Biocompatible materials 14.11.2011

- Determination of biocompatibility by using *in vitro* and *in vivo* methods (cont.)
  - Toxicity tests: Types, Procedures, Results
  - Response tests: Types, Procedures, Results

In vitro Tests – Test and Effects

- Tests for cell-type unspecific functions (basal functions):
  - Disorder of membrane integrity, metabolism (mitochondria, lysosomes)
  - synthesis of cell-type unspecific cell components like DNA and RNA
- Tests for cell-type specific functions:
  - Existence and activity of the alkaline phosphatase, which occurs in high amounts only in active osteoblasts
- Effects
  - General cytotoxic substance: almost all cells react at similar concentrations
  - cell-type specific reaction: only a certain cell-type or a class of cells reacts more sensitive than other cells
    - can refer to basal functions as well as one or more cell-type specific properties
    - can lead to cell death, but also contain a change in the development of special cell components

In vitro Tests

Toxicity Tests (screening tests)	**Reactivity Tests (response tests)**
Result	
result: cells live or die	result: cells survive with different reactions
Cytotoxicity Histotoxicity Haemotoxicity	Cell reaction Blood reaction Tissue reaction Immune reaction Carcinogenesis

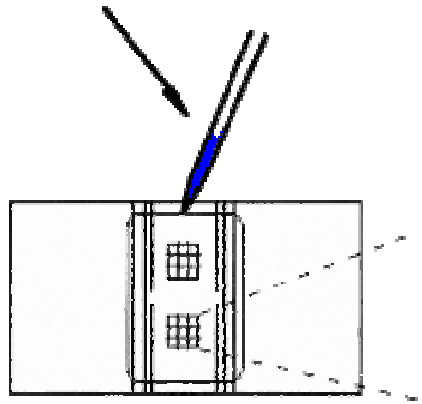
- Short-time or acute tests: registration of influences on cell structures, that are direct accessible for the test substance
- Long-time tests: registration of
  - Effects at the end of a test cascade (delayed toxicity, progressive toxicity) or
  - Effects that are forming after accumulation of the test substance in a special cell (chronic toxicity)

Criteria for In Vitro Biocompatibility in Cell and Tissue Cultures

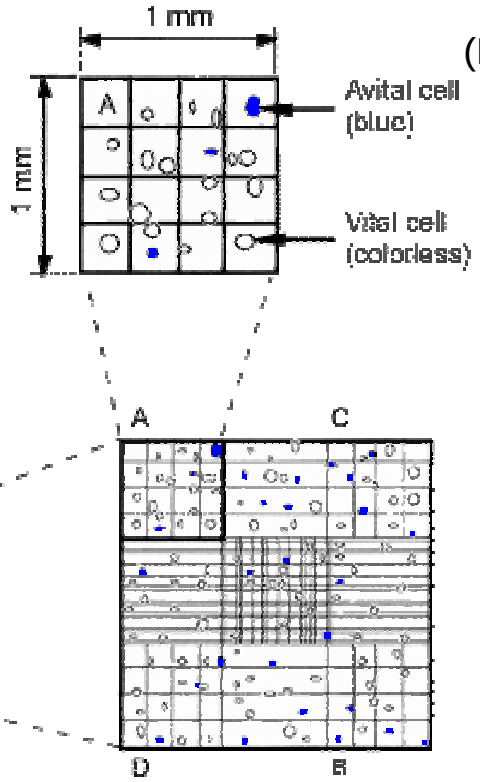
Criterion (cell behaviour)	Increasing Biocompatibility - +	
growth/cell density	die	proliferate
morphology	spherical	extended
adhesion	low	strong
wetting	bad	good
metabolic activity	changed	unchanged

Counter Chamber for Determination of the Cell Number

25	µl	Cell suspension in the medium
25	µl	Trypan blue 0.4% in 0.81% NaCl and 0.06% K_2HPO_4
50	µl	PBS
100	µl	Entire volume



Neubauer counting cell chamber



(E. Wintermantel / S.-W. Ha, 2002)

