In Vitro Biotesting of Nanoparticles on Mammalian Cells

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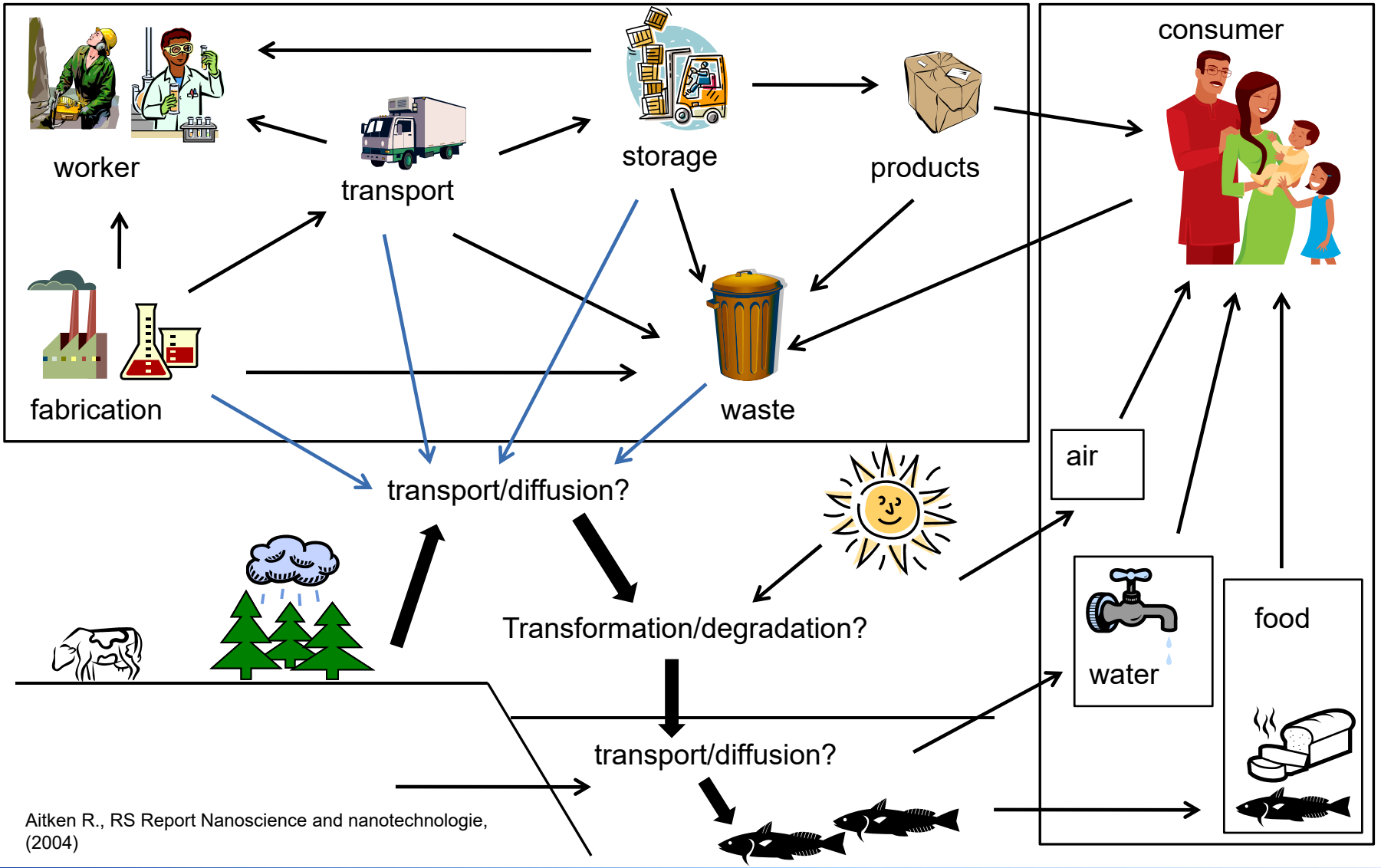
Life Cycle of Nanomaterials

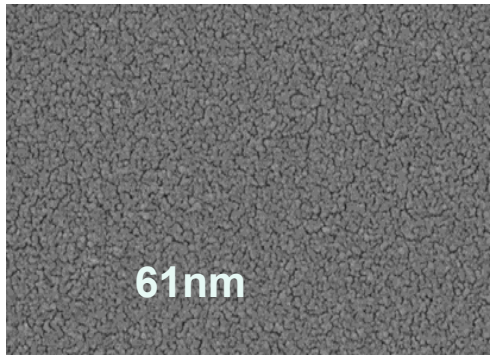


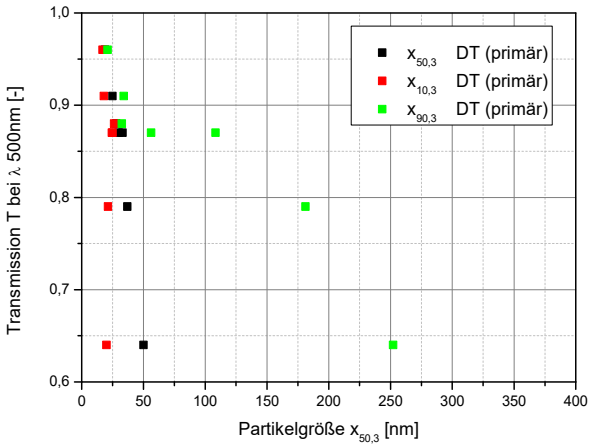
Aitken R., RS Report Nanoscience and nanotechnologie, (2004)

Nanopartikuläre Beschichtungen im Innovationsverbund

iPAT Institut für Partikeltechnik

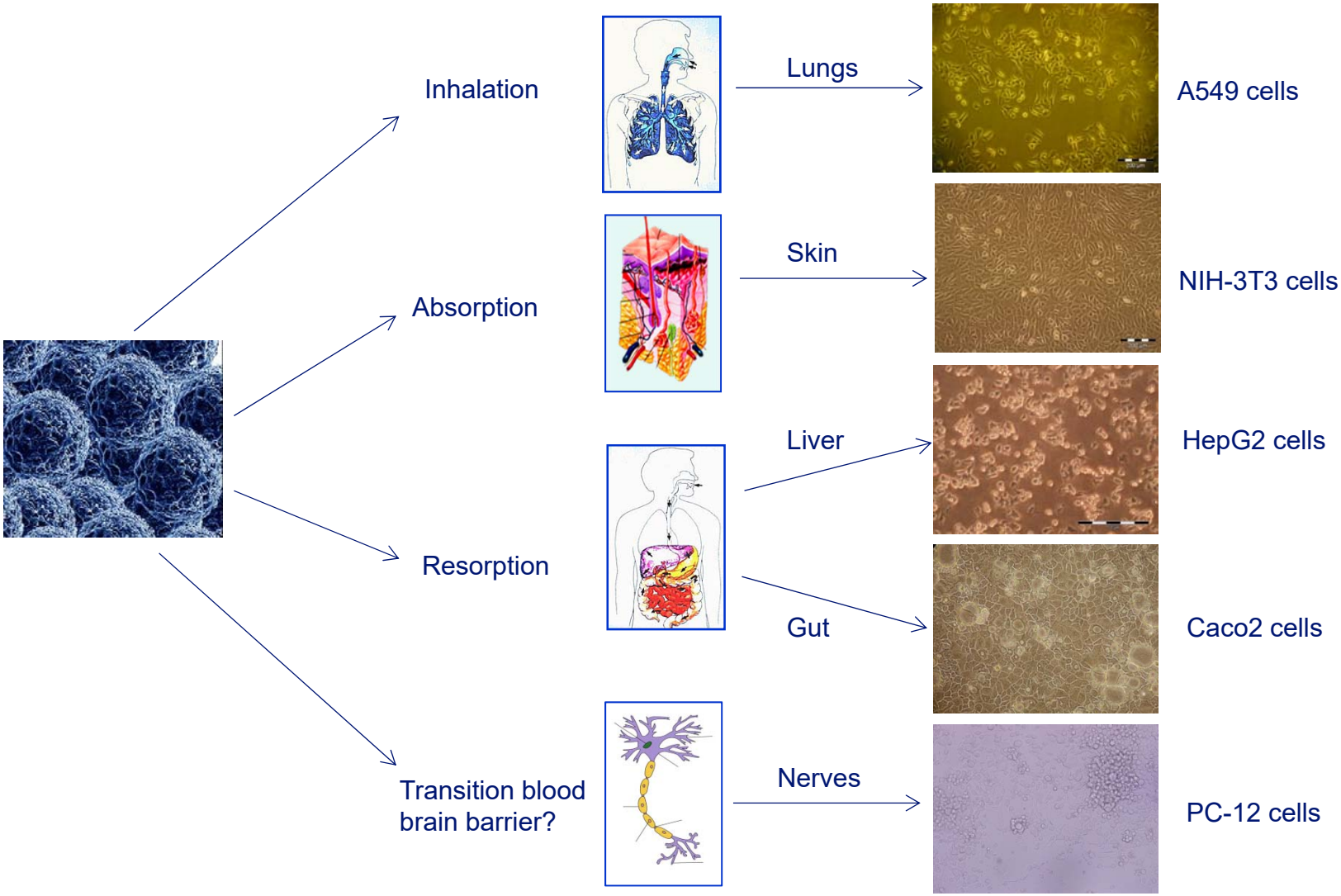
- **Nanopartikuläre Lackbeschichtung**
  - Verbesserung bestehende Rezeptur
  - Herstellung photokatalytisch-aktiver, transparenter Beschichtungen durch Titandioxidpartikel



