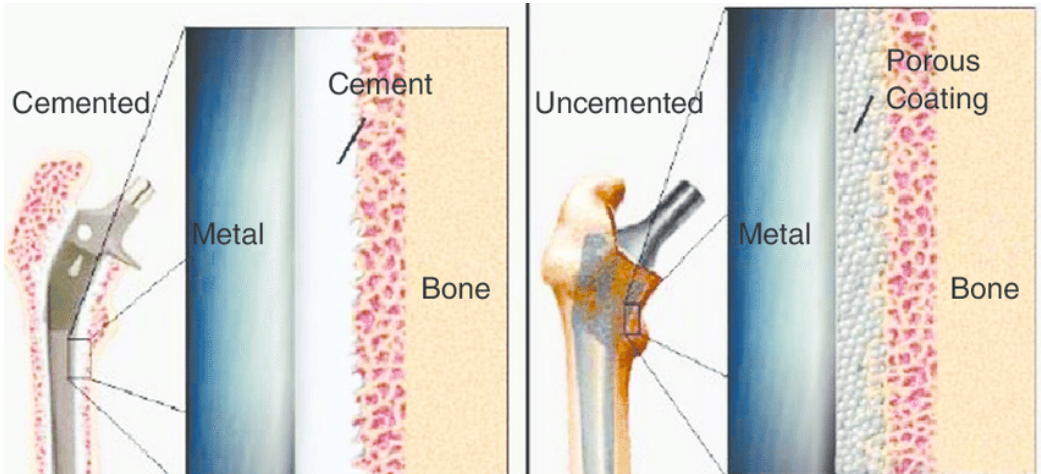
- Bone is living material
- “Bone adapts to the leads under which it is placed”
- Revisited: Stress Shielding!
  - Removal of typical stress from the bone by an implant
  - Leads to a reduction of bone density
  - **Wolff’s Law** → *use it or lose it*



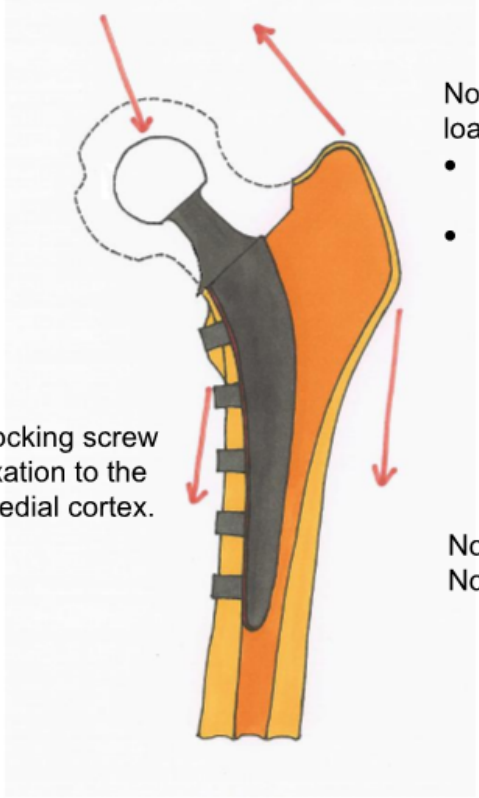
- Total Hip Arthroplasties (THA)
  - Majority of joint replacements
  - Acetabulum and femoral head
  - Hard metal or ceramic femoral head articulating against a (vitamin-E crosslinked) **UHMWPE** acetabular cup
    - Can be fixed *with* or *without* **bone cement** (PMMA)

Cemented vs. Cement-less Implants

- **Cemented (A)**
  - Use bone cement to fill gaps between implant and bone
  - Therefore not in direct contact with bone
  - Must provide inistial axial and rotational stability, sharp corners and non-cylindrical cross section Smooth surfaces to prevent cement abrasion
- **Cement-less (B)**
  - In direct contact with bone, provides primary stability by mechanical locking (press fit!), requires sufficient osseointegration
  - Rough surfaces → more contact area, friction and scaffold
  - Hydroxyapatite coating



Cemented vs. Cement-less vs. Screwed Implants



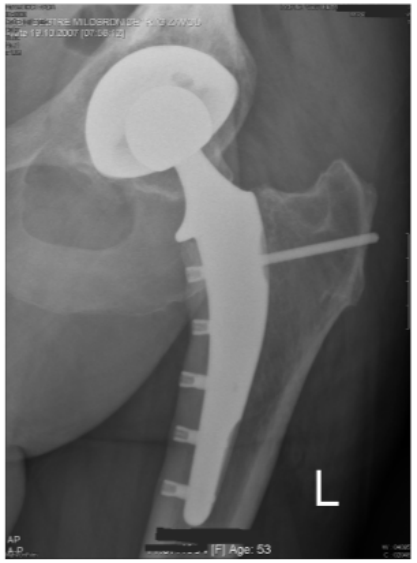
Locking screw fixation to the medial cortex.

