

Introduction and aim

Cells of complex multicellular organisms respond to biochemical and physical stimuli of their immediate surrounding (1). Microstructured surface topographies influence the cell's behavior (contact guidance) (2).

The aim of this project is to build up a migration assay on microstructured elastomer substrates with different cell lines with or without NF1-gene defect (Neurofibromatosis type 1) with a focus on comparing the cell lines with respect to changes in their motility and their morphology in reaction to the groove microstructures.

Cell lines: HP 176 (control), HP 186 (+/-), HP 187B (-/-)
Groove width: 8 μm (structure 6), 10 μm (structure 7)
Distance between the grooves: 5 μm

Pilot-Tests

→ Analysis of the wettability of PDMS-substrates by measuring the contact angle

- Untreated PDMS
- Plasma-treated PDMS
- Plasma- + fibronectin-treated PDMS

Contact angle:

< 90° = wettable (hydrophilic)

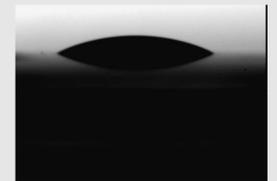
> 90° = not wettable (hydrophobic) (3)



Untreated PDMS; 114,8°



Plasma-treated PDMS; 7,1°



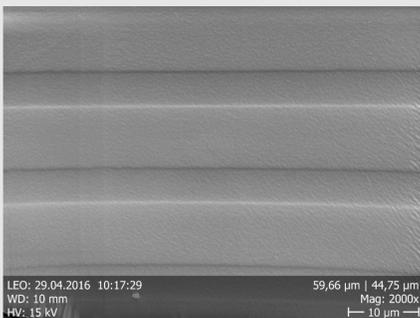
Plasma- + fibronectin-treated PDMS; 23,4°

→ For further experiments plasma-treated PDMS were used

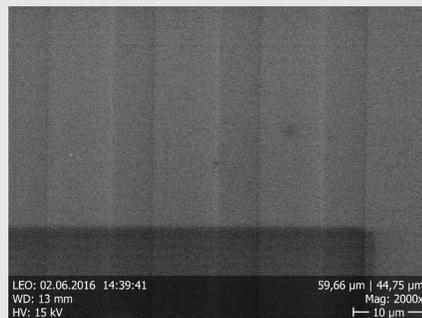
Methods & Results

REM-image of structured PDMS-substrates

Three-time sputtering process and subsequent REM analysis



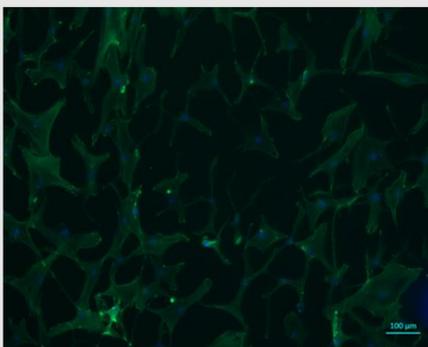
REM image of PDMS-substrate structure 6



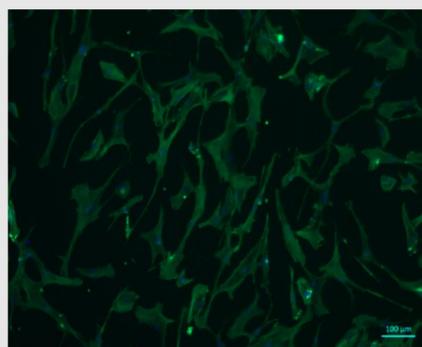
REM image of PDMS-substrate structure 7

Fluorescence image of two different cell lines

- Fixing the cells by Triton-X-solution
- Staining by Nunc Blue and Actin Green



Cell line HP 186 on PDMS-substrate (structure 7)



Cell line HP 176 on PDMS-substrate (structure 7)

→ Slight difference in the cell morphology

Live cell microscopy

- HP 176, HP 186 and HP 187B on structures 6 and 7
- Acquiring one image per hour

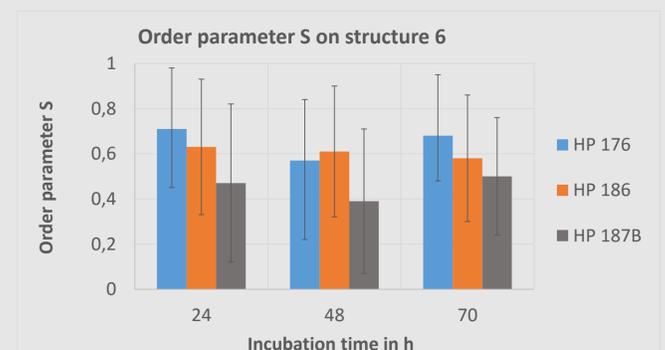
→ Orientation angle along the groove microstructures

Order parameter S:

S = 1: perfect orientation

S = 0: random orientation

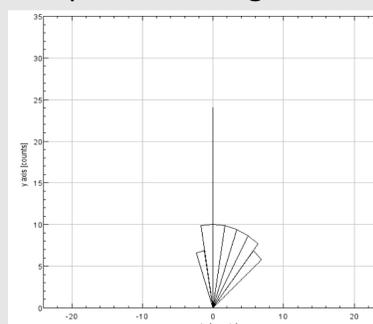
(1)



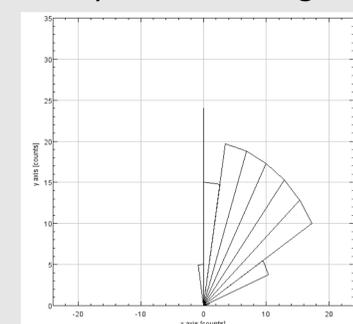
Order parameter S of HP 176 (+/+), HP 186 (+/-) and HP 187B (-/-) on structure 6

→ Cell tracking over a 70 hour period

Rose plots: cell migration in x- and y-direction on groove structures



Cell line HP 176 (+/+) on structure 6



Cell line HP 187B (-/-) on structure 6

→ Differences in orientation and migration flows

Conclusion

- Cell line HP 176 without gene-defect is more orientated toward the grooves than the ones with gene defect
- Rose plots confirm these findings with the healthy cells showing more migration flows towards the y-axis
- Fluorescence images show a slight difference in the cell morphology which supports our suggestion of an influence of the NF1-gene defect on the structure of the actin cytoskeleton and the whole cell morphology

→ Tendency of the cells with gene-defect being different in behaviour and morphology in contrast to healthy ones

Literature:

1. Kaufmann D, Hoesch J, Su Y, Deeg L, Mellert K, Spatz JP, u. a. Partial Blindness to Submicron Topography in NF1 Haploinsufficient Cultured Fibroblasts Indicates a New Function of Neurofibromin in Regulation of Mechanosensory. Mol Syndromol. October 2012;3(4):169–79.
2. Mitchel JA, Hoffman-Kim D. Cellular scale anisotropic topography guides Schwann cell motility. PloS One. 2011;6(9):e24316.
3. Krüss.de