Biological evaluation of metallic materials intended for orthopaedic applications

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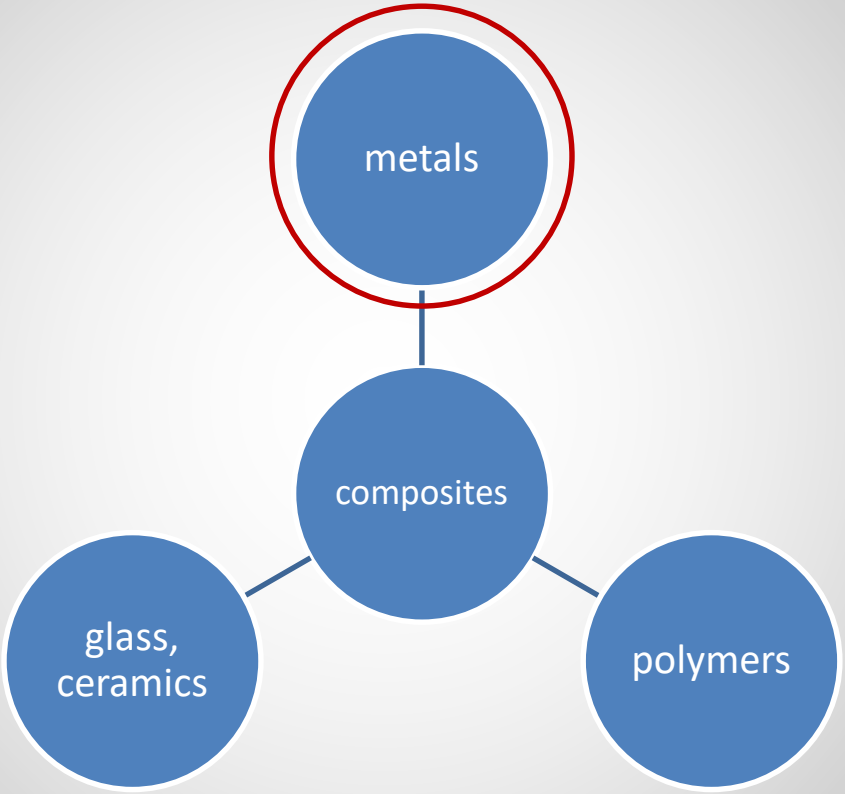
How to (not) get lost in terms such as biocompatibility, cytocompatibility, bioactivity and many more

What is a biomaterial?

- material intended to interface with biological systems to
  - evaluate
  - treat
  - augment
  - replace

tissue, organ or function of the body

Biomaterials



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What should biomaterials do?

- Cause no harm (*be bionert*)

WWII

„Off-the-shelf“ biomaterials
Era of “surgeon heroes“

- To have a beneficial effect
- To have other special properties (combat against bacteria etc.) ..... (*be bioactive*)

What kind of biomaterials?

- **Permanent** implants
  - Improved mechanical properties
  - Antibacterial properties
  - Osseointegrative
- **Degradable** (temporary) implants
  - No need for reoperation

Stress-shielding effect

- Biomechanic (non)compatibility
- Negative phenomenon
- adaptation of the bone to the changed stress distribution

Bone is not loaded properly

→ Thinner bone
→ Less dense bone

Materials with lower elastic modulus

M.P. Staiger et al. / Biomaterials 27 (2006) 1728–1734

Table 1
Summary of the physical and mechanical properties of various implant materials in comparison to natural bone

Properties	Natural bone	Ti alloy	Co–Cr alloy	Stainless steel
Density (g/cm^3)	1.8–2.1	4.4–4.5	8.3–9.2	7.9–8.1
Elastic modulus (Gpa)	3–20	110–117	230	189–205

- 3D –printed porous materials
- Novel titanium alloys (Ti + Nb, Sn, Zr): 40 Gpa
  - Price
  - Non-toxic