Addition of Trypan blue allows the differentiation in viable and non viable cells:

live cells: intact membranes → excludes staining → cell appears colorless and transparent

dead cells: defect membranes → dye penetrates → cell appears blue

Calculation of cell concentration:

Number of cells per unit A, B, C or D

Average cell number of all units
1/4 x (A+B+C+D)

x 4 x 10^4 = Cells per ml suspension

Factor from dilution of cell suspension

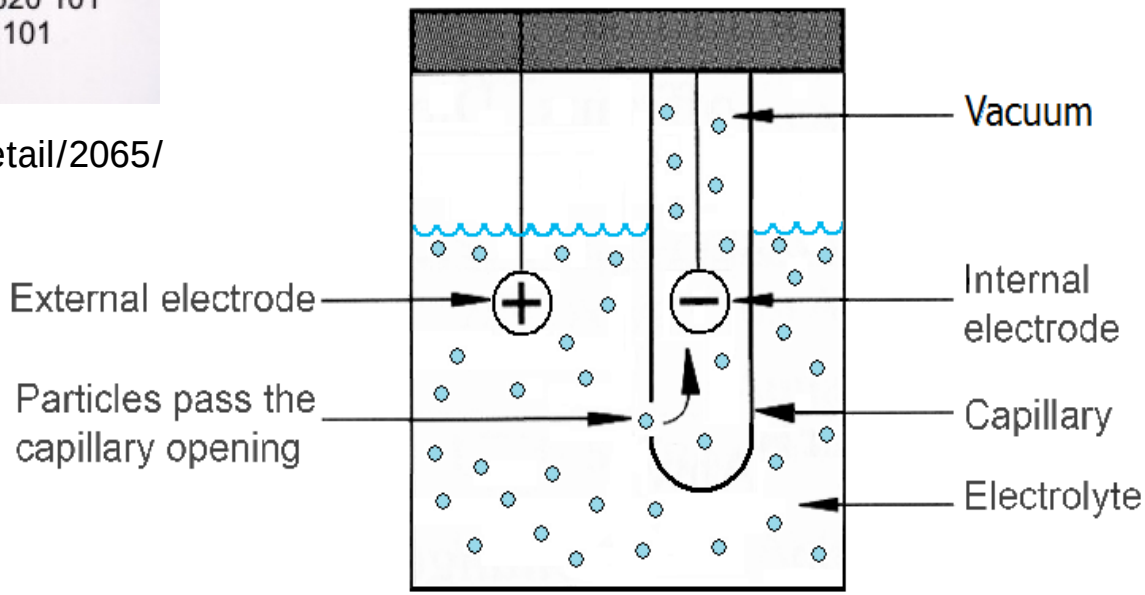
Factor of selected counting chamber volume

http://www.uni-greifswald.de/~immuteach/methods/trypane/trypane.html#

Coulter™-Counter

Analyser Cups for Coulter Counter

us.gbo.com/bioscience/detail/2065/



Indirect determination of the cell number

Among others the following methods can be applied:

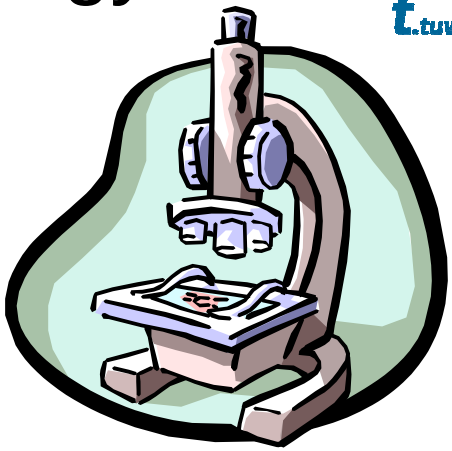
- DNA content of the cells → fluorescence measurement

  http://www.uni-greifswald.de/~immuteach/methods/cell_division/cell_division.html#

- Absolute protein content → colorimetrical method

- Marking with radioactive substances

Determination of Cell Morphology



- Analysis by microscopy
  - Confocal laser scanning microscopy (CLSM)
  - Scanning electron microscopy (SEM)
- Control of the integrity of diverse cell structures or functions by different staining procedures