Normal biomechanical loading preserved:

- Joint forces transferred to medial cortex
- no hoop stress generated as with press-fit stems.

No press fit to lateral cortex
No stress shielding

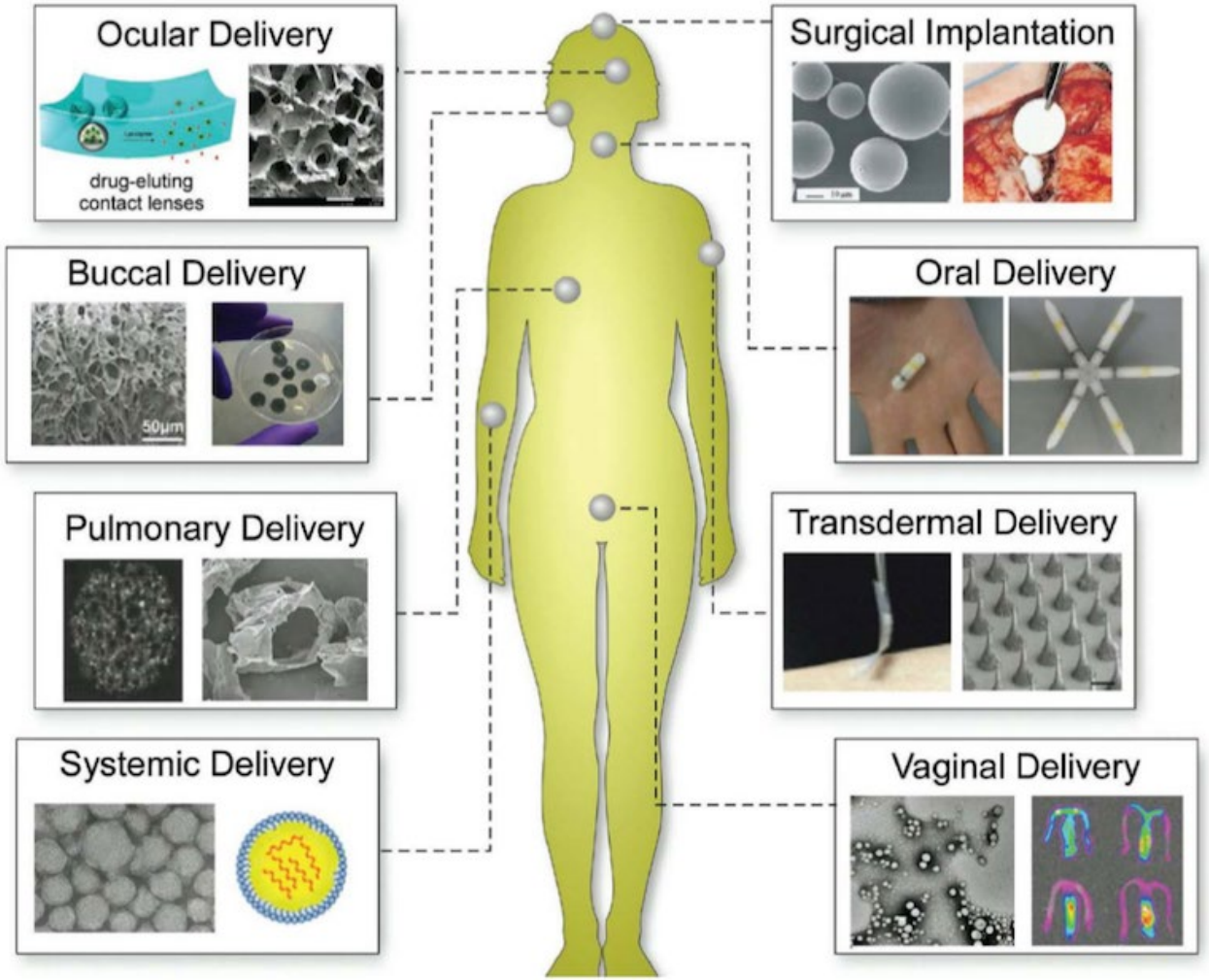
10 month follow-up x-ray with a SCYON THR stem.



