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Influence of microstructures and coating with amorphous diamond-like carbon on the growth of adherent cell lines

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Project leader: Prof. Dr. Petra Radehaus

In Cooperation with the Laser Application Centre

(Prof. Dr. Steffen Weißmantel)

Contents

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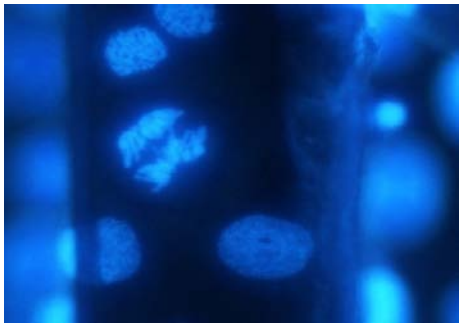
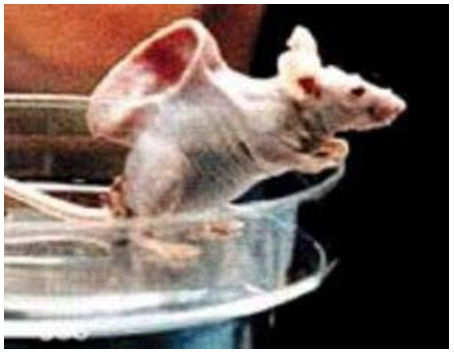
Importance of cell cultures



Epithelial rat cells in division

- Cell as model for basic research
  (cell physiology, growth behaviour, pharmacology, toxicology)



Mouse with ear on back, University of Massachusetts (Photo German Museum)

- Production of medical transplants
  (tissue engineering)

- Testing and optimisation of medical implants, e.g. joint prosthesis, dental implants, stents in blood vessels

Artificial hip joint (Photo Accident Surgery Göttingen)

Optimisation of surfaces – Mechanisms of cell adhesion

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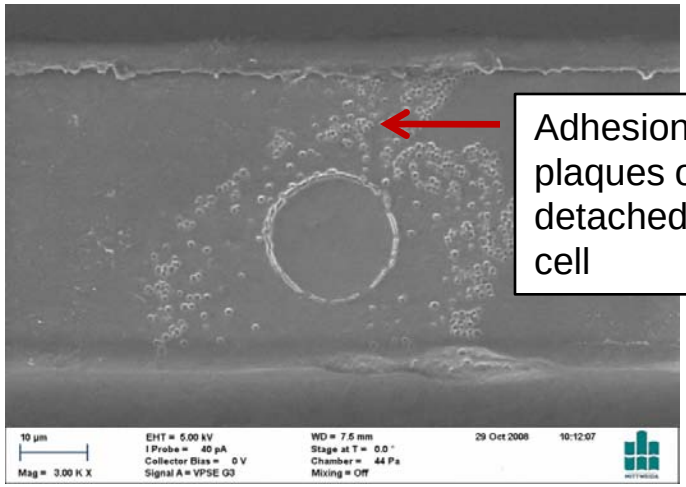
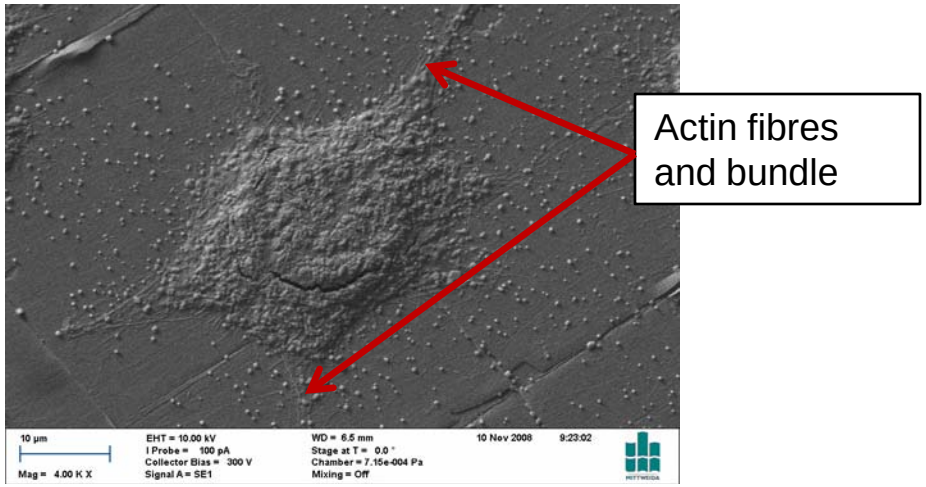
Analysis of the surface-cell-interaction with ***adherent cell lines***