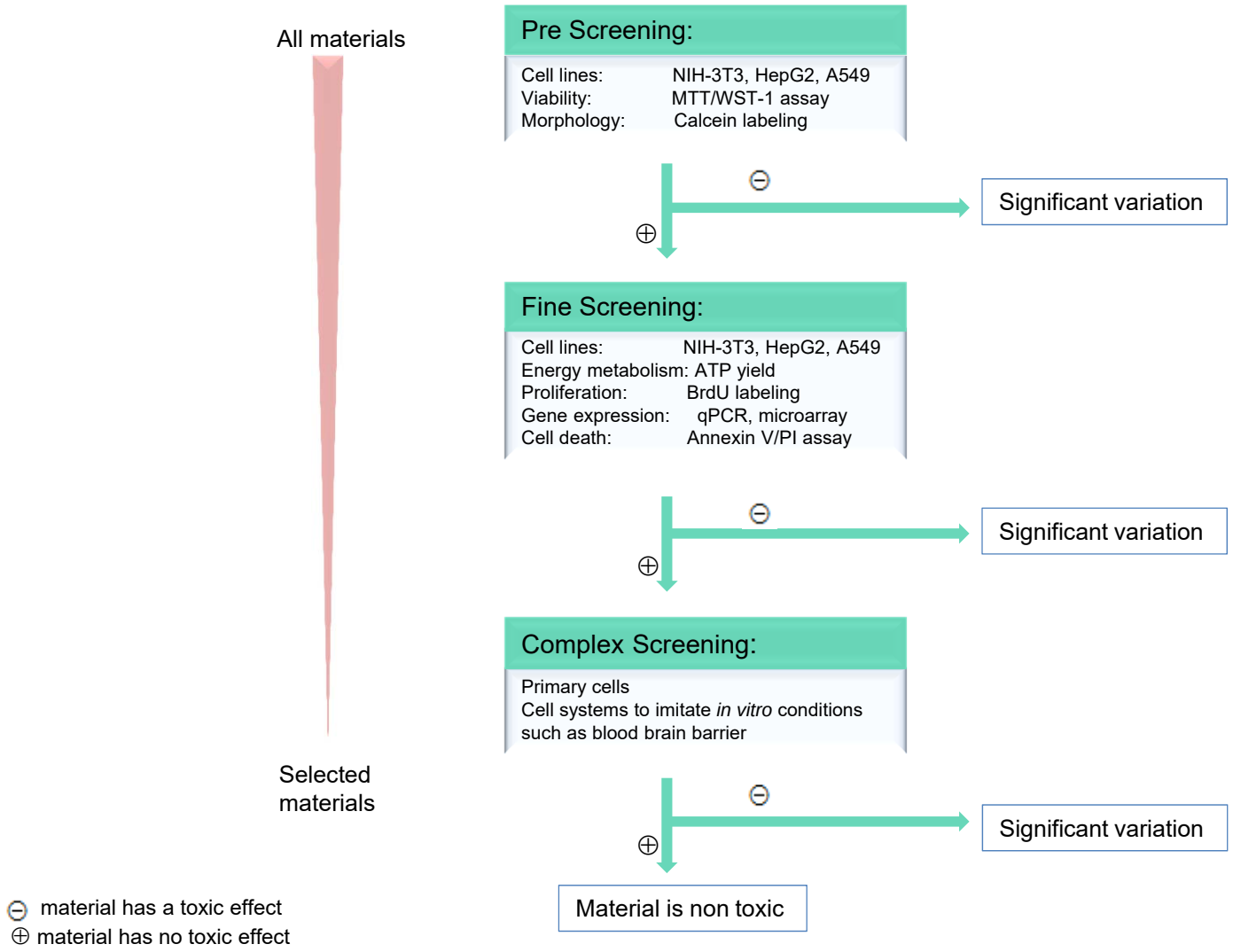


- **Nanopartikuläre Kunstharzoberfläche**
  - Abriebbeständige Beschichtung mit Easy-to-Clean-Oberfläche

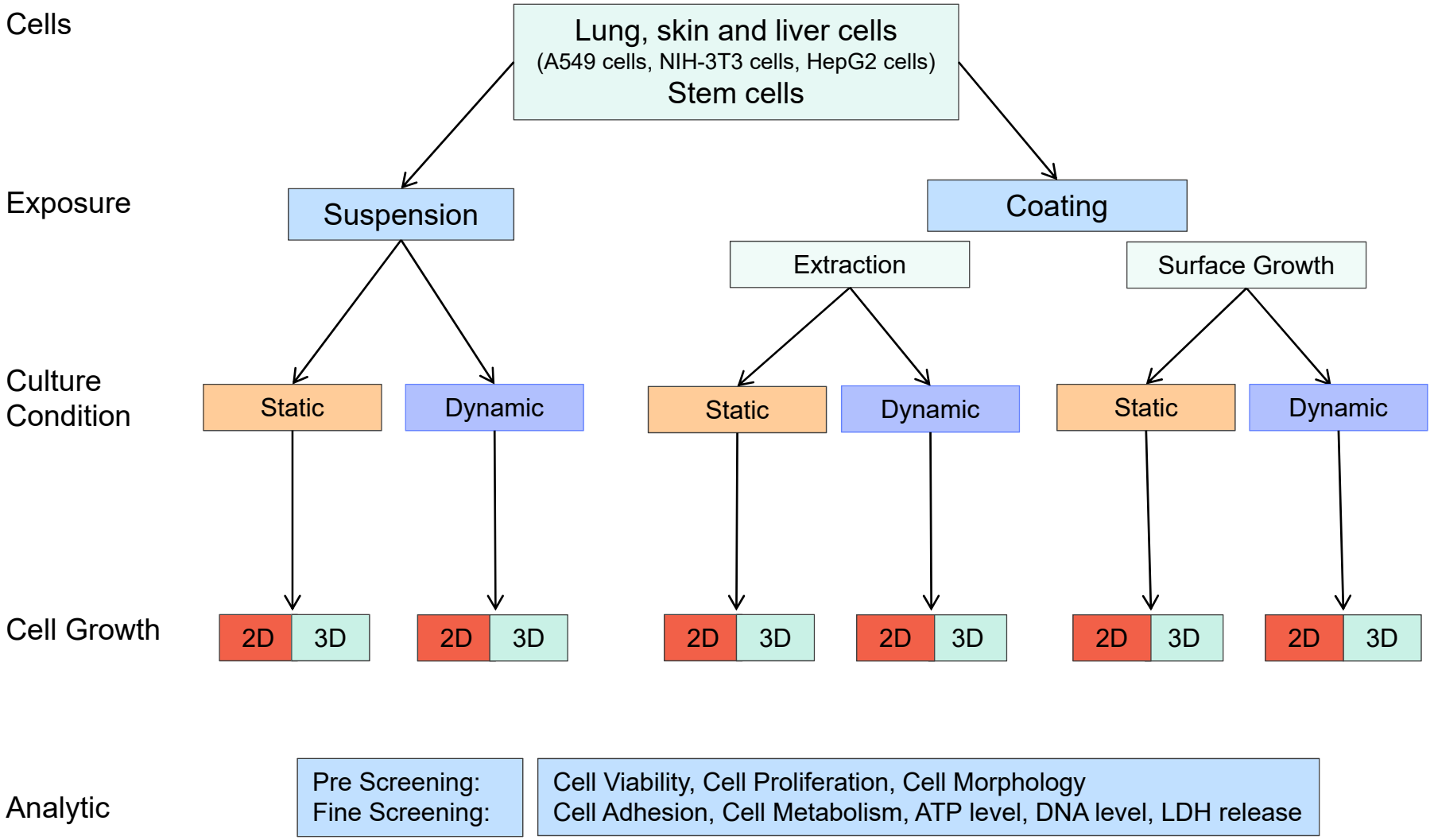
Uptake Possibilities of Nanoparticles



Aim of my Project:
Screening System for Nanoparticle Safety Testing

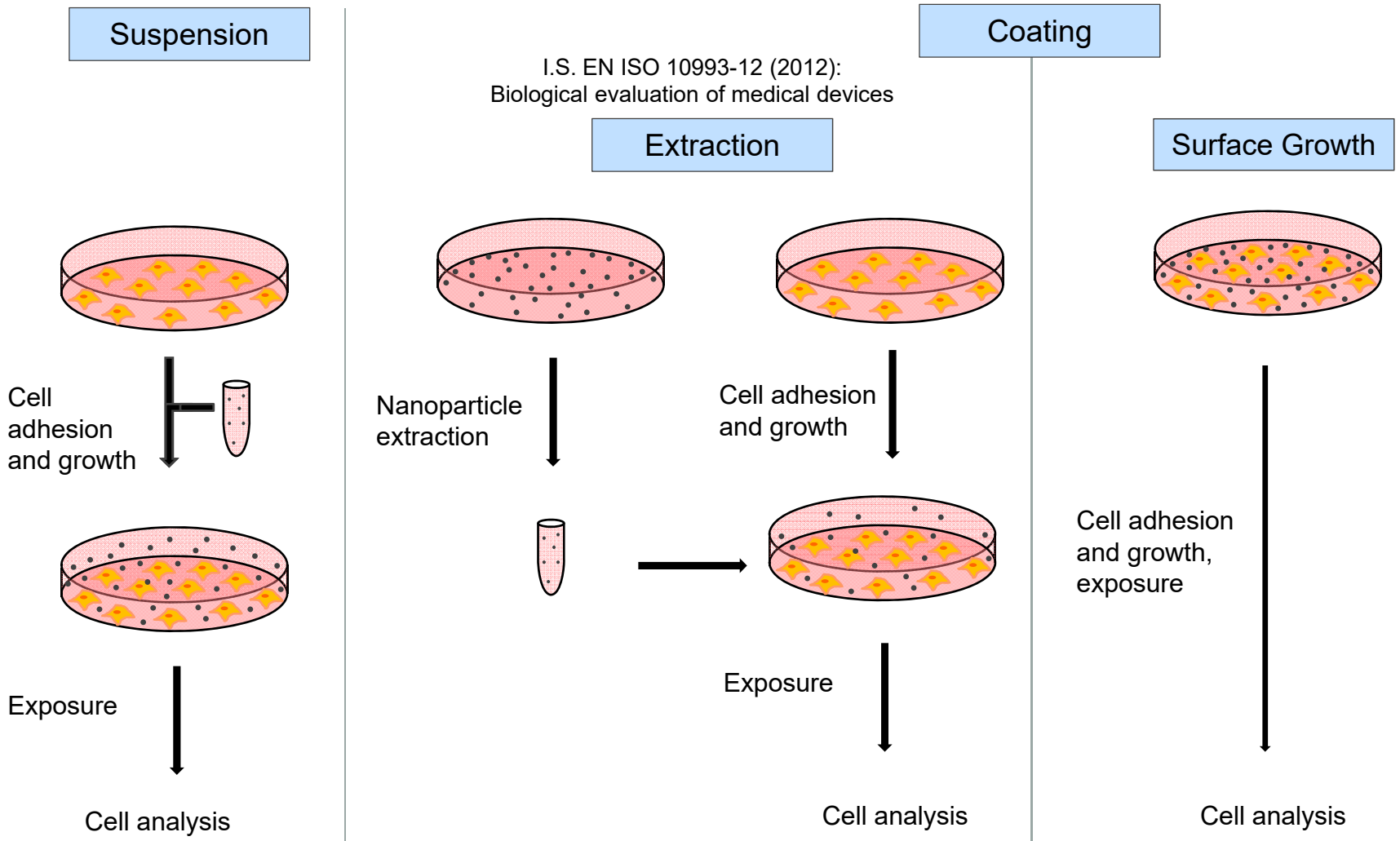


Overview


Cells
Lung, skin and liver cells
(A549 cells, NIH-3T3 cells, HepG2 cells)
Stem cells
Exposure
Suspension
Coating
Extraction
Surface Growth
Culture Condition
Static
Dynamic
Static
Dynamic
Static
Dynamic
Cell Growth
2D
3D
2D
3D
2D
3D
2D
3D
2D
3D
2D
3D
Analytic
Pre Screening:
Fine Screening:
Cell Viability, Cell Proliferation, Cell Morphology
Cell Adhesion, Cell Metabolism, ATP level, DNA level, LDH release