Advantages of Drug Delivery

- Maintain therapeutic level of drug at the site
- Protection of the drug
- Protection of the person
  - (preferably local and not systemic application → less side effects)
- Ease of administration
- Calibrate the drug release profile to the patients needs
- Reach otherwise difficult to reach areas (restricted, such as brain)
- If implant is biodegradable, it does not need to be removed

Routes of Drug Administration



Hydrophobia, Hydrophilia and Polarity

- *Non-polar* (non-ionized) drugs will cross cell membranes easily
- Non-polar drugs are lipid soluble
- In turn however, only polar drugs are soluble in water
- Many drugs are non-polar or hydrophobic and have **poor bioavailability** without drug delivery systems!

Requirements for Drug Delivery System

- Must be safe for clinical use
- Degrade into non-toxic products
- Tunable degradation rate (from days to months)
- Biocompatible

Partition Coefficient

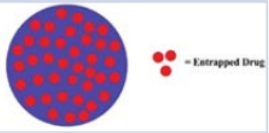
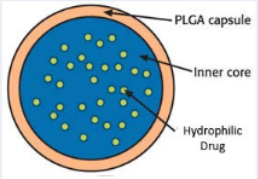
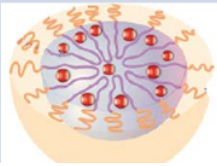
- We define the partition coefficient as

$$\log(P) = \log\left(\frac{\text{amount of drug dissolved in octanol}}{\text{amount of drug dissolved in water}}\right)$$

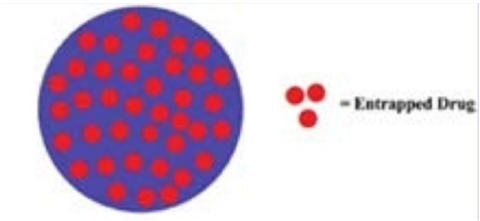
  - log(P) = 1 means 10:1 ratio of organic to aqueous compounds
  - log(P) = 0 means 1:1 ratio
  - log(P) = –1 means 1:10 ratio
- Interpret log(P) *as a measure of hydrophobicity* of a certain drug
- Keep log(P) in mind deciding on strategy for drug encapsulation and subsequent sustained release
- TAMOXIFEN (log *P* = 5.93) is therefore *hydrophobic*!

Drug Encapsulation Methods

- Methods for fabrication of **drug-releasing microspheres** from polymers
- Encapsulation methods depend on many factors, such as
  - Polymer, solubility and stability of molecule to be incorporated
- Organic solvents used to dissolve polymer → affects activity of molecule

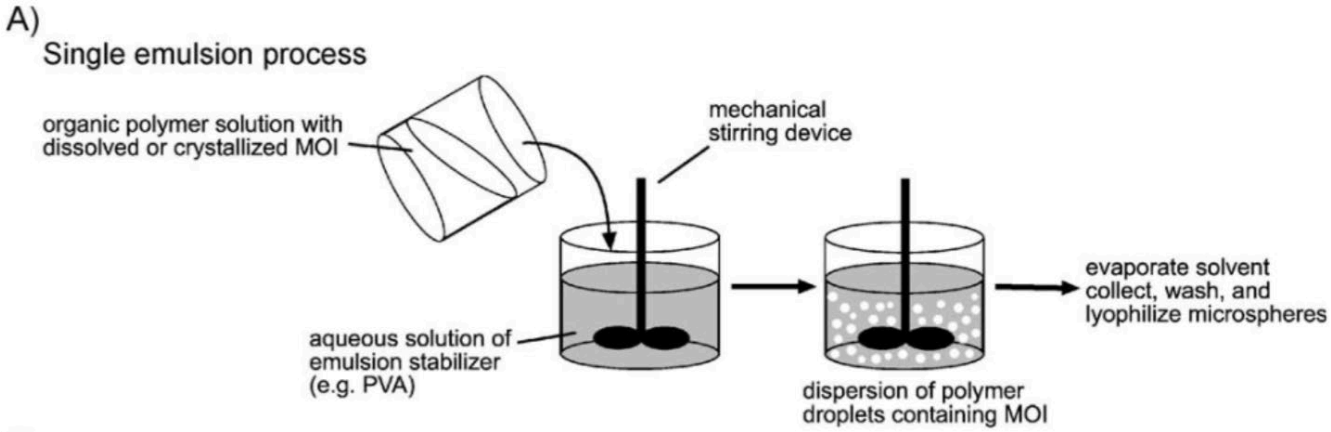
Drug	Polymer	Method
Hydrophobic	Hydrophobic	Single Emulsion 
Hydrophilic	Hydrophobic	Double Emulsion 
Hydrophobic	Hydrophilic/ Hydrophobic Block Co-Polymer	Self Asssembly 