Adherence:

> Cell lines growing as monolayers, which firmly adhere at the surface

> Anchorage with filopods with characteristic actin fibres and adhesion plaques





SEM-photos of adherent cells (L929) on ta-C covered polystyrene, 3000-4000x scanning electron micrograph Carl Zeiss EVO 40 XVP

Optimisation of surfaces – Selection of cell lines

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Interaction cell <> surface can be influenced by
- topography of surface
- chemical composition

Diversification of surfaces possible for
- increase of growth
- inhibition of growth

- **Most properties analogue to human body functions can be found in human primary cells**

- In vitro culture of **human primary cells**

  Disadvantage: often short stability and dedifferentiation

- **permanent tumour cell lines** (e.g. Hela cells)

  Disadvantage: changed cell characteristics

- Spontaneously **immortalised mouse cells**

  = compromise

Optimisation of surfaces – Selection of cell lines

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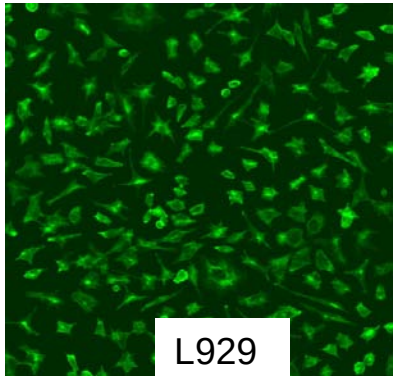
Fibroblasts (cells of connective tissue)

>> different functions, e.g. lesion closure,

>> cells are mobile and migrate into lesions (with filopods)

L929 - cells of connective tissue from subcutaneous connective tissue of mice

NIH3T3 - cells of connective tissue from embryonic mice, contact inhibited

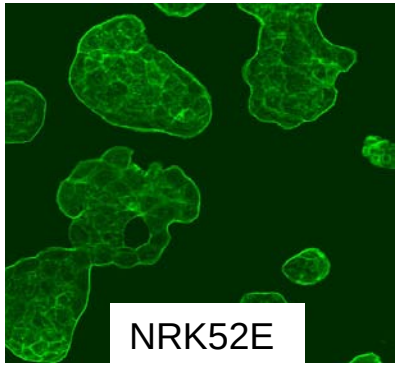

L929

Epithelial cells

>> cover tissue from viscera, cells connected with special structures (tight junctions)

NRK52E – epithel like cells from rat kidney

(all cell lines from the German Collection of Microorganisms and Cell Cultures)


NRK52E

Laboratory equipment

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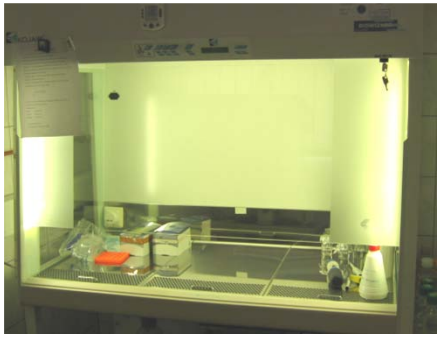
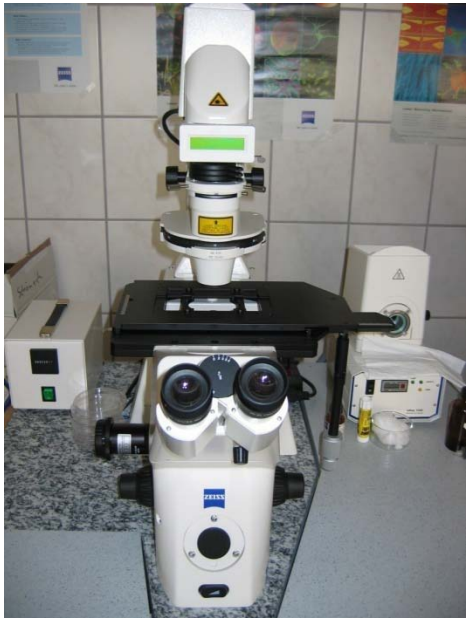


Clean bench

Water bath

Carbon dioxide incubator



Laser scanning microscope

Liquid nitrogen storage

State of knowledge at the beginning of the research project
Goal: improvement of cell adhesion

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Conventional methods:

- Coating of surfaces with gels, proteins etc.
  >> expensive, short stability
- Plasma treatment for formation of functional groups on surface
  >> only suggestive with polymer plastics