The Department of Metals and Corrosion Engineering, UCT

Implant and bone tissue

soft tissue interface

implant bone

osseointegration

implant bone

Antibacterial coatings of biomaterials

- Reducing the risk of postoperative infection
- Antibacterial agent: silver

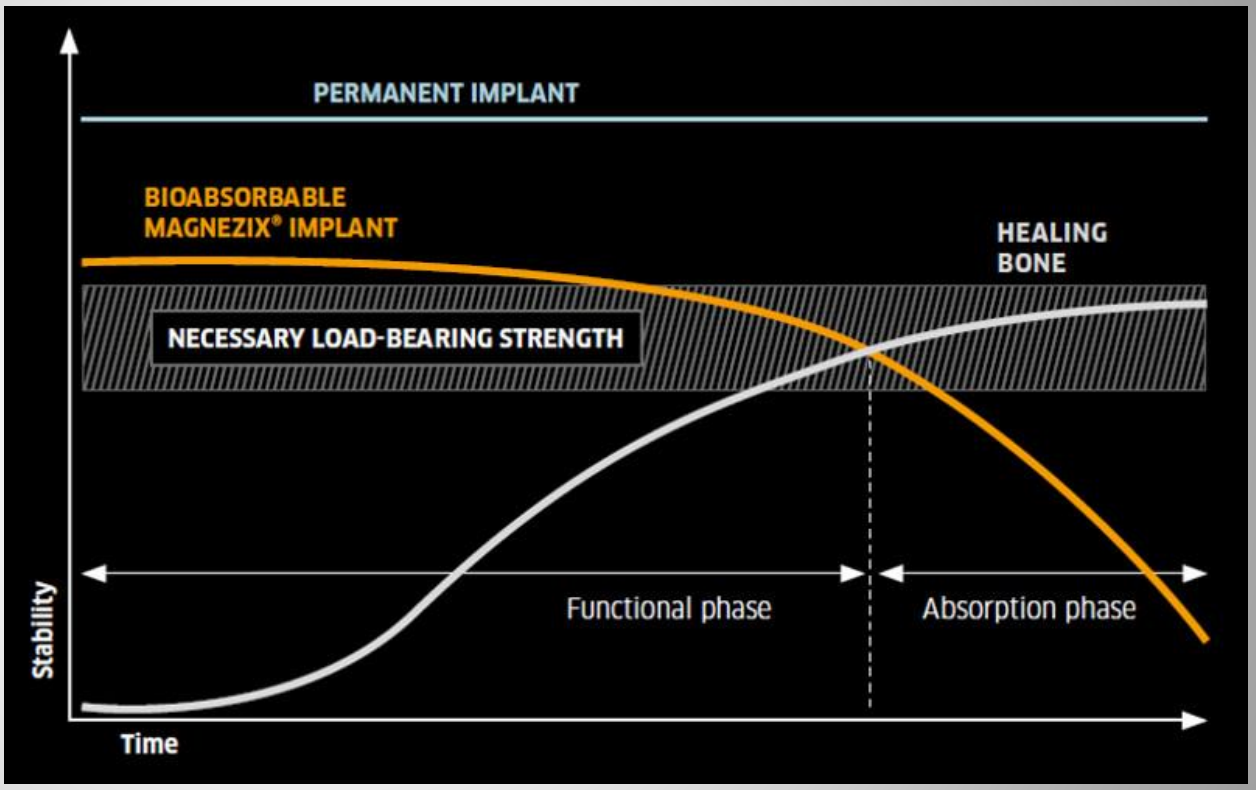
http://www.accentus-medical.com/products-agluna.asp
https://www.implantcast.de/en/company/technology/silver-coating/

Antibacterial bioactive coatings

- Sol-gel method
  - + Ag
  - + Ca+P

Department of Glass and Ceramics, UCT

Degradable implants



https://www.syntellix.de/en/home.html

Magnesium alloys

- How to decrease corrosion rate?
  - Alloying elements: Al, Li, Y and other rare earth elements
  - Preparation and processing
  - Surface treatment