Drug Encapsulation Methods

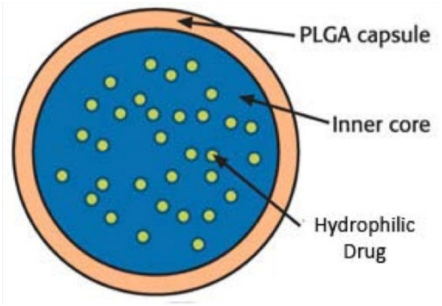


1. Single Emulsion

- drug and polymer are hydrophobic
- Use when molecule of interest can be dissolved in organic solvent or is stabile in crystalline form when dispersed in organic solvent
- **Water-Oil** single emulsion method
- The organic solution is emulsified with a stabilizer, such as polyvinyl alcohol (PVA), which prevents the organic droplets from coalescing

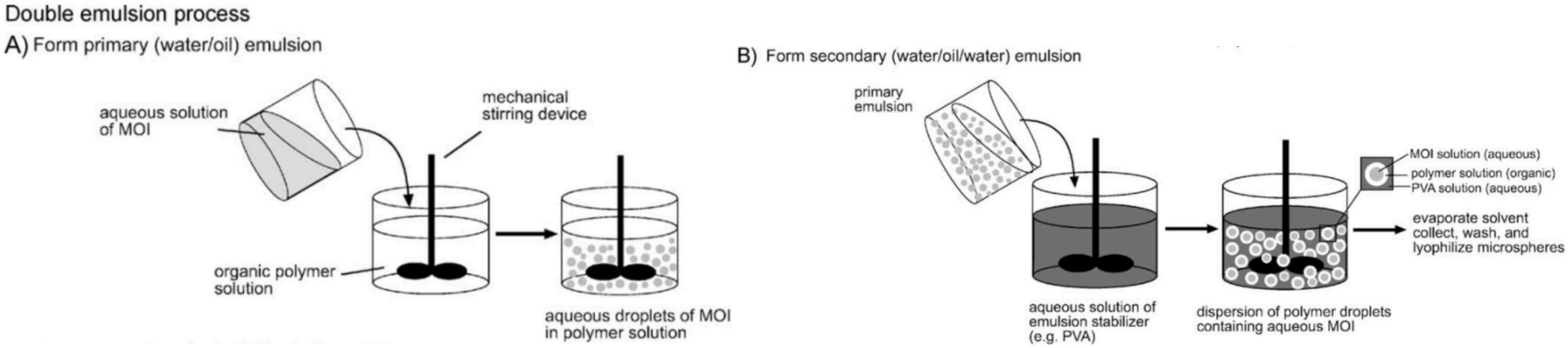


Drug Encapsulation Methods