Promising methods:

- Surface wrinkling by etching, chemical modification, structuring, covering
  >> change of wettability behaviour and improvement of biocompatibility

Implementation in the research project:

- structuring of plastic surfaces with laser treatment
- Pulsed laser deposition (PLD) of a strong amorphous diamond like carbon layer (ta-C)

Generation of microstructures and coating

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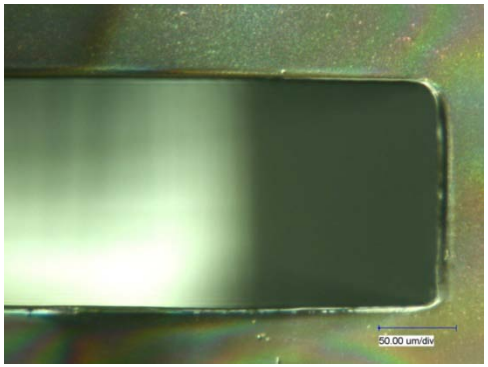
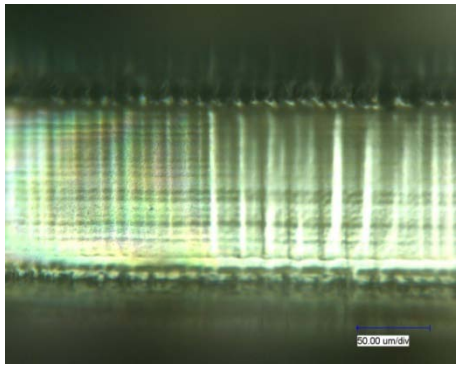
Treatment of polystyrene with eximer laser

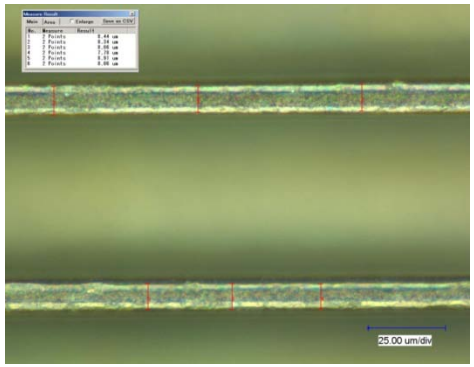
➢Generation of square profiles on 10 x10 mm polystyrene with masks using Femto second laser



Material surface
Digital microscope, 1000x



Bottom



Grid surface

Coating with amorphous carbon layers with PLD plant

Characteristics:

➢High fraction of diamond like bonding up to 85%
➢High hardness up to 65 Gpa
➢Low surface roughness
➢Low friction coefficients

Investigated structure options

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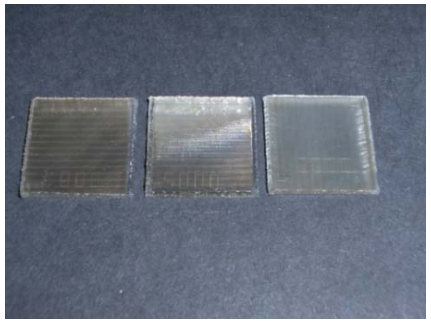
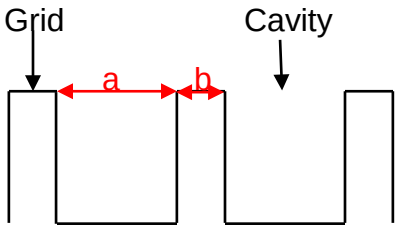
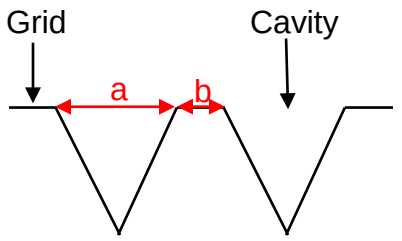
- All samples cut from original cell culture bottle (polystyrene, uncoated, edge length approx. 10 mmx10 mm
- Each version examined 3-fold to 6-fold