Toxicity Tests (screening tests)

The tests are classified with respect to the contact between the cells and the sample:

- Direct test: Settlement test
- Indirect test: Agar diffusion test
- Test with extracts: Extraction test

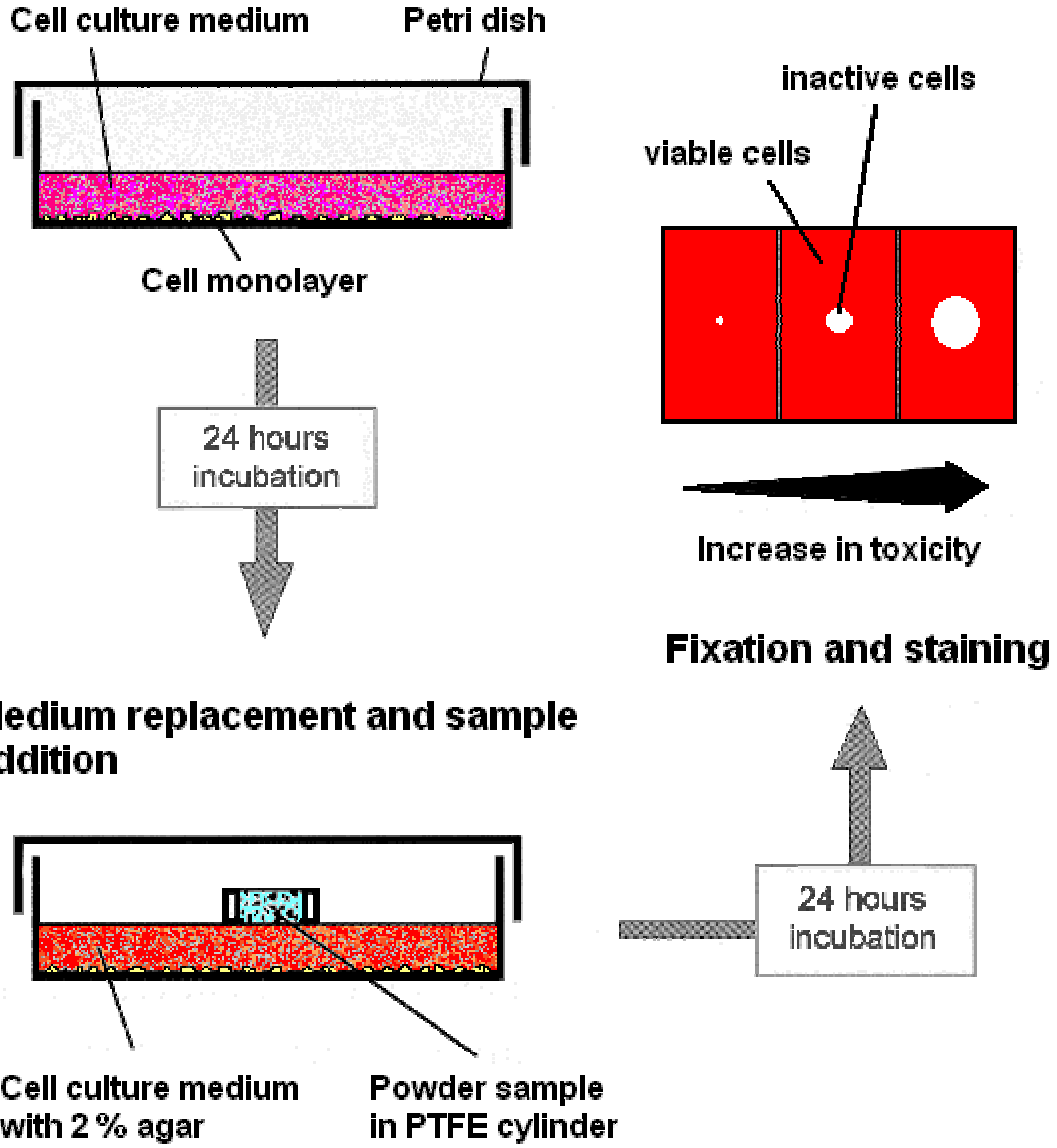
Direct Tests

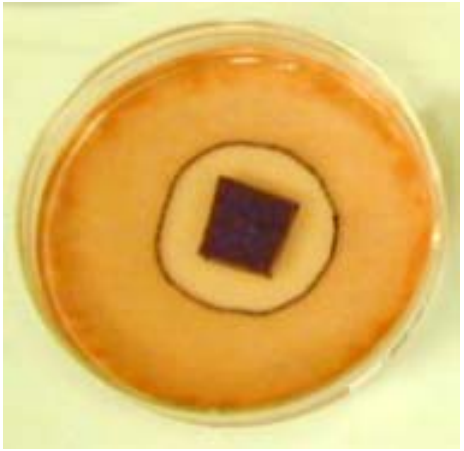
There are two methods to bring the cells in direct contact with the material:

1. Material is directly settled with the cells

2. Petri dish is settled with cells, the material is applied on the cell layer

Indirect Test – Agar-Diffusion Test





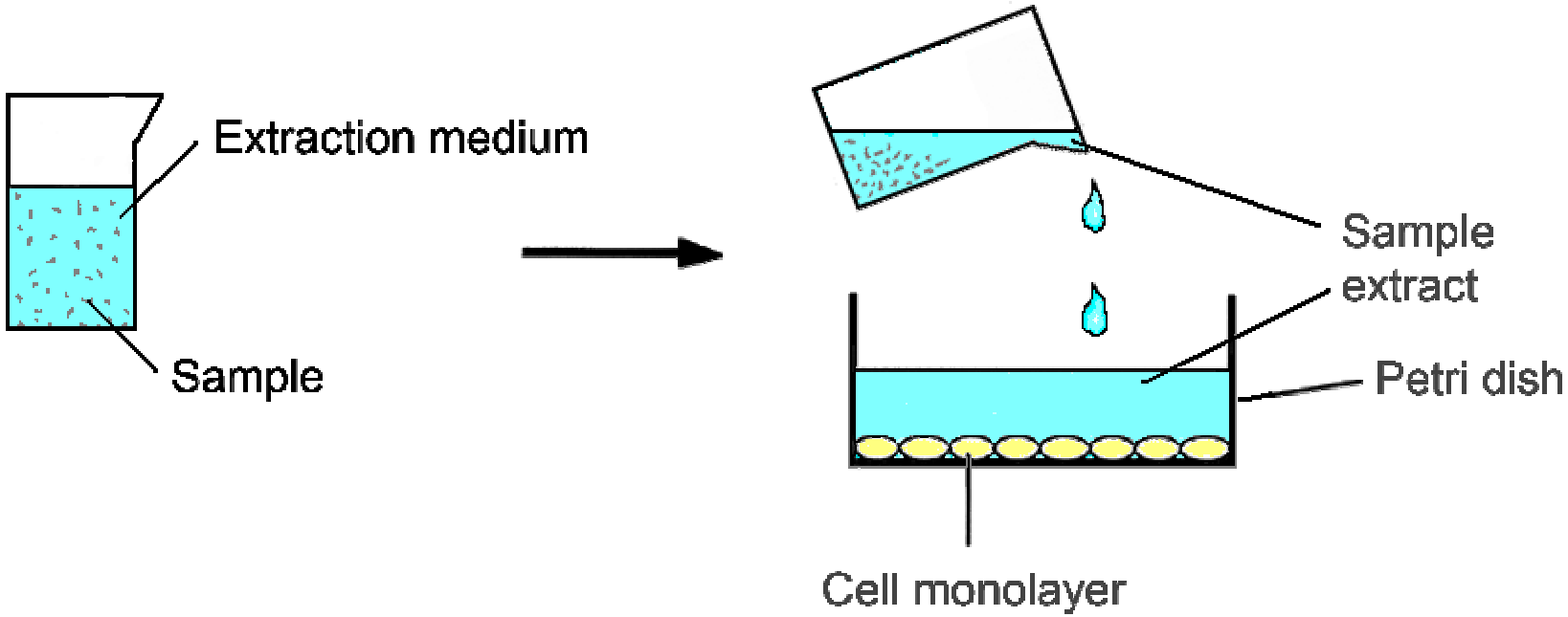
www.mbresearch.com

Graded scale for agar diffusion and direct contact tests

Grade	Type of reactivity	Comments on reactivity zone
0	None	No detectable reactivity zone
1	Minimal	Very few degenerated or malformed cells next to sample
2	Mild	Reactive zone limited to area adjacent to sample
3	Moderate	Zone may extended to 1 cm beyond sample
4	Severe	Zone extends beyond 1 cm around the sample

(S.A. Guelcher et al., 2006)