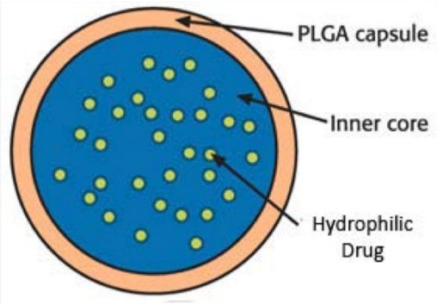


2. Double Emulsion

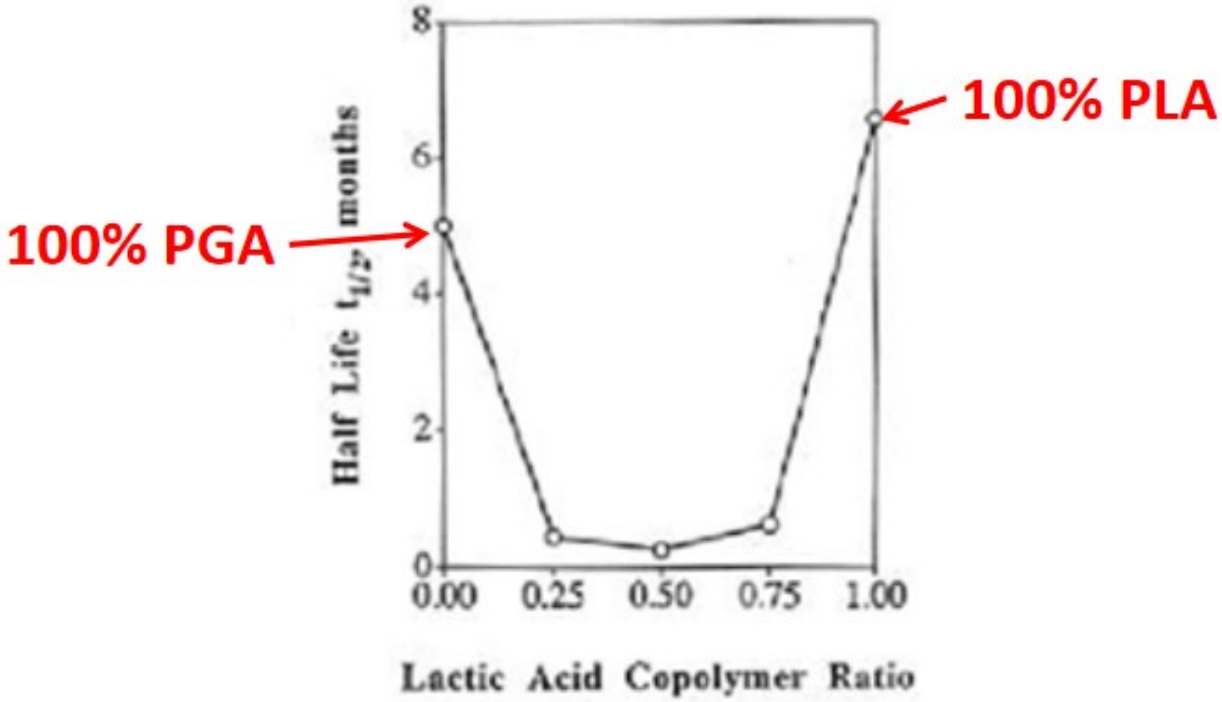
- drug is hydrophilic and polymer is hydrophobic
- Use when dealing with water-soluble molecules: may become inactivated by direct exposure to solvent → Water-Oil-Water double emulsion
- More technically challenging, but diversifies the range of bioactive molecules in drug delivery system



Drug Encapsulation Methods



Degradation of PLGA

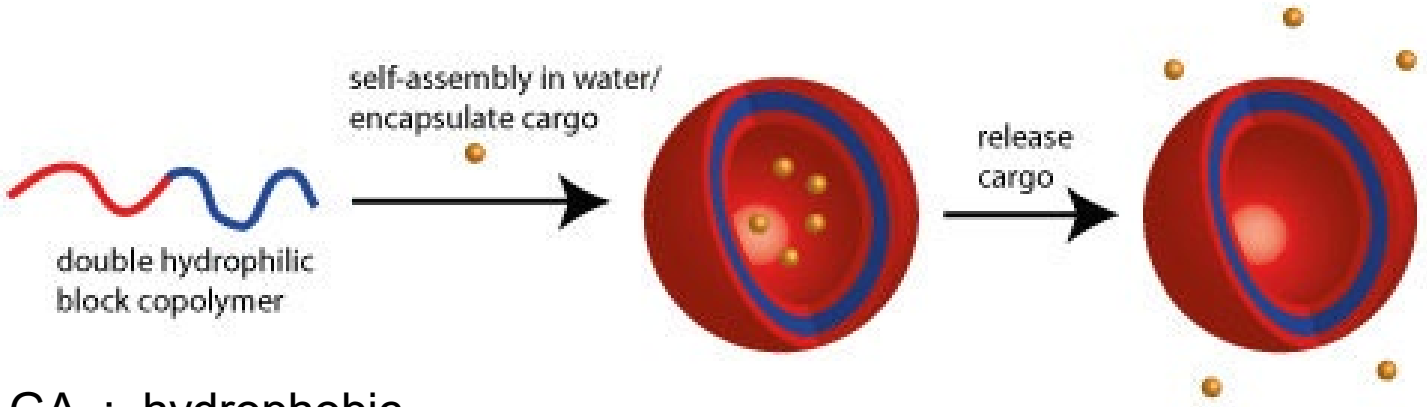


Hydrolysis!