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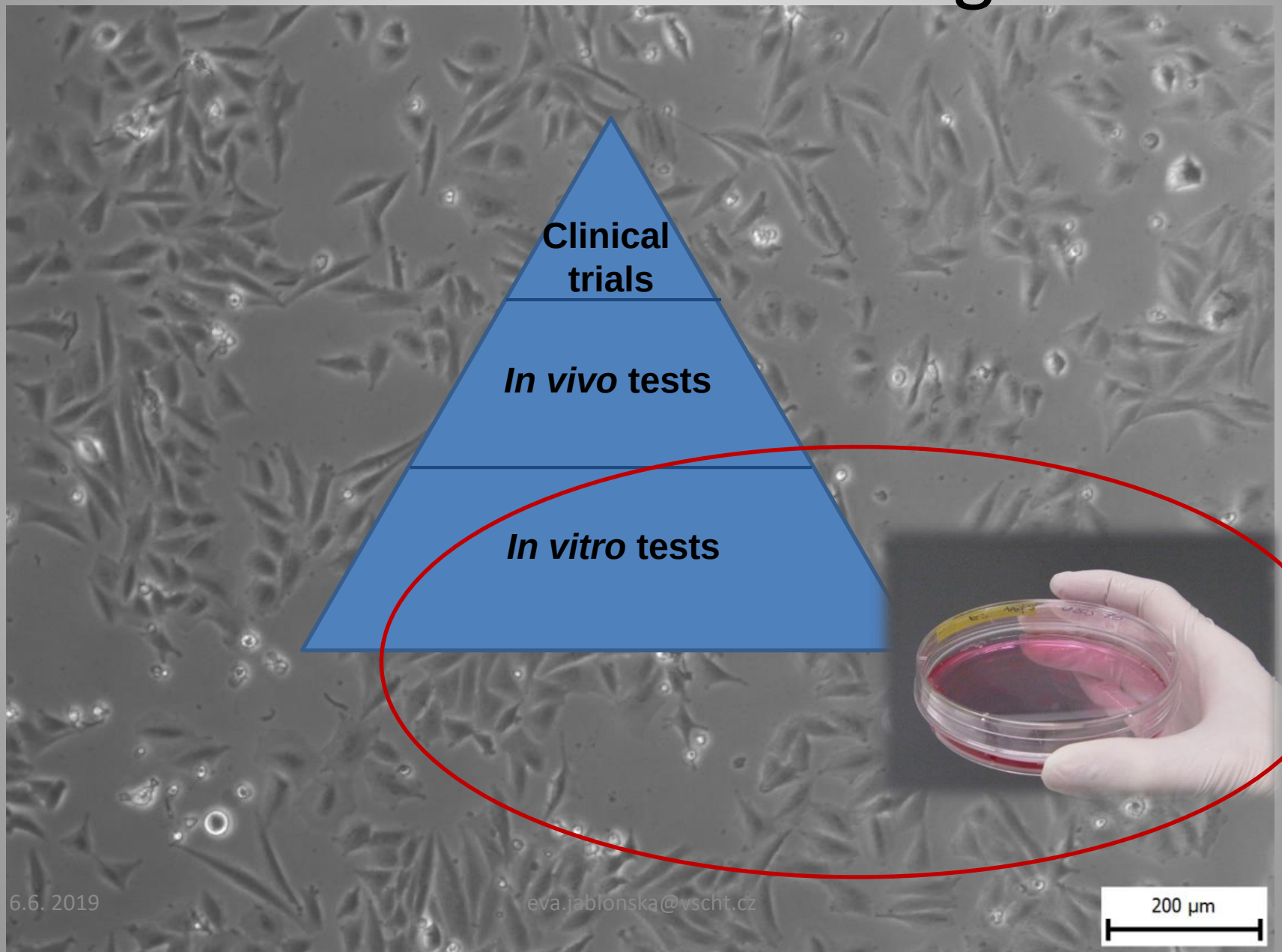
Department of Physics of Materials (Charles University)

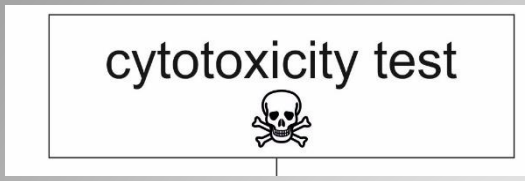
Biomaterials testing



In vitro tests

- Cytotoxicity tests
  - ISO 10993-5 standard
  - „Biological evaluation of medical devices -Tests for in vitro cytotoxicity“

„We need to test biocompatibility, cytotoxicity, viability, metabolic activity and proliferation“