Sample	Cavity width	Grid width	Depth	ta-C-layer
control	untreated			
ta-C 1	unstructured			100 nm
3	50 µm	8 µm	50 µm	
6	50 µm	8 µm	50 µm	50 -100 nm
5	40 µm	20 µm	50 µm	
12	50 µm	40 µm	50 µm	
15	50 µm	40 µm	50 µm	50 -100 nm
18	100 µm	100µm	60 µm	







a… Cavity width
b… Grid width

For comparison: Dimension of cells 15-40 µm

Results: Structures

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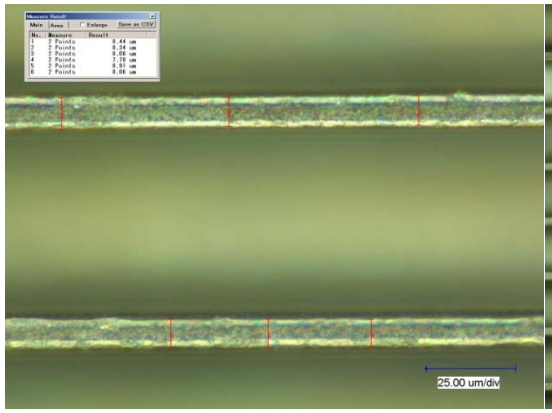
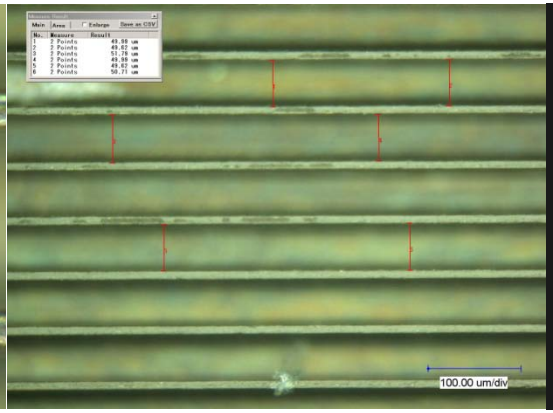
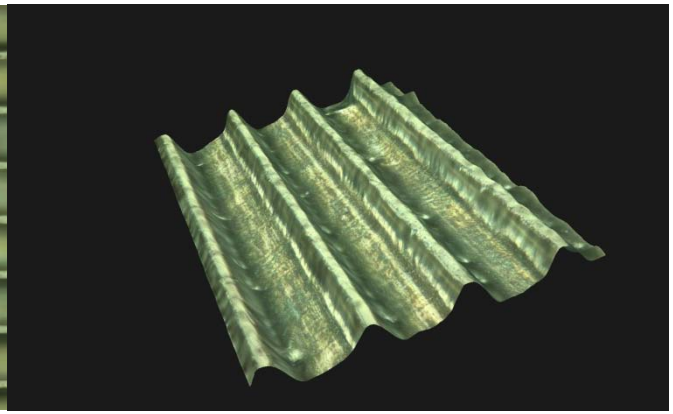
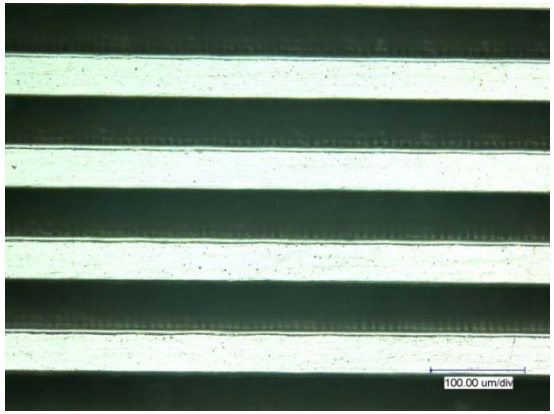
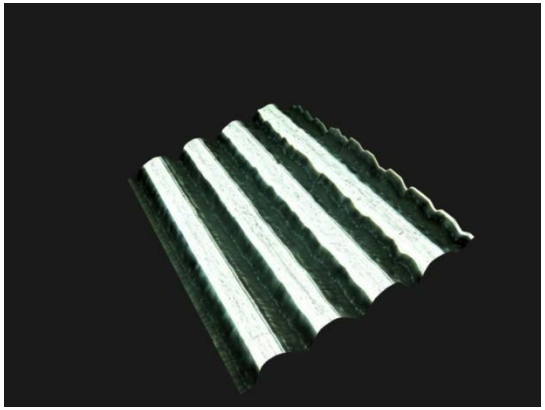