Drug Encapsulation Methods

3. Self-Assembly

- drug is hydrophobic and polymer is hydrophilic/hydrophobic block co-polymer
- Spontaneous organization of molecular units into well defined, dynamic structures → most often driven by non-covalent interactions



PLGA : hydrophobic
PEG : hydrophilic

Biocompatibility Testing Protocols

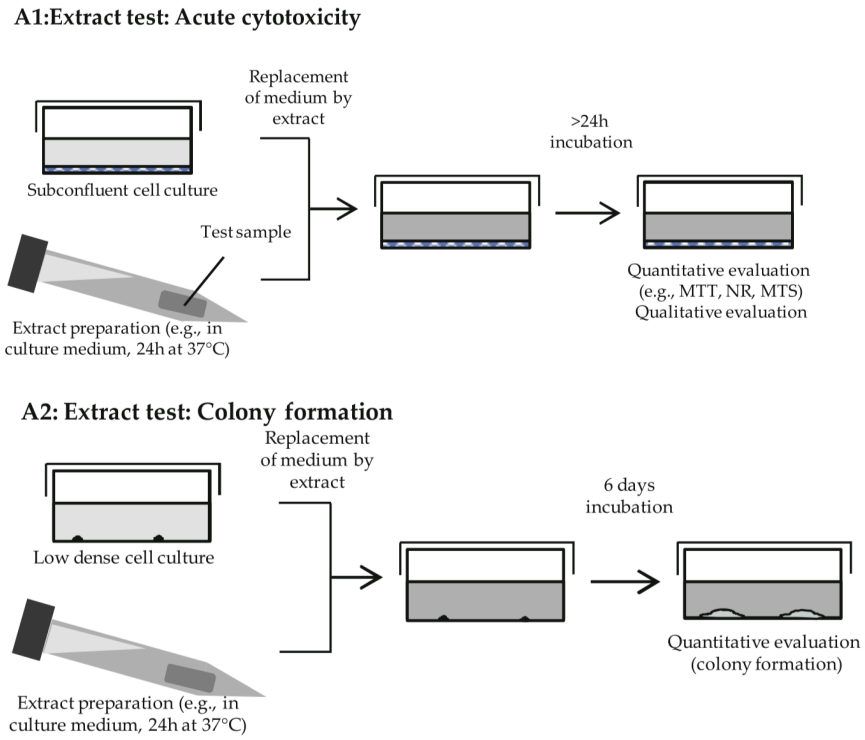
- Three categories of tests are proposed for assessing the cytotoxicity of potentially released materials in *ISO 10993-5*
  1. Extract Tests
     - Normally based on a so-called extract obtained by exposing cell culture medium to the test material or compound of interest for 24 h at 37 °C. Subconfluent cell cultures are treated by measuring effects on cell functionality typically after 24 h, or low-density cultures are revealed by measuring effects after a prolonged time period of six days.
  2. Direct Contact Test
     - A test sample covering about 10% of the subconfluent cell layer is placed on top of that layer, while in the agar diffusion test an agar layer covers the cells instead of cell culture medium and the test samples are placed on top of the agar layer. In both tests, the sample is removed after 24–72 h exposure time and the cells are qualitatively and quantitatively assessed below and adjacent to the test samples.
  3. Indirect Contact Test
     - For this cells are cultured until confluency on one side of the filter, which is then placed with the cell side on top of an agar layer. Subsequently, the test material is placed on the other side of the filter. Effects on cells are qualitatively assessed after 2 h exposure time.