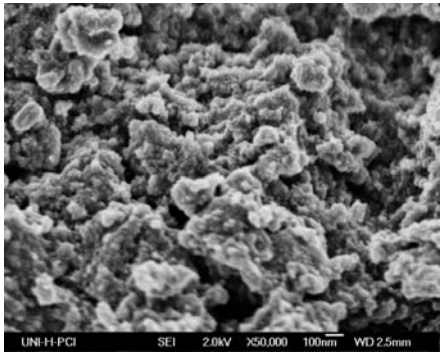
Stage of Work

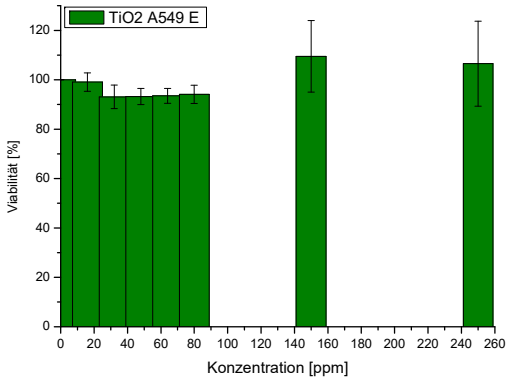
Comparison of Different Ways of Exposure



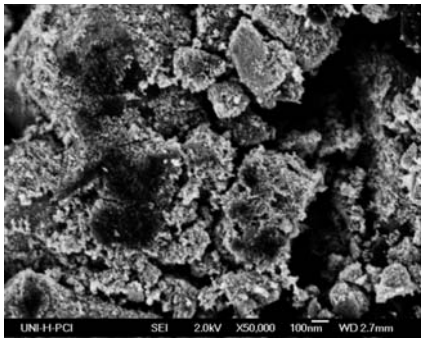
Benchmark-System

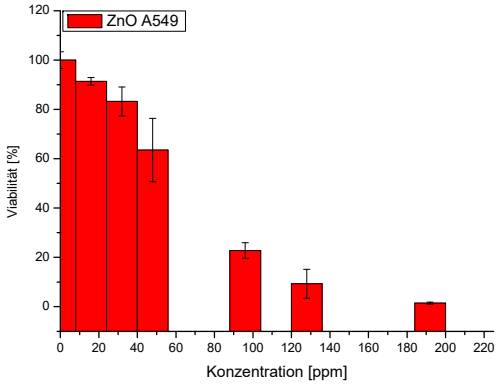
Non-toxic
Titanium dioxide nanoparticles
(UV 100)





Toxic
Zinc oxide nanoparticles
(0.1 % Ru)



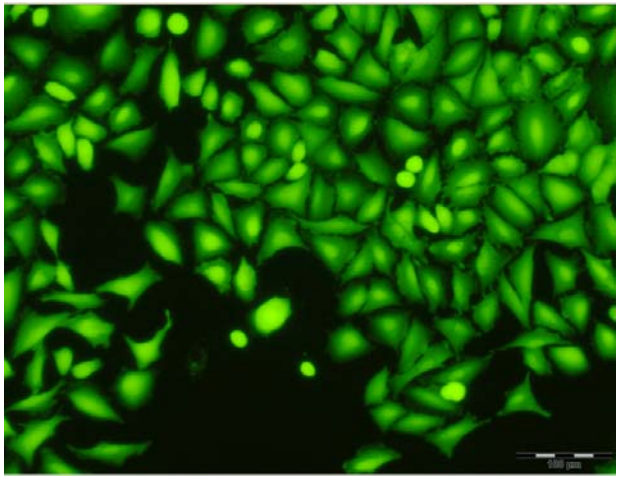


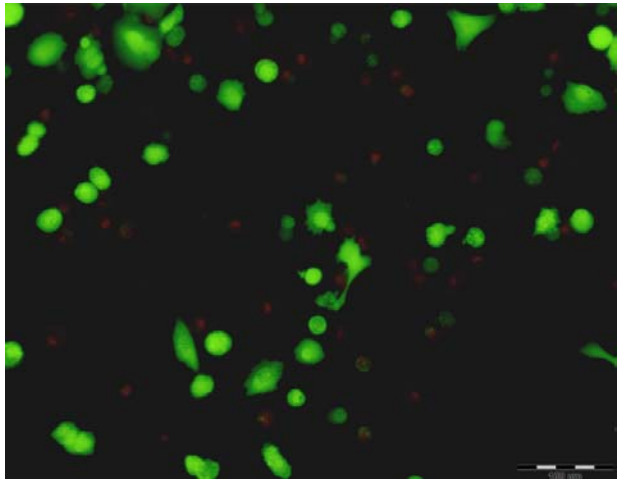
Screening-System
Benchmark-System: Lebend-Tod-Färbung (Calcein-AM/Pi-Färbung)

Kontrolle

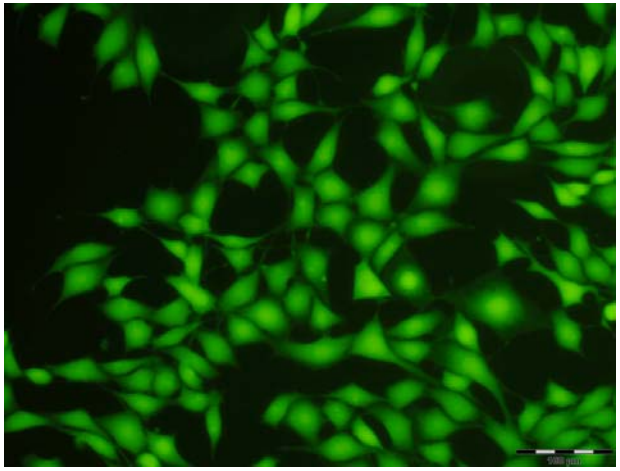
ZnO 0,1 % Ru

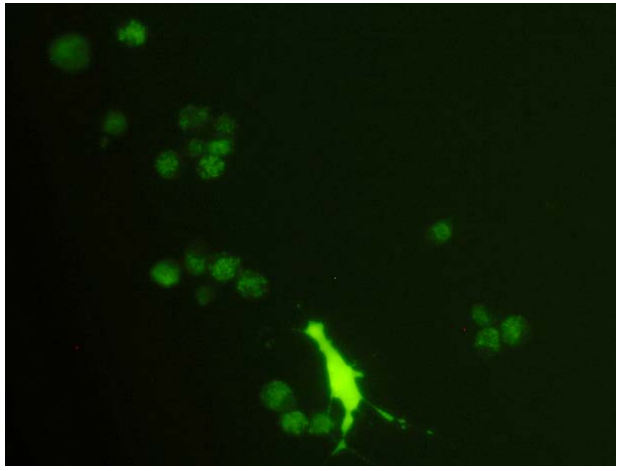
A549-Zellen



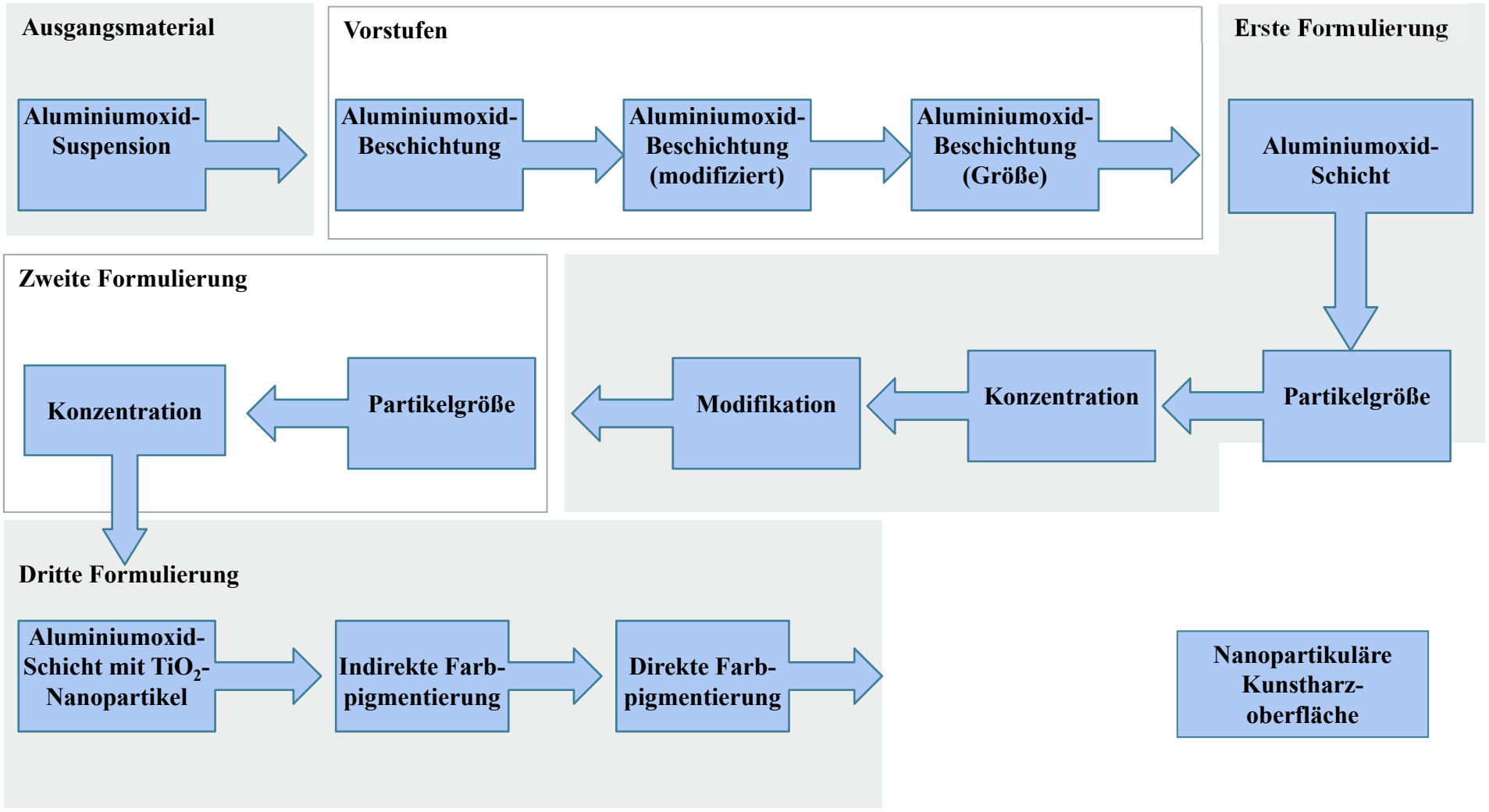


NIH-3T3-Zellen





Prozesskette der nanopartikuläre Beschichtung (Aluminiumoxid –Nanopartikel)