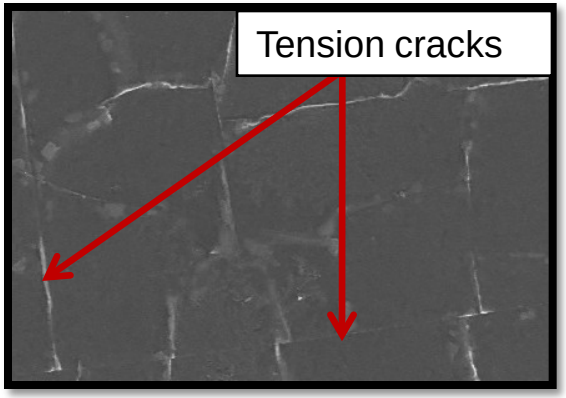


Sample 1: 2000x, Grid width 8 µm, Cavity width 50 µm



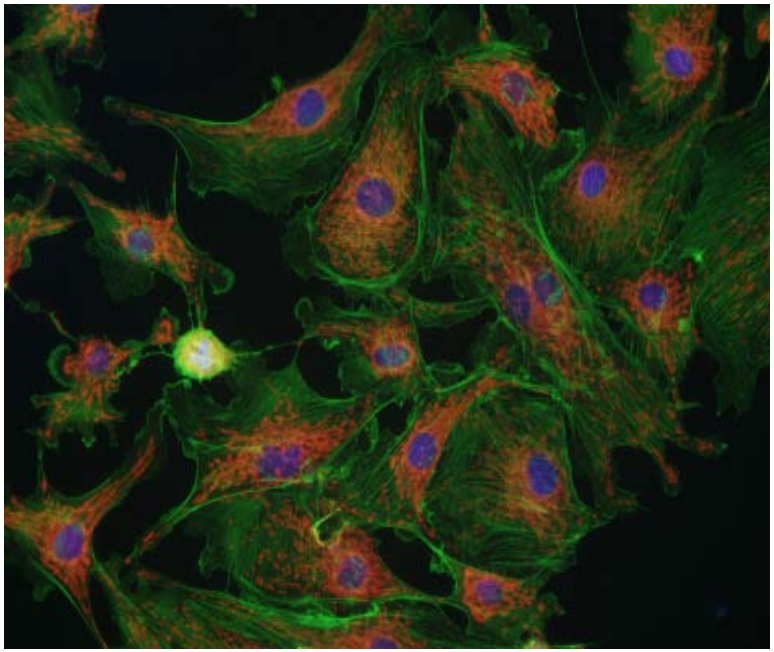


Sample13: 500x, Grid width 40 µm, Cavity width 50 µm

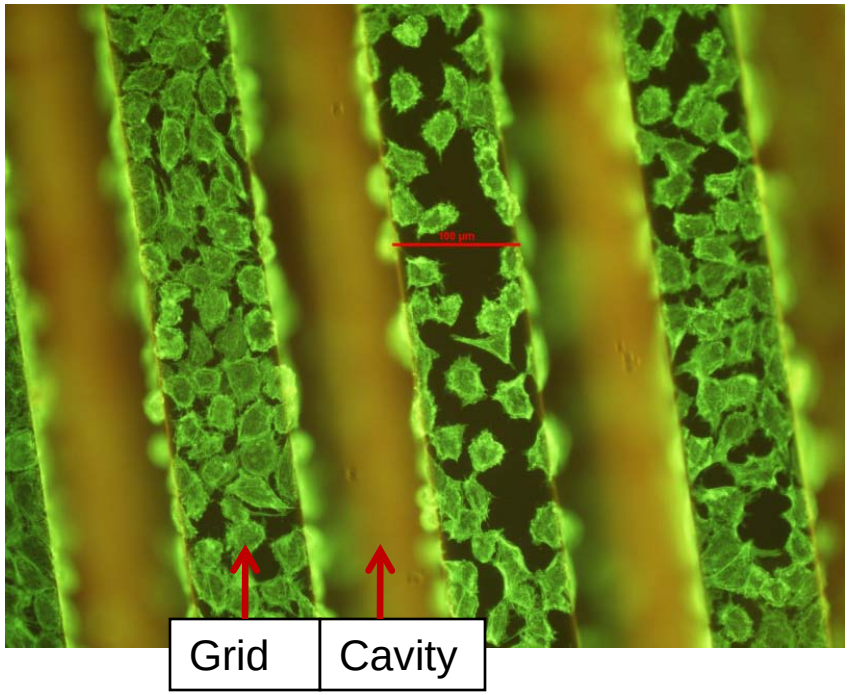


SEM-photo of ta-C-layer on polystyrene, 800x

Results: Visualisation of cells



L929-Cells on untreated polystyrene,
fluorescence staining, in triplicate
400x Zeiss Axiovert M



High cell density of mouse fibroblasts (L929) on laser treated grids (Grid width 100µm, Cavity width 100 µm),
Fluorescence staining Alexafluor, 200x Zeiss Axiovert M