Tests with Extracts (determination of leachables)



Aim: Determination of the dilution concentration that leads to 5 % or 50 % change in comparison with standard values

Graded scale for extract dilution (Elution) test

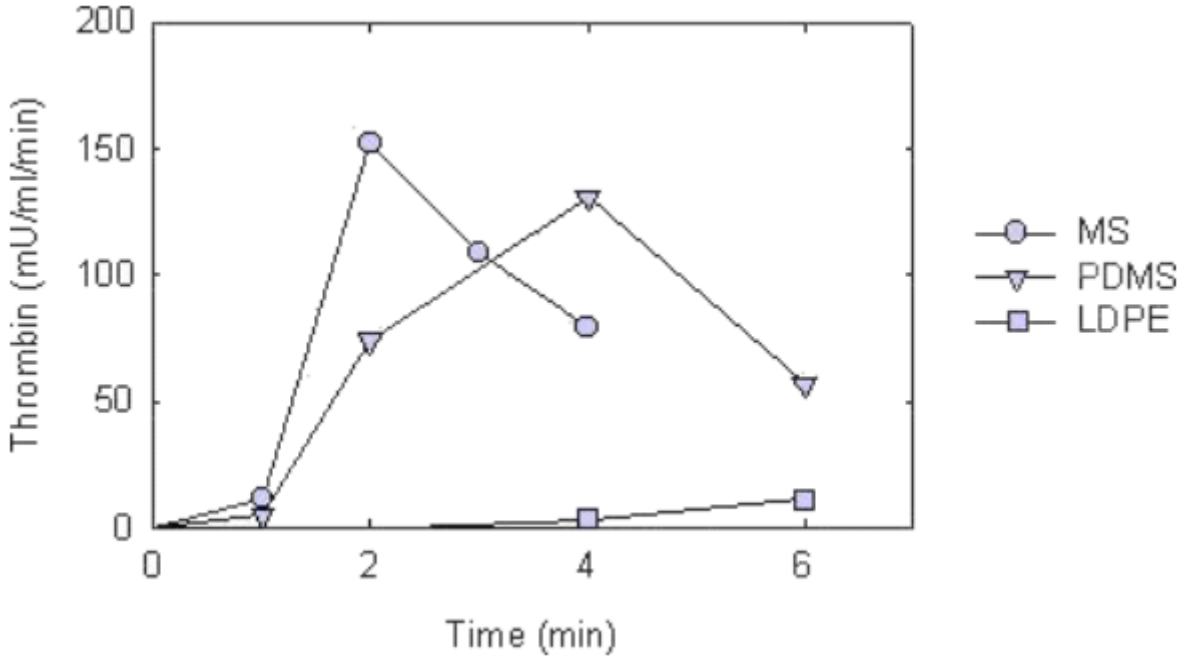
Grade	Type of cell response	Comments on cellular response
0	None	No detectable adverse cellular response
1	Minimal	≤ 20 % of the cells display adverse response
2	Mild	≤ 50 % of the cells display adverse response
3	Moderate	≤ 70 % of the cells display adverse response
4	Severe	Cells display near complete adverse response

(S.A. Guelcher et al., 2006)

Reactivity Tests (Response Tests)