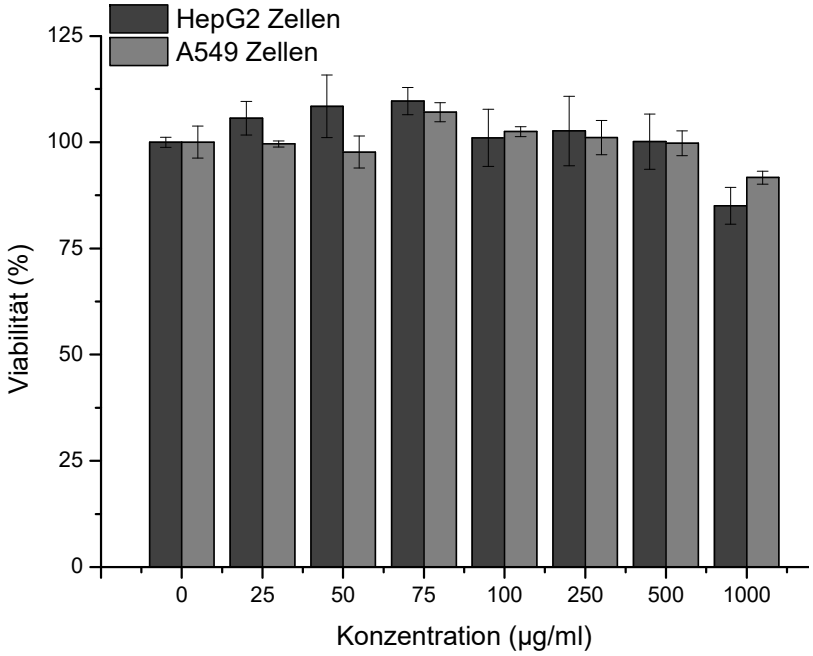


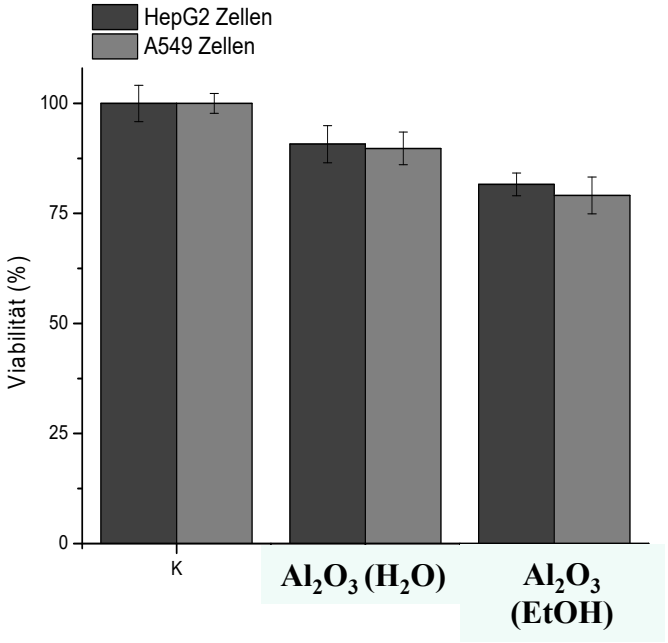
Toxikologische Charakterisierung: Ausgangsmaterials und Vorstufen

Ausgangsmaterial
Aluminiumoxid-Suspension

- **Aluminiumoxid (11 ± 2 nm (REM))**
- **Wässrige Suspension, pH 7**

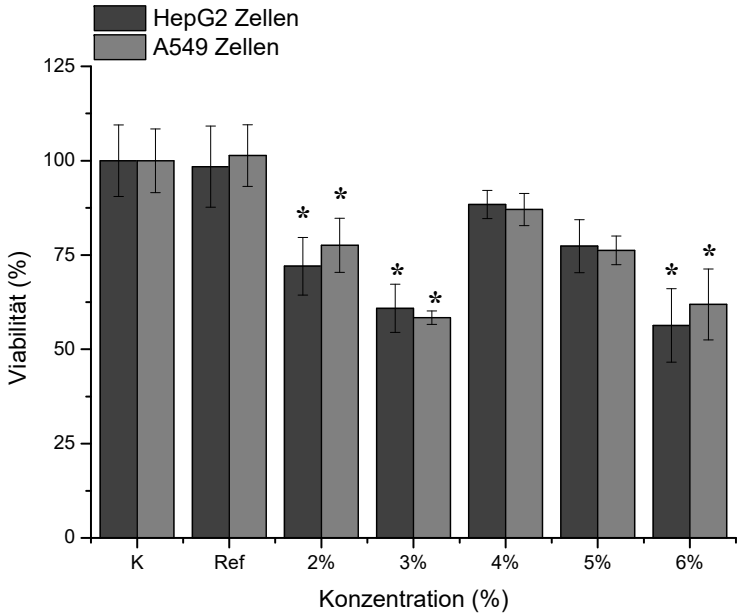
Vorstufe:
Aluminiumoxid-Beschichtung
Suspension: Aluminiumoxid (EtOH): 500 nm (DLS)
Aluminiumoxid (H_2O): 130 nm (DLS)
Extraktionsbedingungen:
72 h, 37 ° C, 5 % CO_2
3 cm²/ml DMEM (10 % FKS)
10 % w/w Beschichtung



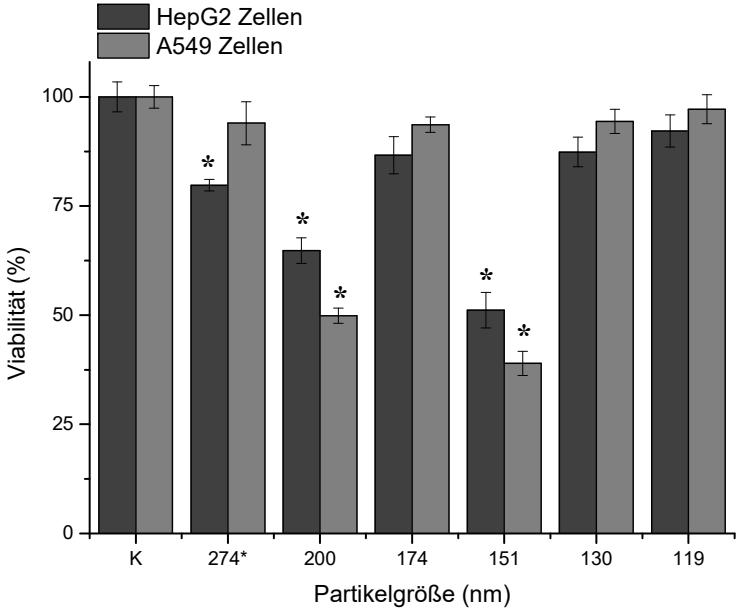


Toxikologische Charakterisierung: Konzentration und Partikelgröße (zweite Formulierung)

Zweite Formulierung
Aluminiumoxid-Schichten
Vergleich von verschiedenen Konzentrationen in der Schicht

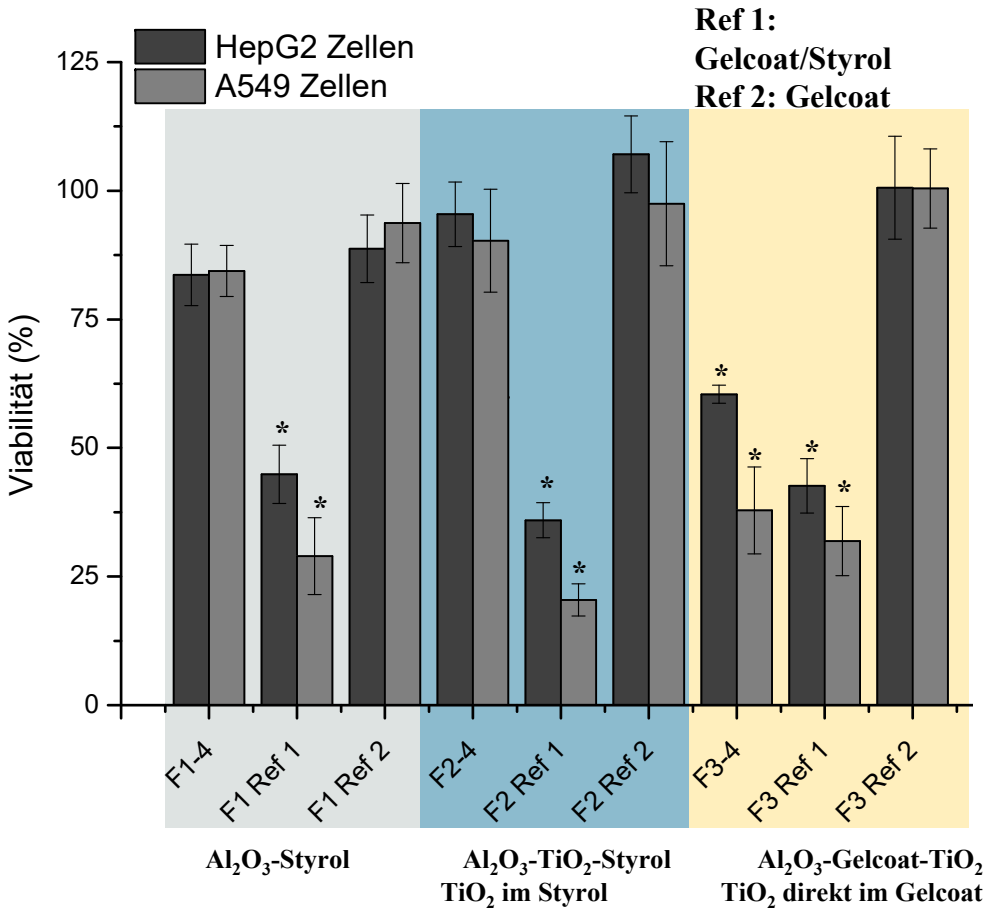


Zweite Formulierung
Aluminiumoxid-Schichten
Vergleich von verschiedenen Partikelgrößen in der Schicht