Vitality Assay/ Quantification of cells

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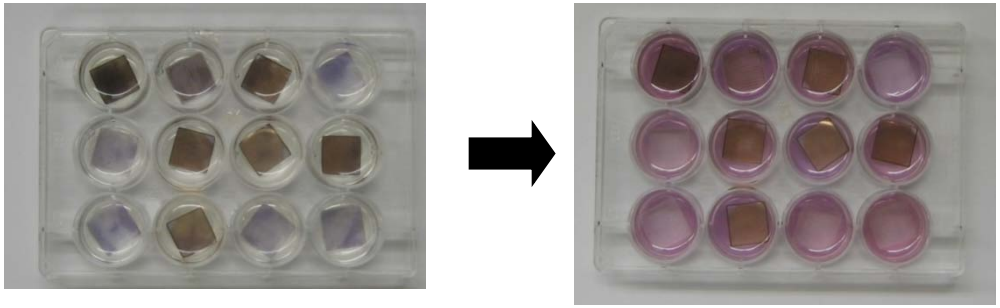
MTT-Assay:

Stain: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide = MTT (yellow)

- in viable cells the stain transforms to blue formazan
- Measuring of extinction with photometer at 570 nm

>> Quantity of accumulated formazan is directly proportional to the number of active cells



Left - blue Formazan enclosed in cells

Right – after addition of solvent Formazan discharged in solution (photometric measurable)

Procedure:

- Sterilisation of samples with H_2O_2 and ethanol
- Inoculation of samples with cells and incubation for 24-72h
- Incubation with MTT-stain for 2h
- Extraction and quantification of formazan in solution

Results:
Cell growth on modified surfaces

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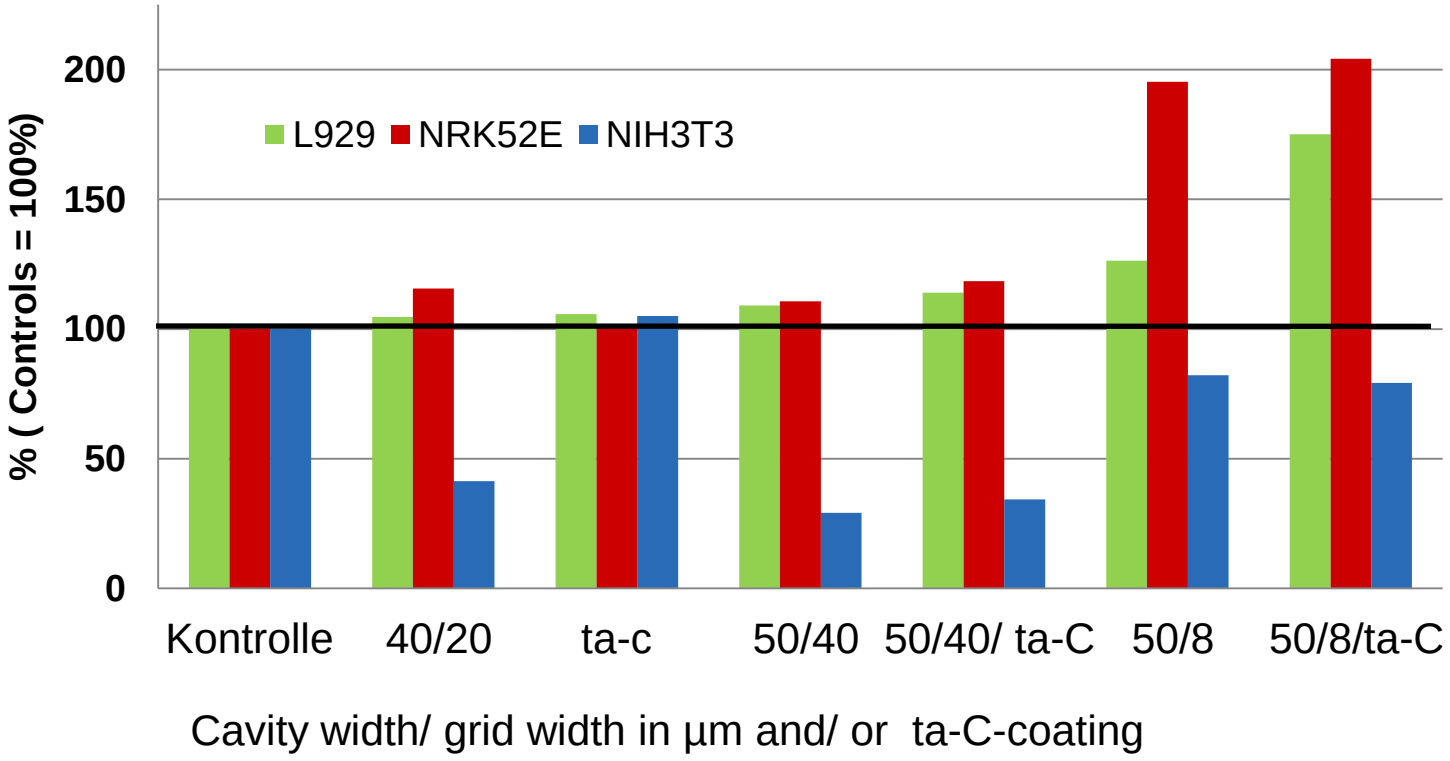
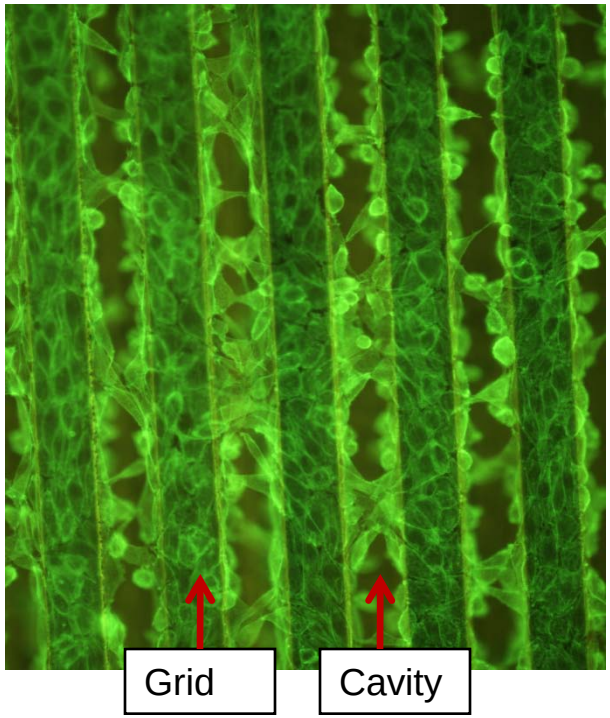
MTT-Test : Active cells after 48 h growth – Mouse – and rat cells on laser modified structures in relation to untreated polystyrene controls