Material is not cytotoxic → is **cyto**compatible

Biocompatibility – broader term

Biomaterials 35 (2014) 10009–10014

Contents lists available at ScienceDirect

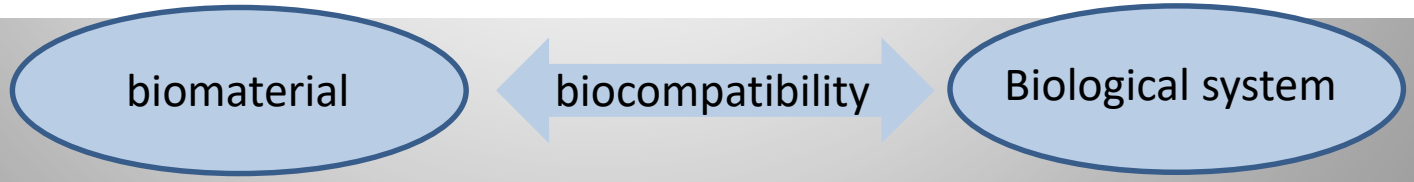
Biomaterials

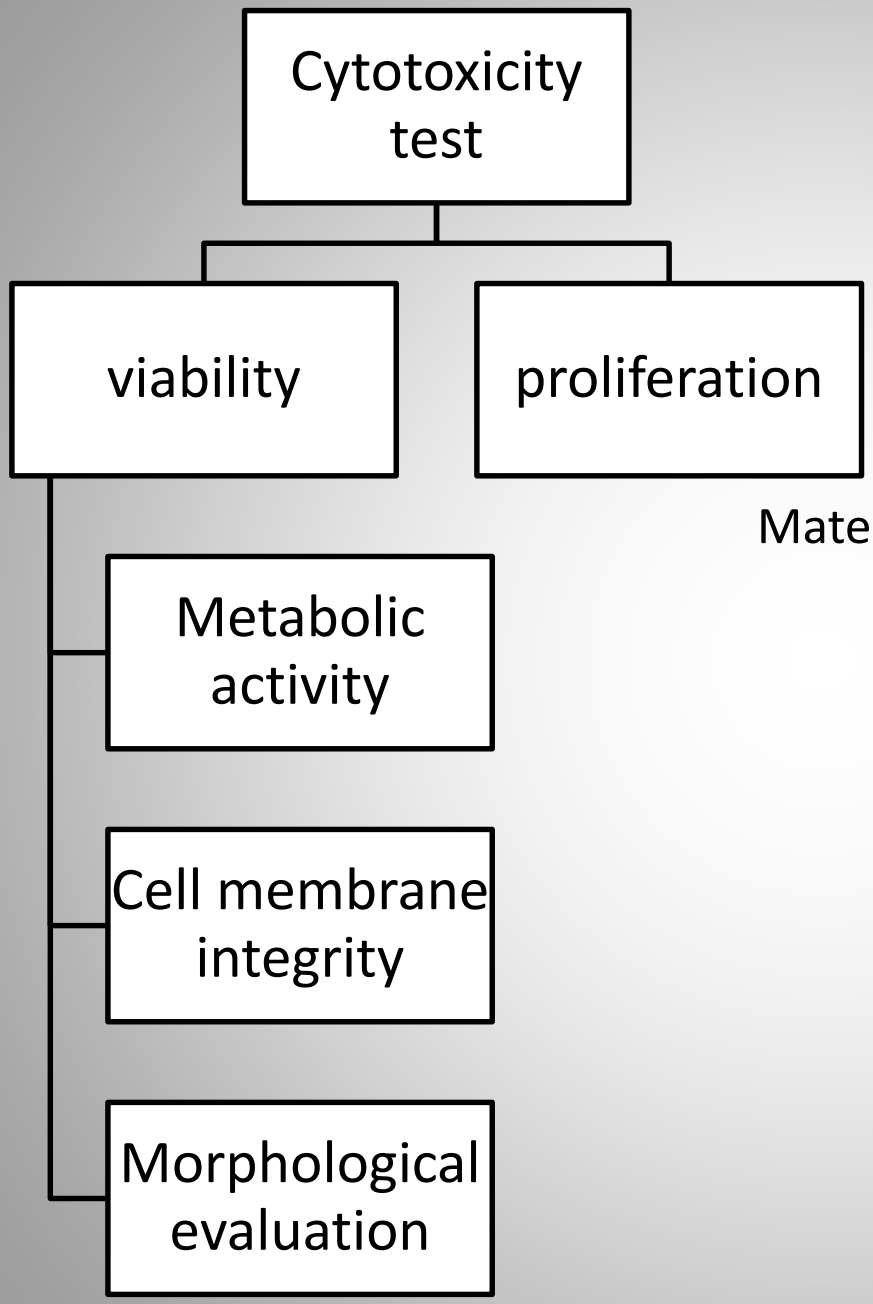
journal homepage: www.elsevier.com/locate/biomaterials