Aluminiumoxid mit Silan, 4% in der Schicht (Jutta Hesselbach)
Aluminiumoxid, perfluoriert (Alexander Kockmann), 274*

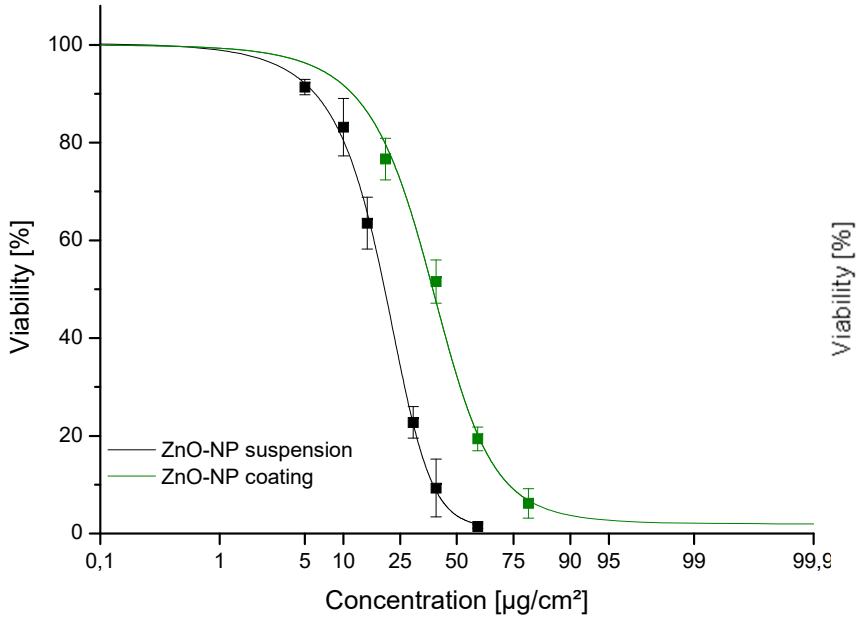
Dritte Formulierung
Aluminiumoxid-Schichten
Vergleich von verschiedenen Farbpigmentierungen



Stage of Work

Coating vs. Suspension: 2D Static Culture (Surface Growth)

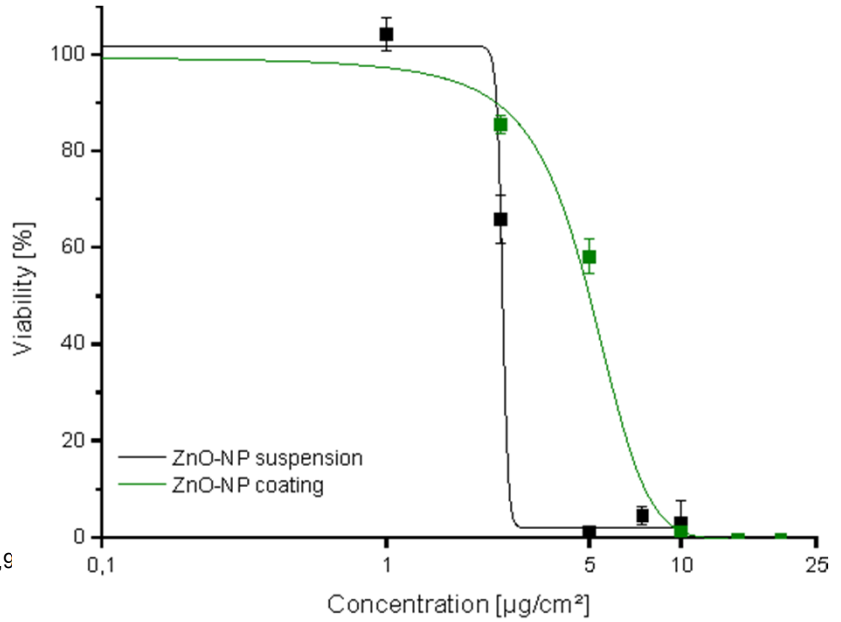
Lung cells (A549 cells)



Nanoparticle: ZnO (0,1 % Ru)

Suspension: IC_{50}= 18 µg/cm²
Coating: IC_{50}= 35 µg/cm²

Skin cells (NIH-3T3 cells

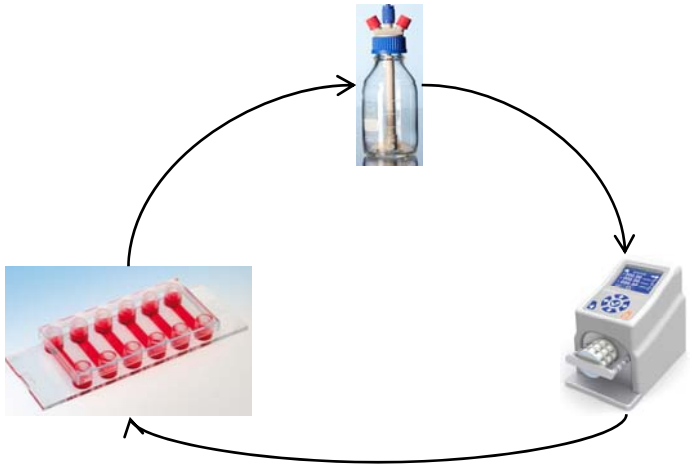


Nanoparticle: ZnO (0,1 % Ru)

Suspension: IC_{50}= 3 µg/cm²
Coating: IC_{50}= 8 µg/cm²

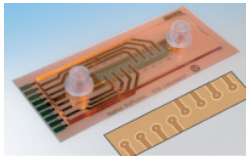
Stage of Work

Suspension: 2D Dynamic Culture



- Dynamic culture condition
- More physiological conditions (comparable with blood stream)
- Well-defined shear stress

- Cell viability
- Cell metabolism
- ATP level
- Cell adhesion (ECIS)

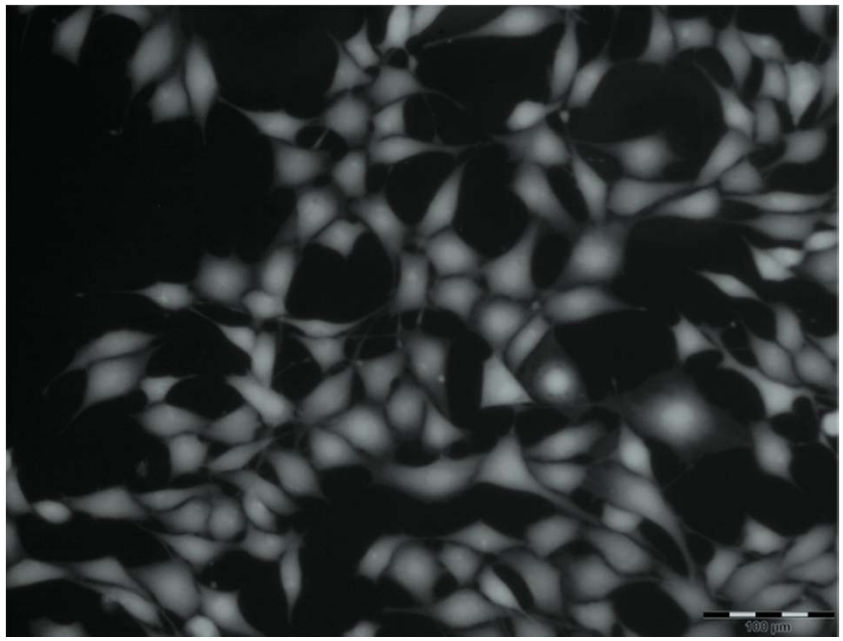


Stage of Work

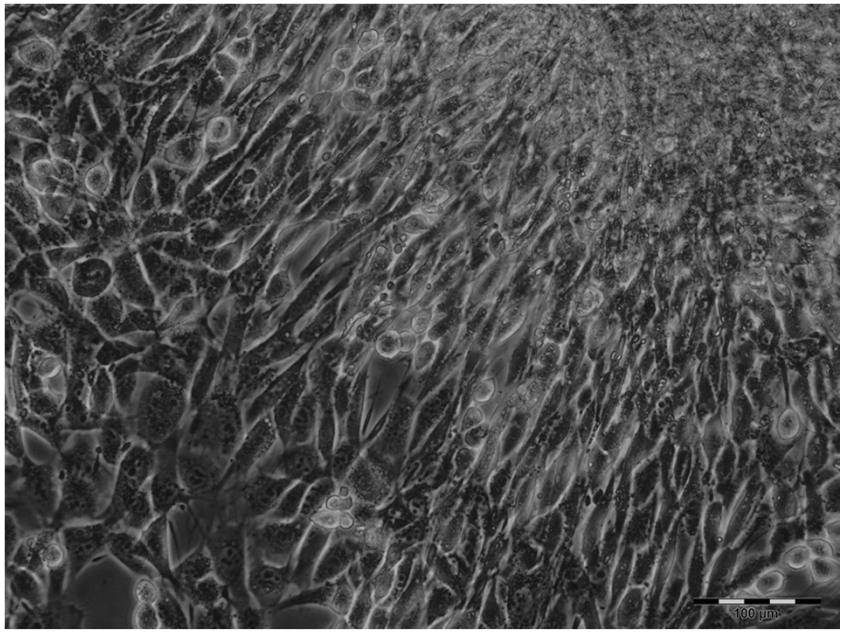
Static vs. Dynamic Culture Condition

Cell Morphologhy of Skin Cells (NIH-3T3 cells)