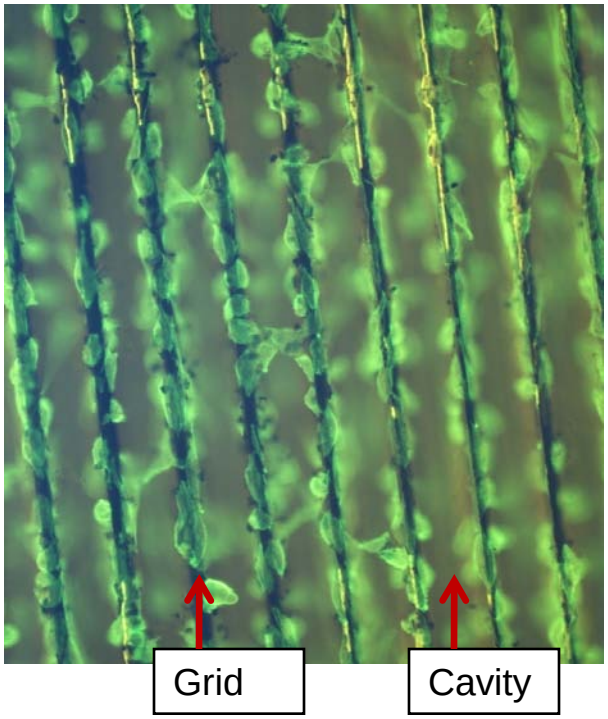
Results: Visualisation of cells



High degree of cross linking of the L929 - cells (fibroblasts)

grid width in cell dimension (20-40 µm)
on laser created structures
(grid width **50 µm**, cavity width **40 µm**)