Leading opinion

There is no such thing as a biocompatible material

David F. Williams [a, b, c, d, e, f, g, *]



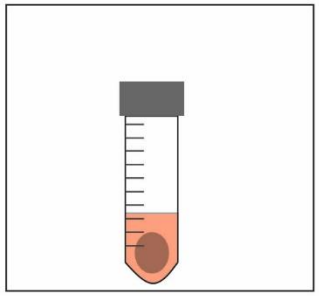


Material is not cytotoxic → is cytocompatible

Type of exposition

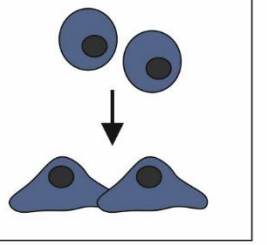
test layout (interaction of the cells with the material)			
test with extracts (elution test) according to ISO 10993-5 qualitative or quantitative	**direct contact** according to ISO 10993-5 qualitative or quantitative	**indirect test** (diffusion test) - filter - agar according to ISO 10993-5 qualitative only	seeding cells directly on the materials NOT specified in ISO 10993-5

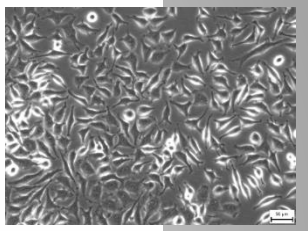
In vitro test for cytotoxicity - extracts

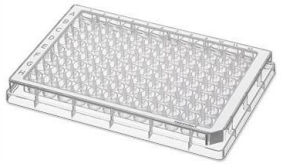


Material extraction
Medium

Cell preparation

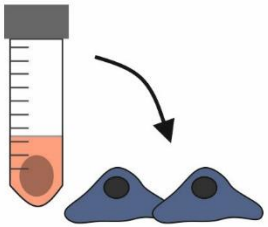






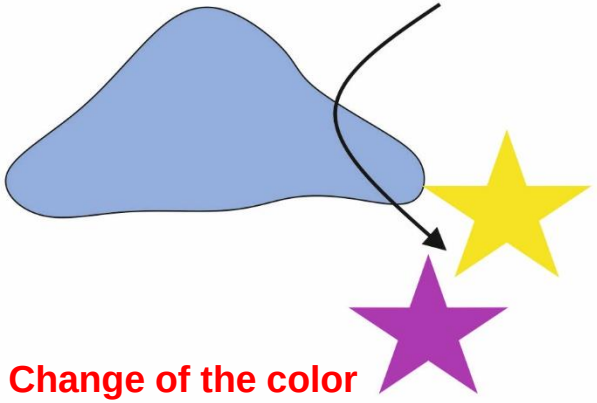
96-well plate

Addition of extract

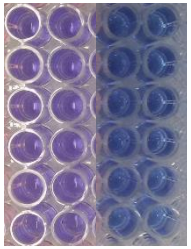


Incubation

Test for toxicity



Change of the color



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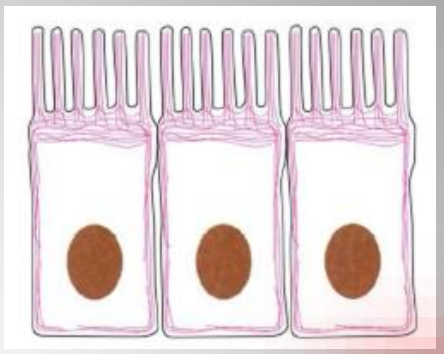
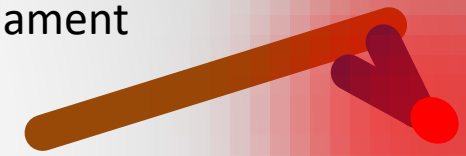
Cell shape

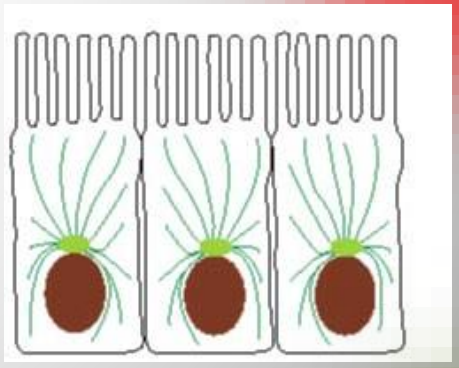
- Cytoskeleton
  - **Actin (microfilaments)**
  - Tubulin (microtubules)