Static Culture

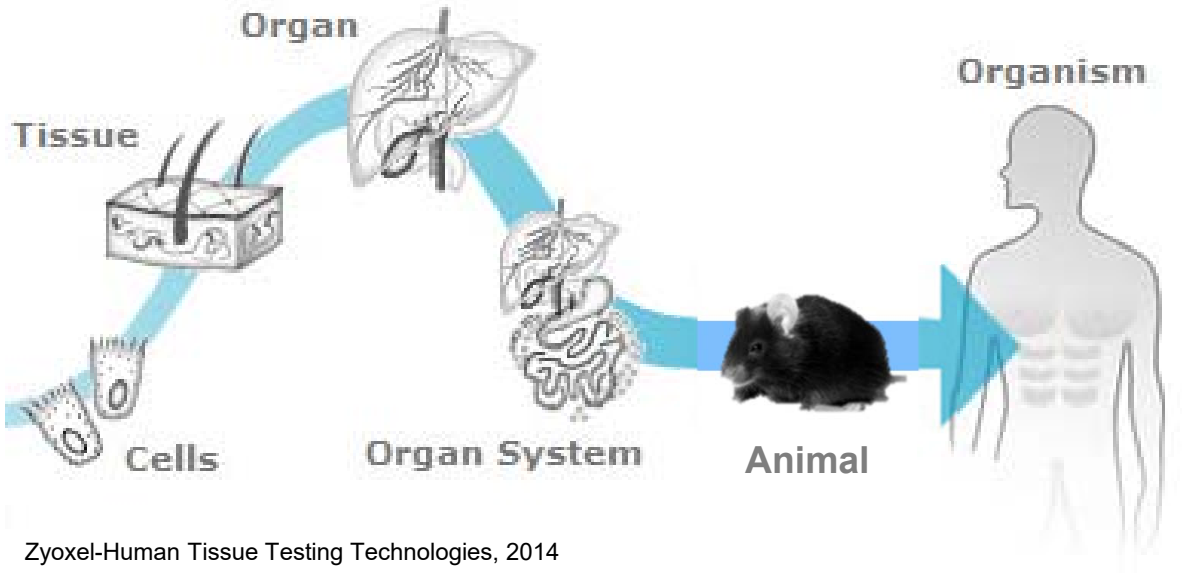


Dynamic Culture



Stage of Work

2D vs. 3D Cell Culture

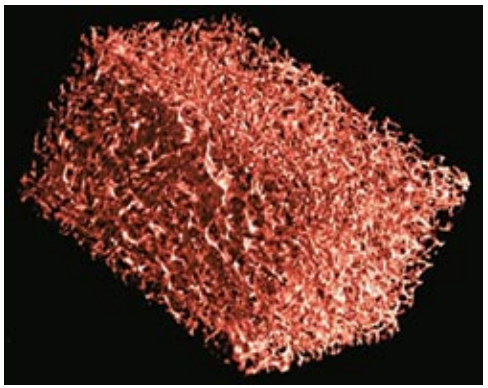


Zyoxel-Human Tissue Testing Technologies, 2014

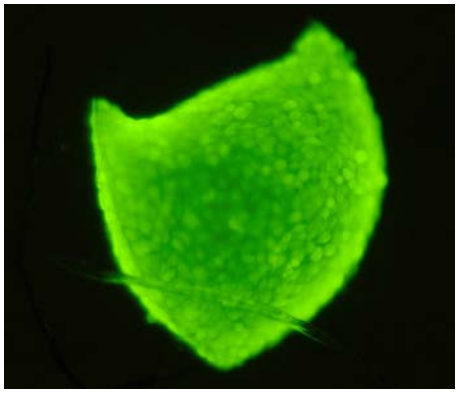
- 2D static cell culture is unrepresentative model
- Cell-cell and cell-matrix interactions
  → significant role in cellular response to drugs and toxins

Stage of Work

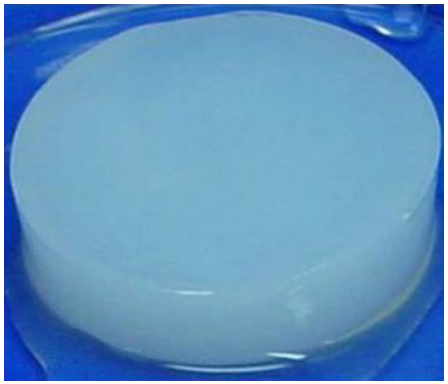
Suspension: 3D Cell Culture Modells



Cell Adhesion on
Scaffolds

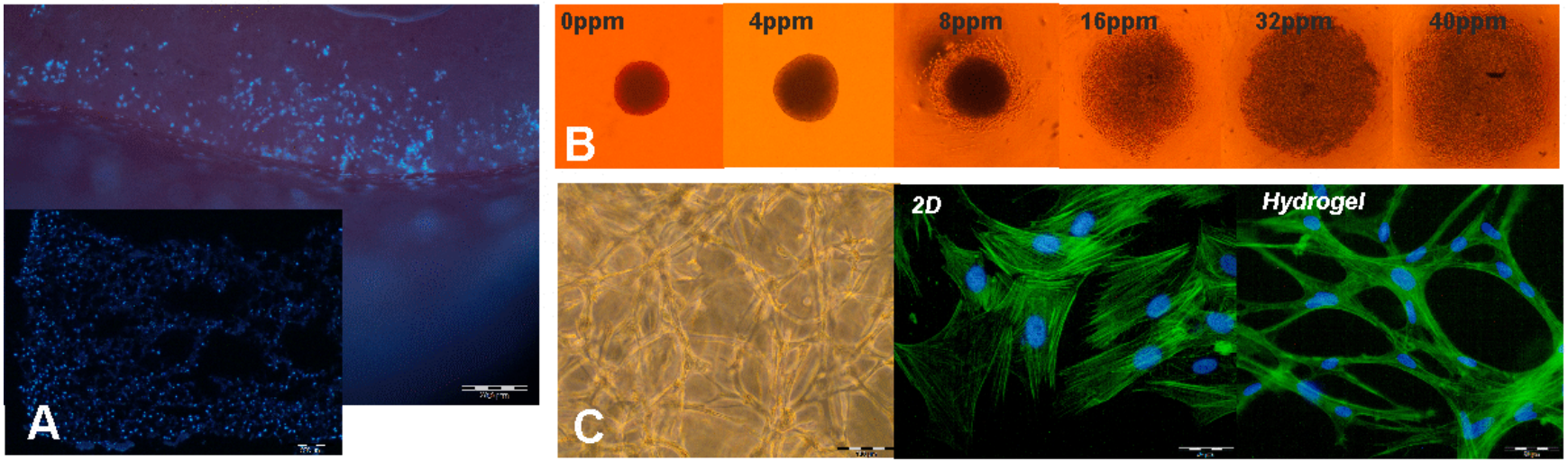


Cell Aggregation,
Spheroid Formation

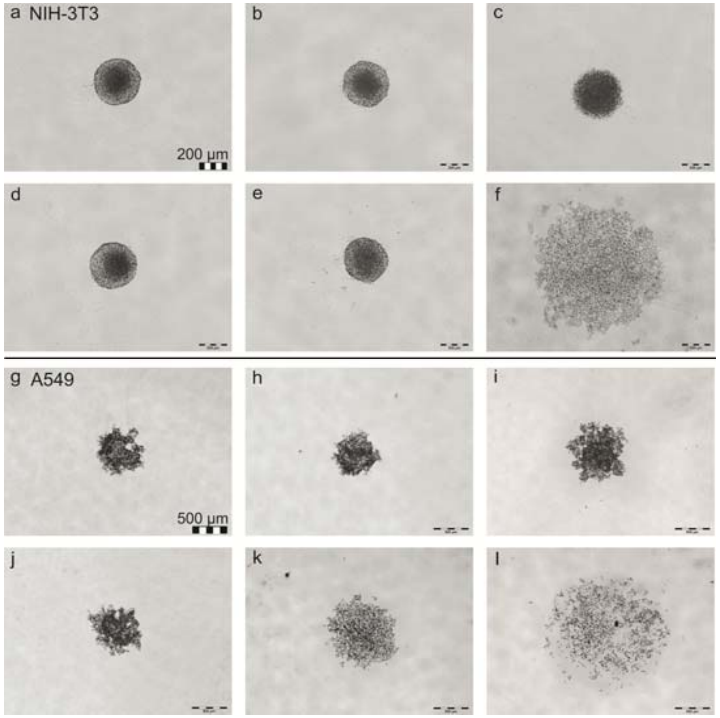


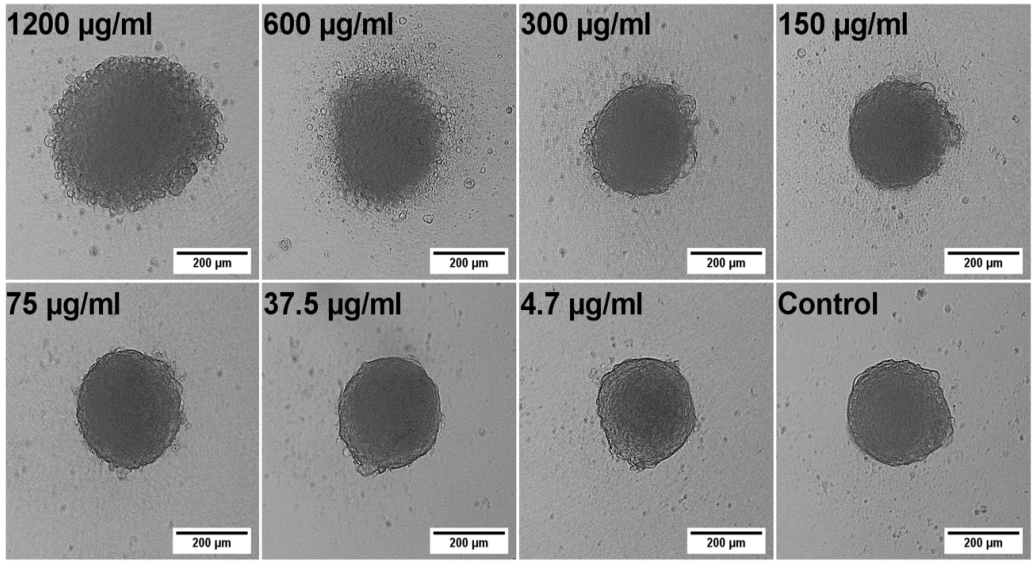
Cell Immobilization in
Hydrogel

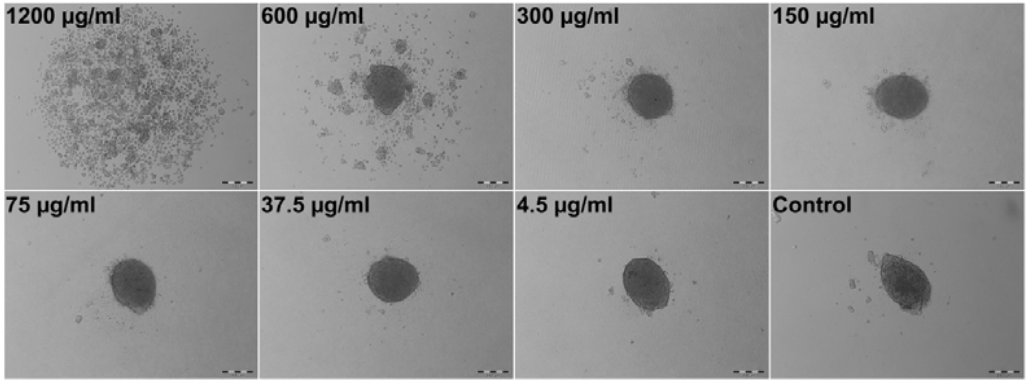
Establishment of 3D Cell Cultures: 3 Models