Fluorescence staining Alexafluor, 200x Zeiss Axiovert M

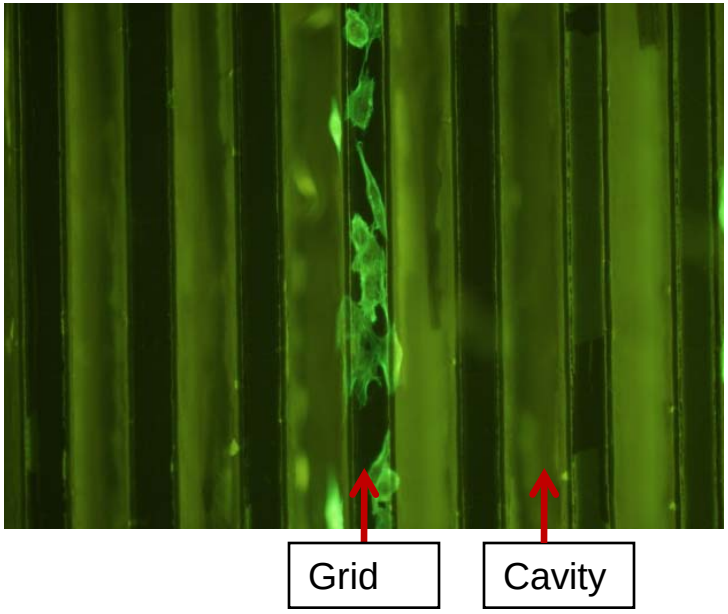


L929-cells

Overgrowth on narrow grids,
laser created structures
(grid width **8 µm**, cavity width **50 µm**)

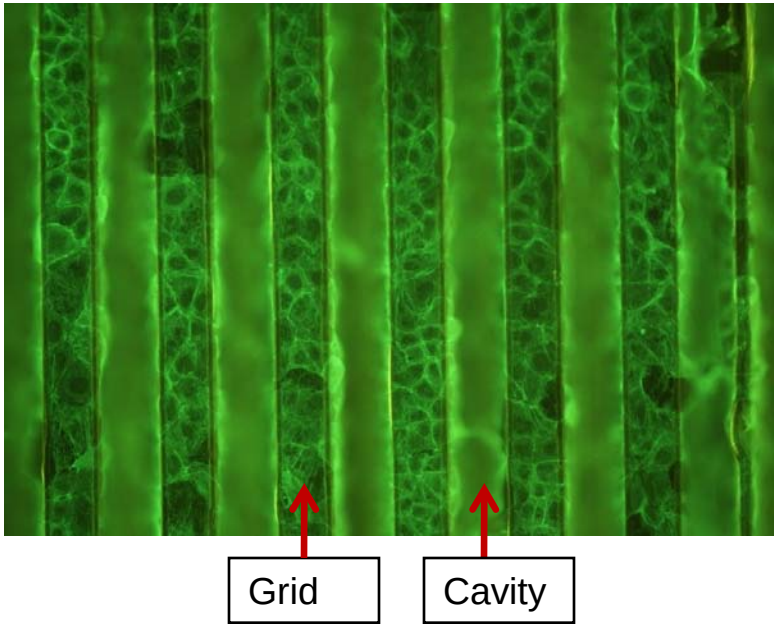
Fluorescence staining Alexafluor, 200x Zeiss Axiovert M

Results: Visualisation of cells



NIH3T3 cells
(Fibroblasts, contact inhibited)
grid width in cell dimension (20-40 µm)
on laser created structures
(grid width **50 µm**, cavity width **40 µm**)

Fluorescence staining Alexafluor, 200x Zeiss Axiovert M



NRK52E cells
(Epithelial cells)
grid width in cell dimension (20-40 µm)
on laser created structures
(grid width **50 µm**, cavity width **40 µm**)

Fluorescence staining Alexafluor, 200x Zeiss Axiovert M

Conclusions and Summary

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Investigation of growth behaviour of animal cells on structured and covered plastic surfaces:

- Strong amorphous diamond like carbon layers (ta-C) are biocompatible and inert.
- Laser structured surfaces are biocompatible.
- Highest growth density and adhesion of cells (by two from three tested cell lines) were detected by combination of:
  - structuring
  - covering with ta-C
  - very narrow grids
- Strong alignment and cross linking of cells with grids in cell dimension (20-40 µm)

Perspectives

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- Application of combined structuring and coating with ta-C on surgical implants possible

  Goal: rapid and better covering of materials with the body´s own cells and better integration of implants in tissues

- Application on other materials like glass and metals

- Decrease of cell growth after surface coating may be relevant for special cell lines (e.g. at implants for blood vessels)

- Doping the coating layer with biocide atoms could be important for implants or sensors, where cell covering is undesirable)

Continuation of research project in the ESF- funded young academics research group

Pulsed laser deposition of layers and laser structuring of solid body materials ´LAMIS´

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Thanks for listening!