- Blood reactions – haemocompatibility
  - Blood coagulation test
  - Adhesion of blood platelets and proteins
  - Thrombogenicity
- Immune reactions
- Carcinogenicity (Ames Test)

Thrombogenicity



MS … medical steel

PDMS … polydimethylsiloxane

LDPE … low density polyethylene

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Reactivity Tests (Response Tests)

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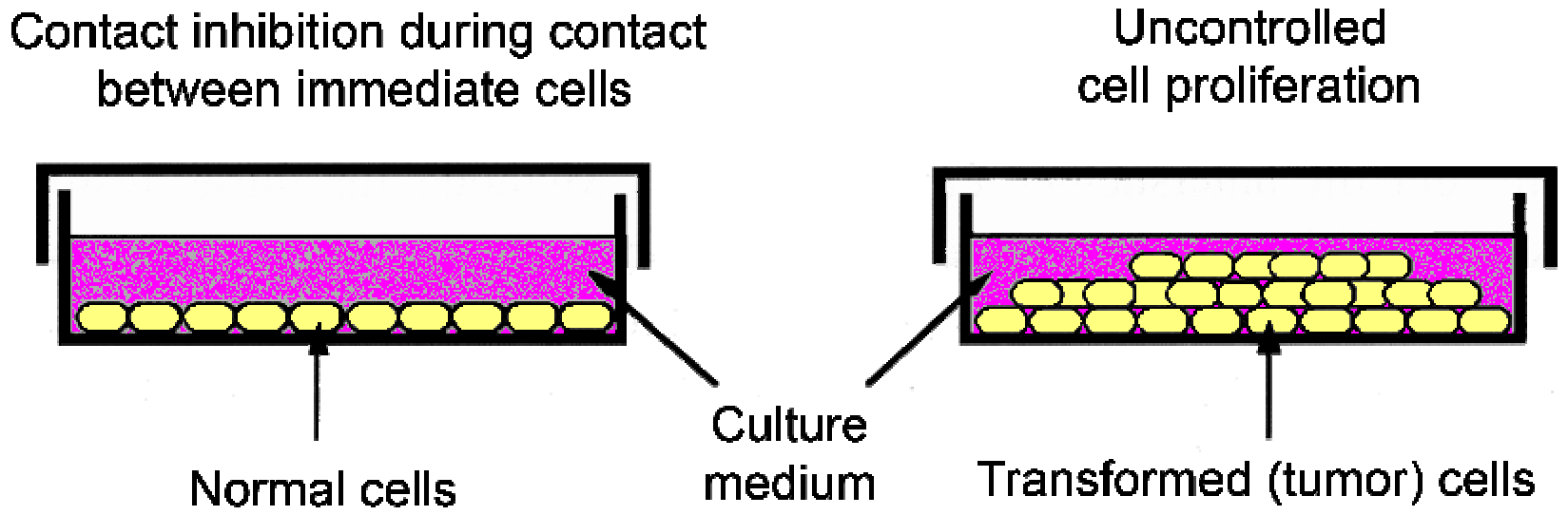
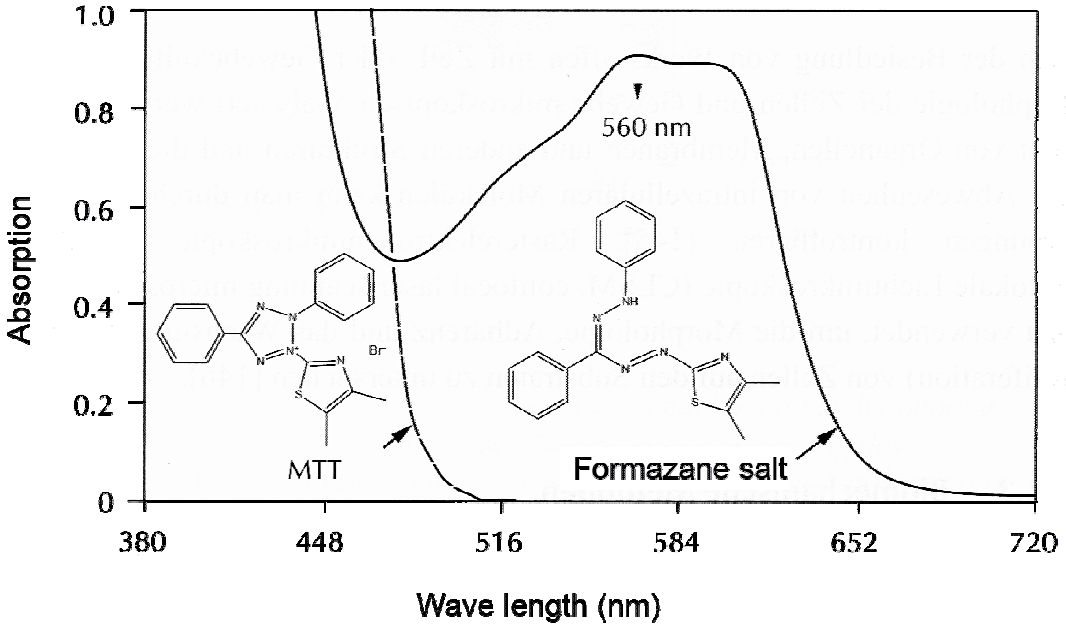
Carcinogenicity (Ames test)