Establishment of 3D Cell Cultures : Microtissues / Cell Spheroids

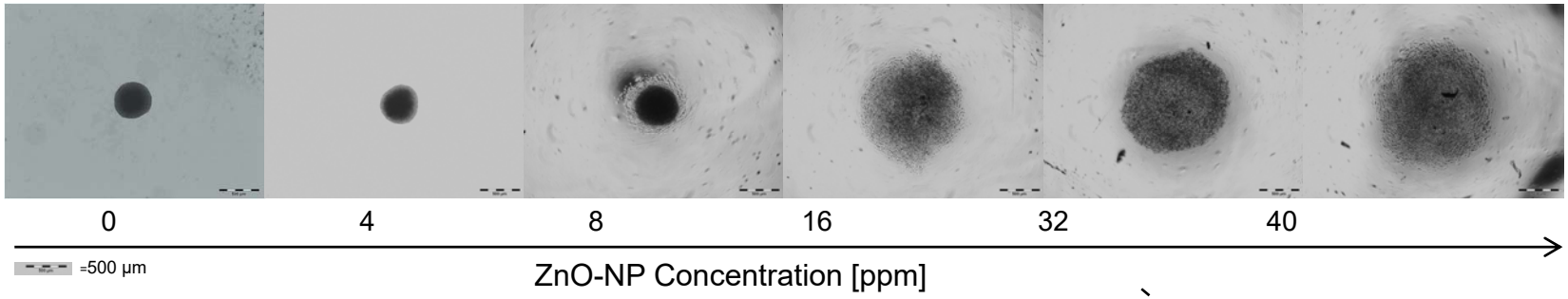


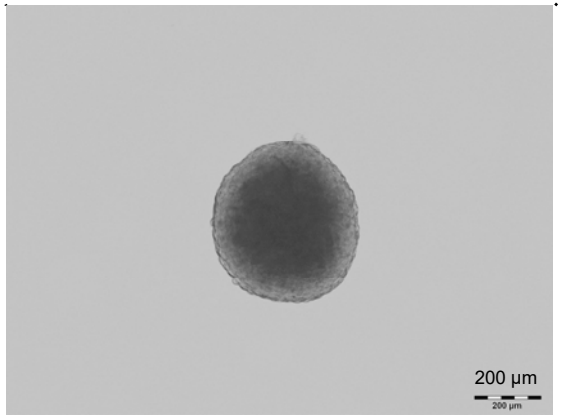




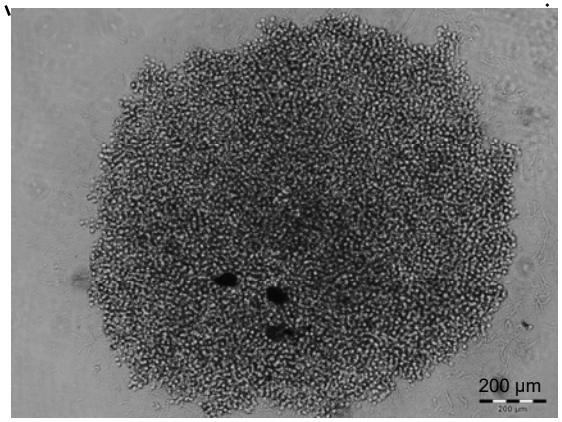
Stage of Work
Suspension: 3D Static (Speroid)

Skin Cells (NIH-3T3 cells) Cell Morphology





0 ppm



16 ppm

Leibniz Universität Hannover

Spheroid Formation of NIH-3T3 Cells

24 hour time lapse video

Institute of Technical Chemistry

Leibniz University Hannover

Franziska Sambale

www.tci.uni-hannover.de

Leibniz Universität Hannover

Spheroid Formation of NIH-3T3 Cells with Titanium Dioxide Nanoparticles

24 hour time lapse video
50 µg/ml titanium dioxide nanoparticles
Nanoparticle addition without equilibration

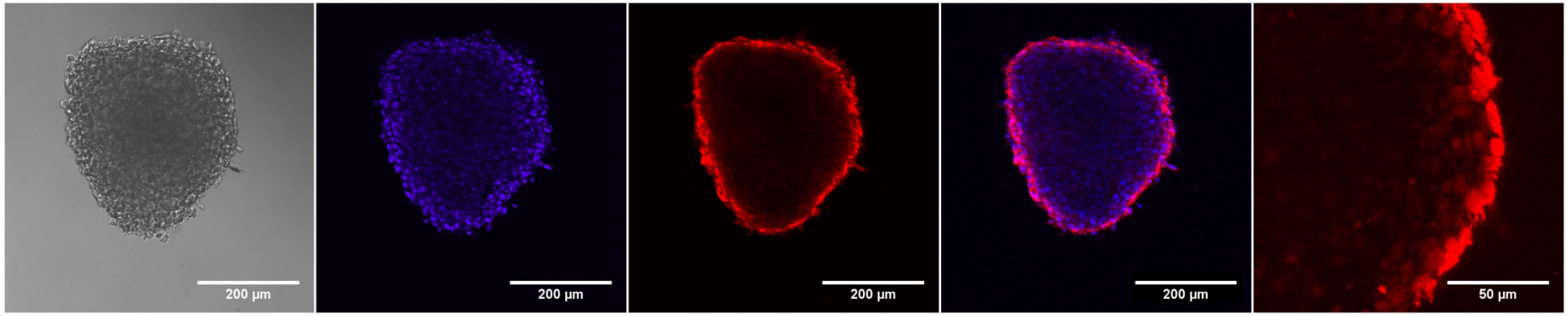
Institute of Technical Chemistry

Leibniz University Hannover

Franziska Sambale

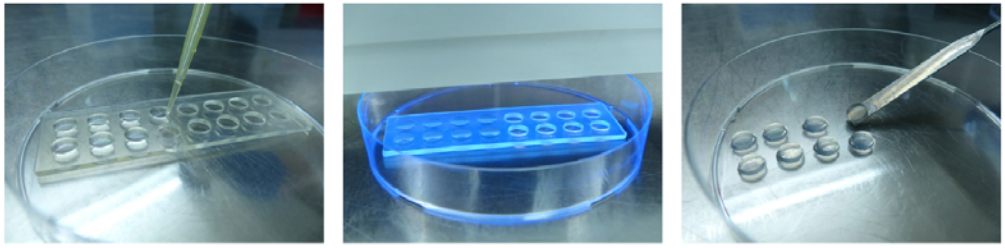
www.tci.uni-hannover.de

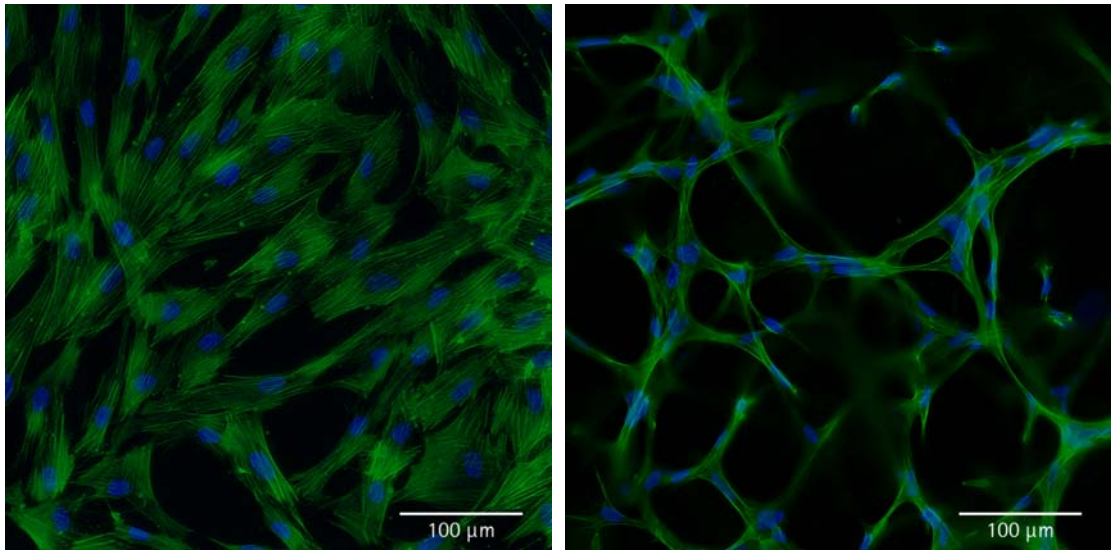
Konfokalmikroskopie, nach 4h Inkubation mit QDots