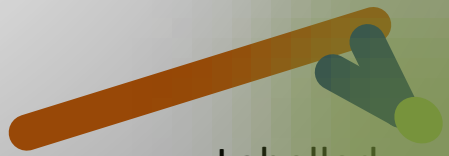
Actin filament



Labelled phalloidin

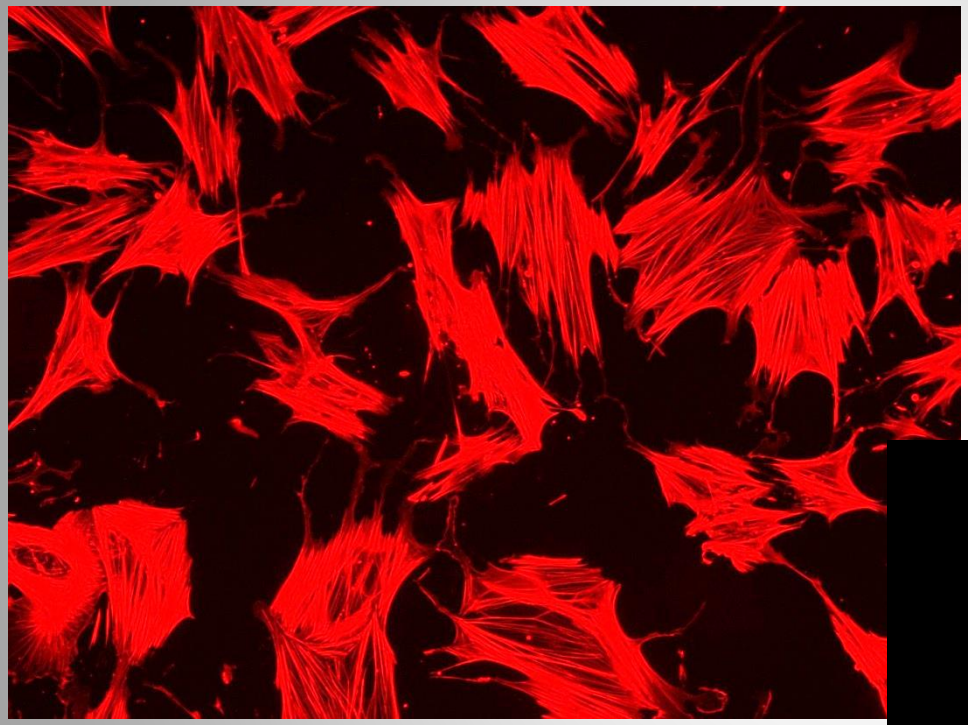


Tubulin



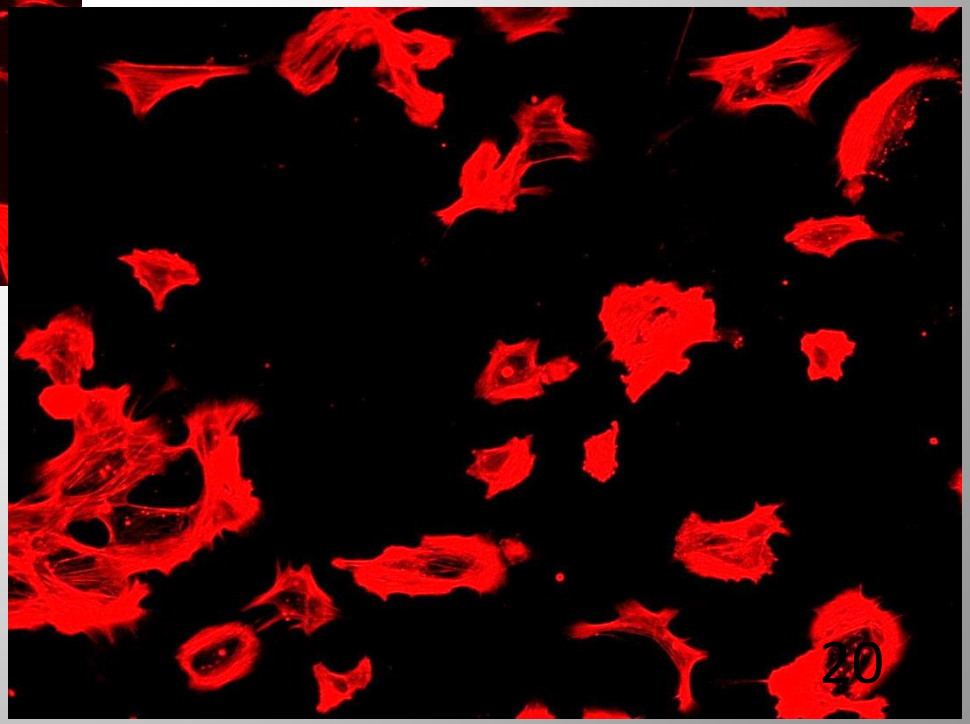
Labelled antibody

Cell shape



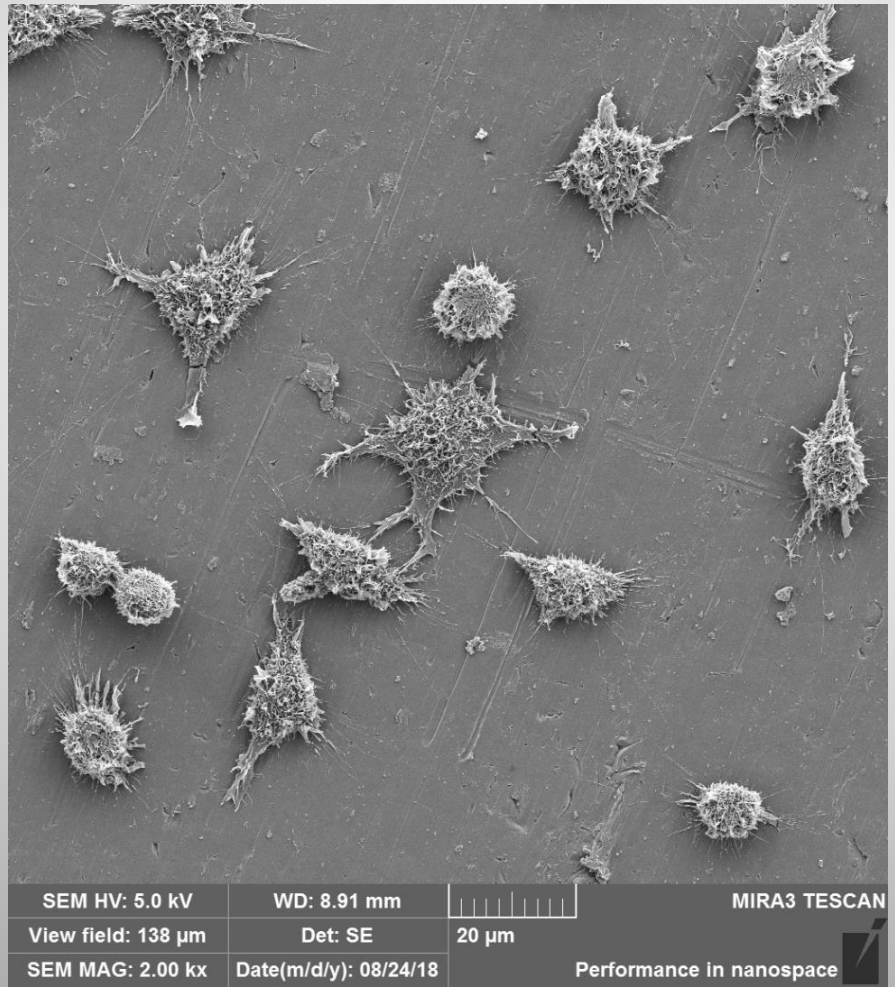
Happy cells

Less happy cells



Cell shape

- Scanning electron microscopy



Live/Dead Assay

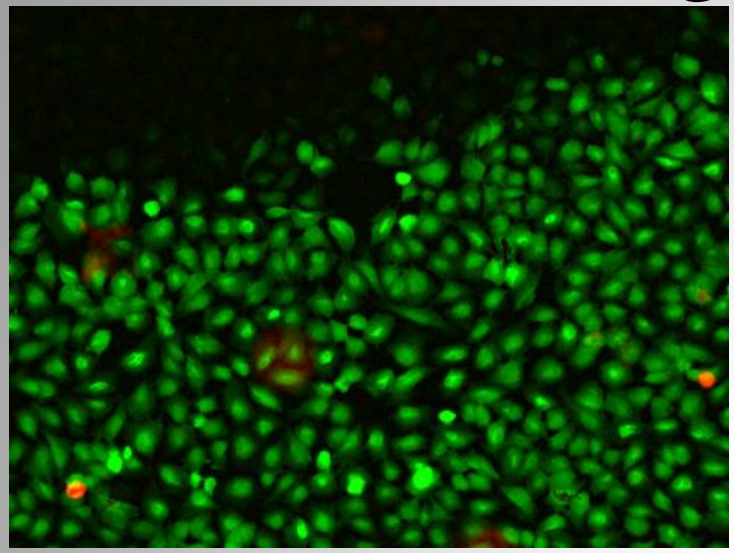