Biocompatibility Testing Protocols

- **Extract Test : Colony Forming Assay**
  - Cytotoxicity of leachable substances released from test item is assessed
  - Measure ability of colony formation after treatment (plating efficiency)
  - If cell activity is reduced by $\geq 30\% \rightarrow$ **toxic**!
  - Standard Conditions
    - Extraction Vehicle (6 cm^2/ml)

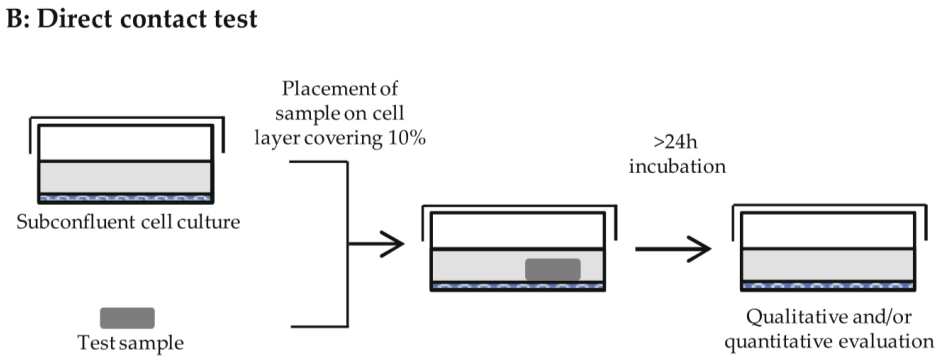
    a) Culture medium with serum
    b) Physiological saline buffer
    c) Pure water or dimethyl sulfoxide
  - Possible Extraction conditions

    i. 24 ± 2 hours at 37±1 °C
    ii. 72 ± 2 hours at 50±2 °C
    iii. 24 ± 2 hours at 70±1 °C
    iv. 1 ± 0.2 hours at 121±2 °C



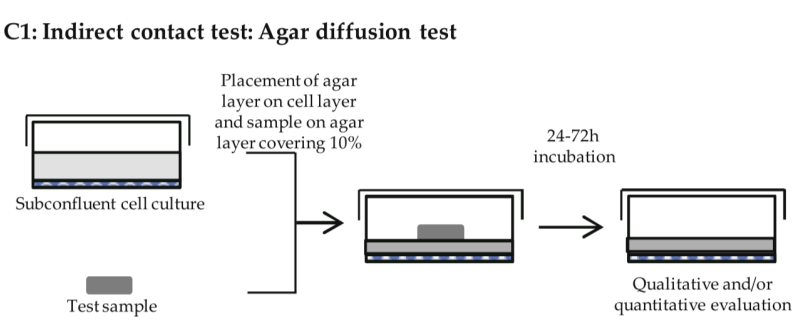
Biocompatibility Testing Protocols

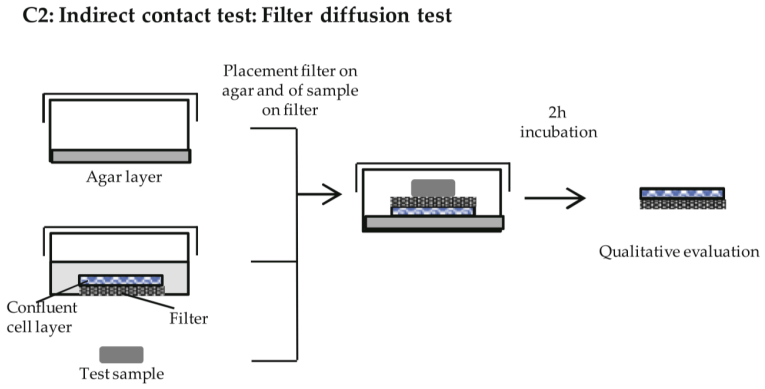
- **Direct Contact Test**
- Material must cover 10% of area
  - Piece of test material is placed directly onto cells growing on culture medium → the cells are then incubated.
  - During incubation, leachable chemicals in the test material can diffuse into the culture medium and contact the cell layer.
  - Malformation, degeneration and lysis of cells around test material indicate reactivity of test sample



Biocompatibility Testing Protocols

- **Indirect Contact Test:** *Agar and Filter Diffusion Test*
  - Often used for high density materials. Thin layer of nutrient-supplemented agar is placed over the cultured cells.
  - Test material or an *extract dried on filter paper* is then placed on top of the agar layer, and the cells are incubated.
  - Zone of malformed, degenerative or lysed cells under and around the test material indicates cytotoxicity.





Limitations of Discussed Protocols

- **Most striking limitation of all tests is the short test period**
  - 2 hours for filter diffusion
  - 24 hours for extract acute cytotoxicity
  - 24-72 hours for agar diffusion
  - Very limiting regarding the informative value and the kind of effects that can be assessed
  - Effects based on accumulation and delayed/progressive effects will not be detected!
  - Second limitation is use of cell lines that may not be relevant for the proposed use of the biomaterial

Questions?