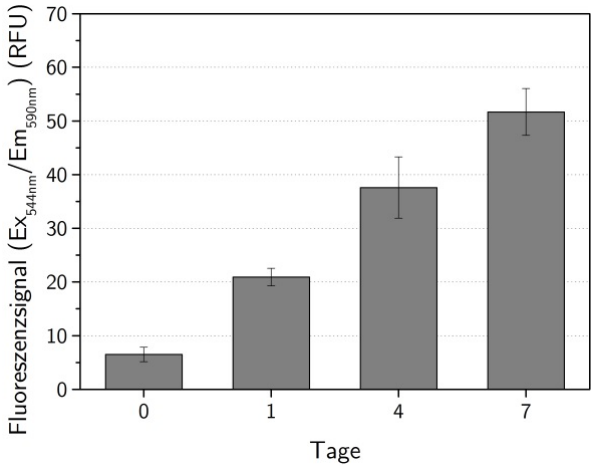


Bright field **DAPI** **QDots** **Qdots+DAPI** **Magnified spheroid**

Establishment of 3D Cell Cultures : Hydrogel-based cell cultures

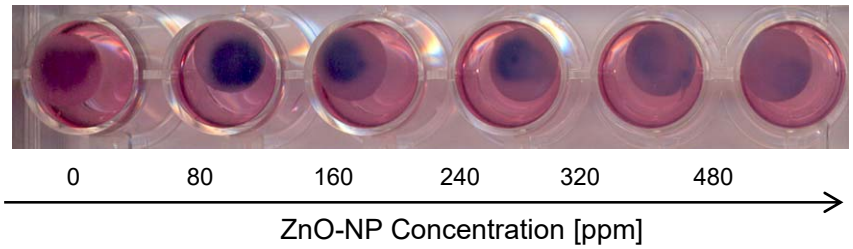


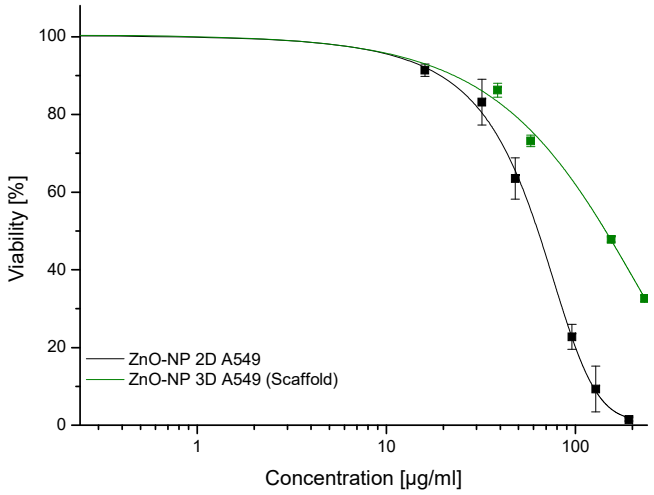


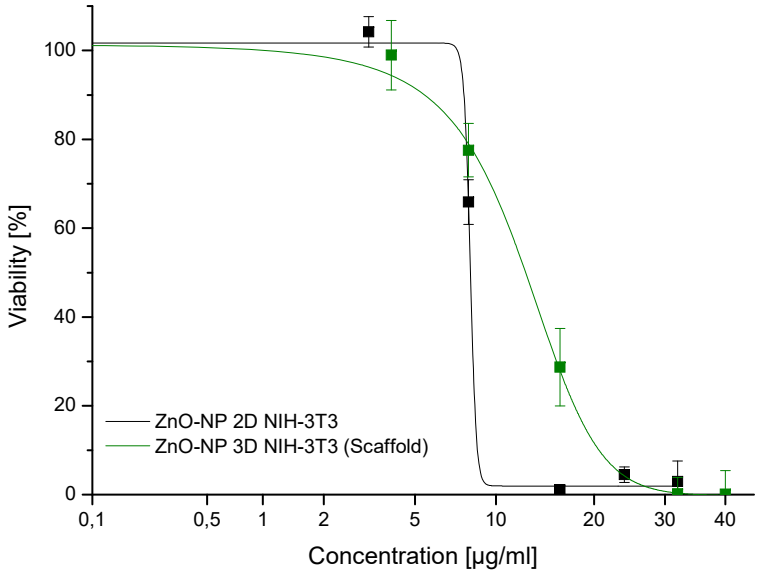



Pluronic F127-Fibrinogen
RTG Properties
TIMER 1
TIMER 2
CLOCK
H
M
S
CLEAR
START
MEMORY
STOP

Stage of Work
Suspension: 3D Static (Scaffold)







Nanoparticle: ZnO (0,1 % Ru)

2D:IC_{50} = 56 µg/ml
3D: IC_{50}= 202 µg/ml

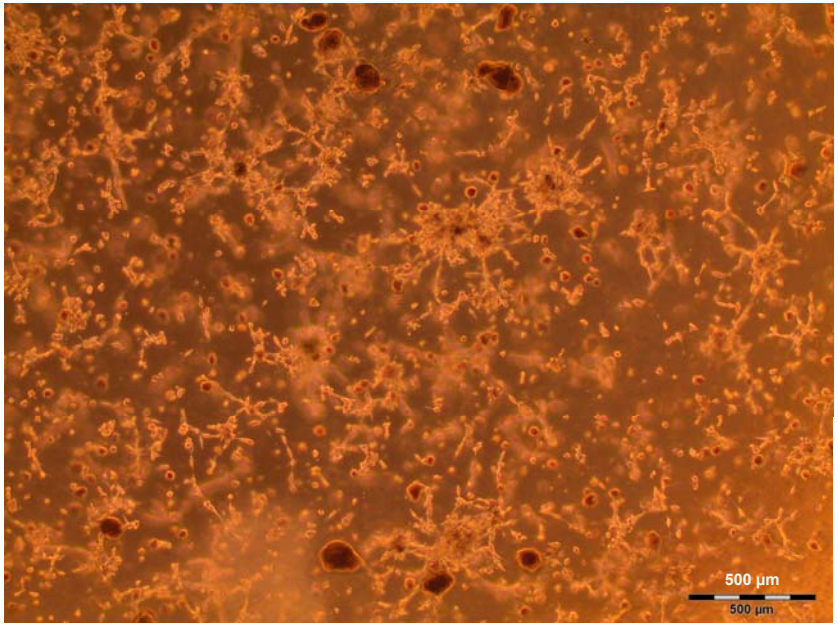
Nanoparticle: ZnO (0,1 % Ru)

2D:IC_{50} = 8 µg/ml
3D: IC_{50}= 12 µg/ml

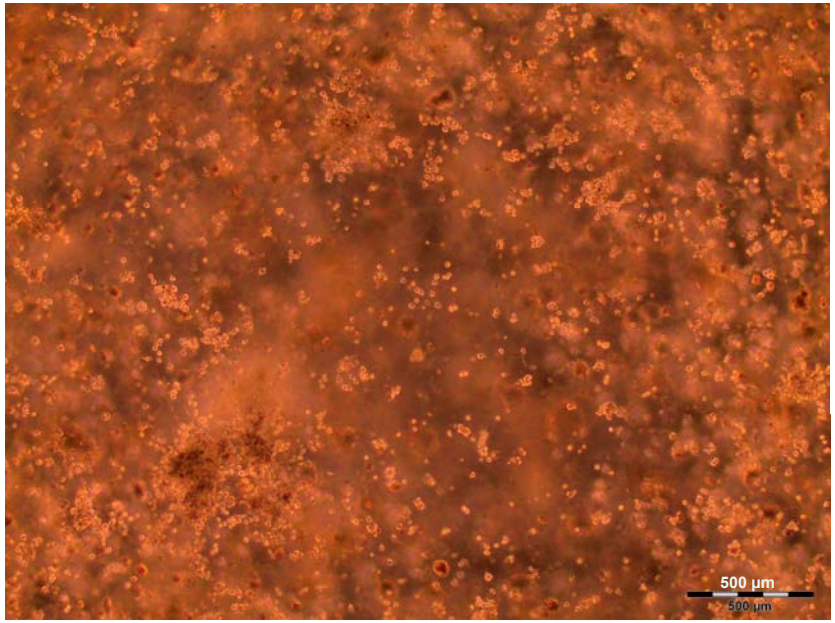
Stage of Work

2D vs. 3D Cell Morphology (Hydrogel)

Skin Cells (NIH-3t3 cells)



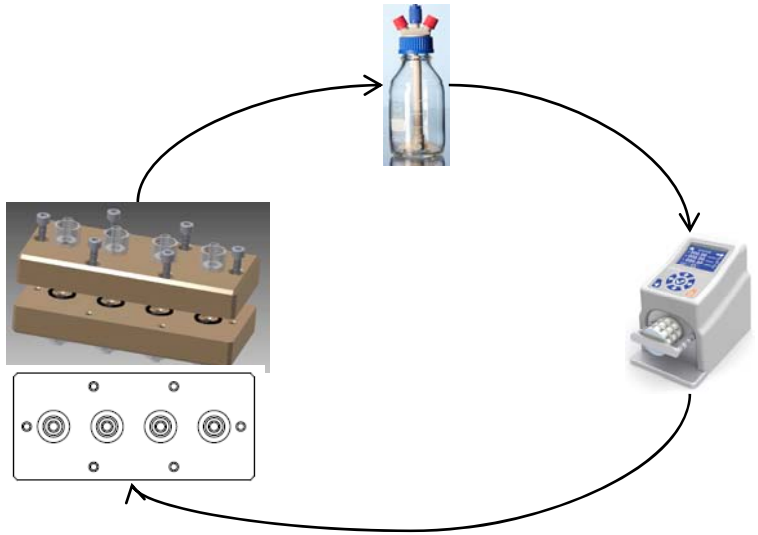
Control



40 ppm ZnO-NP

Stage of Work

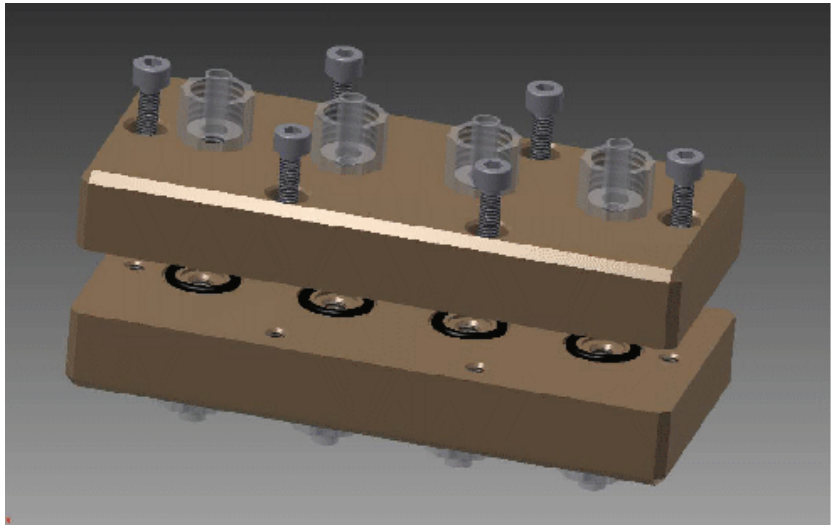
Suspension: 3D Dynamic Culture

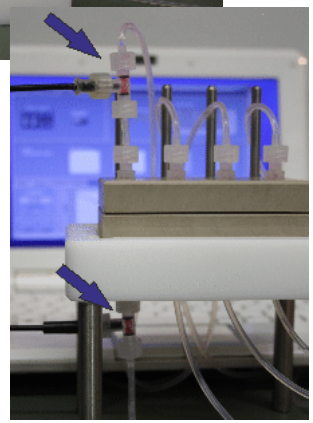


- Dynamic culture conditions
- Well-defined shear stress
- 3D cell growth
- More physiological condition (comparable with blood stream)

- Cell viability
- Cell metabolism
- ATP level

Dynamic 3D Cell Culture





Summary and Outlook

Current *in vitro* nanotoxicity research needs optimization
Nanoparticle coating instead of nanoparticle suspension
2D cells are more sensitive
3D cell culture is a better model (cell-cell interactions)

Further experiments

- Compare different 3D cell models
- Development of dynamic cultivation
- Combine both ideas with a 3D dynamic model

Thank you for your attention.