viability measurement while evaluating:

- Cell membrane integrity
- metabolic activity
- morfology

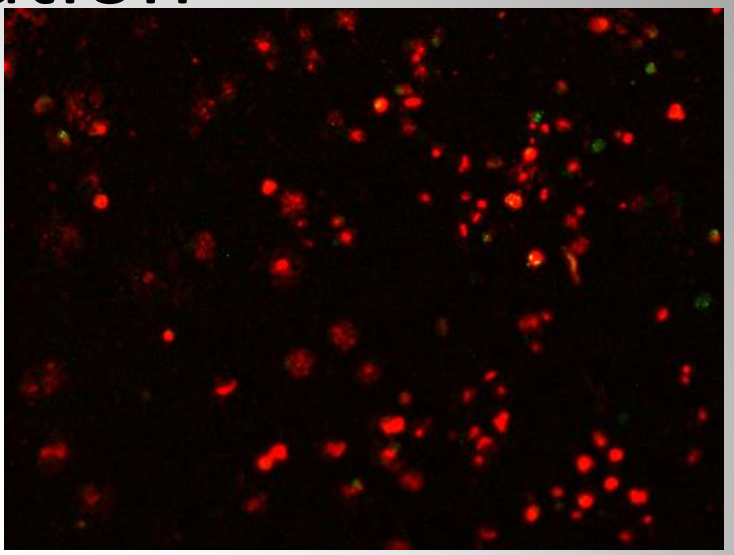
principle:

- Two dyes:
  - Ethidium homodimer enters into dead cells and stains nuclei **RED**
  - Calcein-AM enters live cells and is hydrolysed staining cytoplasm **GREEN**

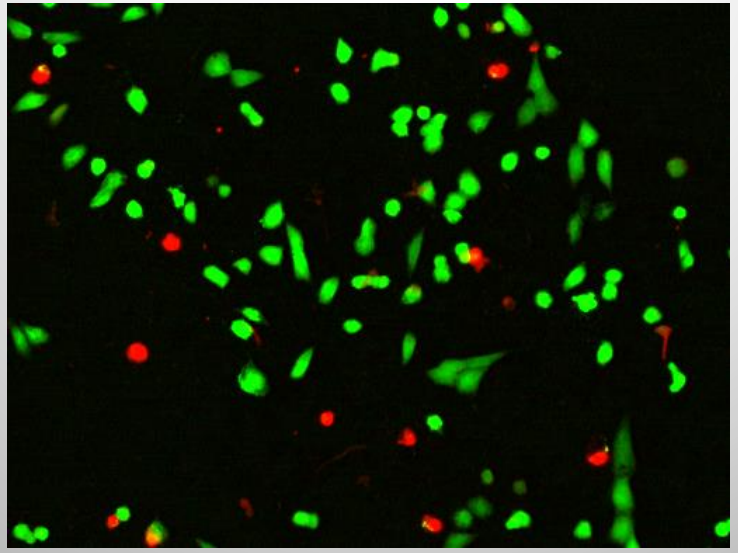
Live/Dead assay – microscopic evaluation



Non-toxic material



Very toxic material



Moderately toxic material

TE 01020390 - Centrum vývoje moderních kovových biomateriálů pro lékařské implantáty (2012 - 2018; Technologická agentura ČR, Centrum kompetence)
Perspektivní hořčíkové slitiny se zlepšenými korozními, biologickými a mechanickými vlastnostmi (2016-2018; Grantová agentura ČR)