Division behaviour of cells



Biochemical Methods

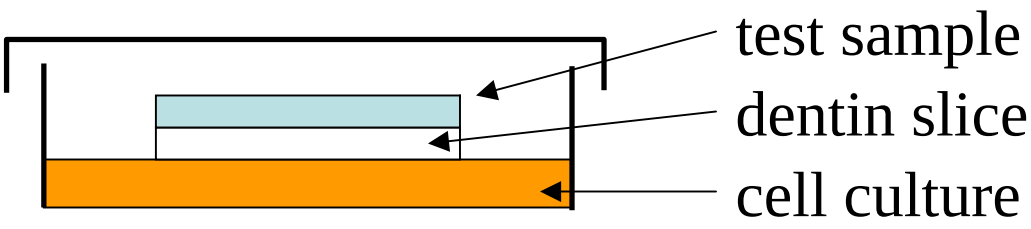
- Neutral red assay
- MTT (MTS) assay



- Lactate Dehydrogenase (LDH) assay
- Marking with radioactive chromium ($Na_2{}^{51}CrO_4$)

Evaluation of restorative dental materials

- Dentin barrier tests
  - slices of dentin are incorporated between a test sample and the cells in culture



Biocompatibility tests with cell cultures, Why?

- Investigation of the toxic potential
- Evaluation of the release of potential harmful substances
- Investigation of the interactions between implant surface and surrounding tissue
- Quality control
- ...

Advantages of cell and tissue cultures

- The vitality of individual cells can be quantified
- A controlled environment
- Investigation of the influence of biocompatible materials on specific cell types
- Reproducible results are obtained very fast
- Cell and tissue techniques allow experiments with human tissue
- ...

Disadvantages of cell and tissue cultures

- In vitro environments reflect only a simplified part of the complex *in vivo* mechanism
- Cell and tissue cultures have not detoxification and excretion possibilities
- It is impossible to reproduce all *in vivo* effects in cell cultures
- Implant induced tissue reactions are complex
- ...

In vivo tests – animal experimentation

- Determination of *in vivo* compatibility of biomaterials or medical devices prior to human testing
- Should be only performed after appropriate *in vitro* tests have been carried out with already evaluated results
- The selection and design of animal tests has to be appropriate to address the specific scientific objectives of the study
- Require appropriate animal care before, during and after the experiments -> minimising pain, suffering, distress or lasting harm that might be inflicted
- The results are complex and sometimes difficult to interpret, and require experienced personnel -> Tests are expensive
- Alternatives to animal experimentation -> working with multiple cell types (e.g., human skin equivalent EpiDerm™ or EpiSkin™)

Lessons learned

- Toxicity tests: types, procedures, results
- Response tests: types, procedures, results
- Biochemical methods
- Pros & cons of cell and tissue cultures
- Pros & cons of animal experimentations