Kinetic Studies of Apoptosis on Single Cell Arrays in High-Througput

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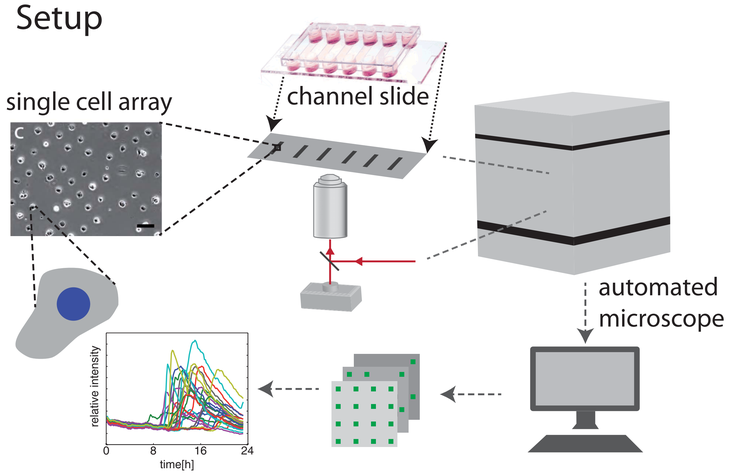
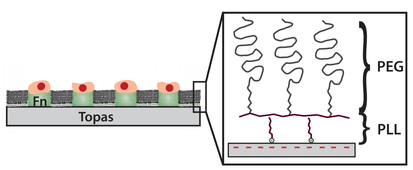
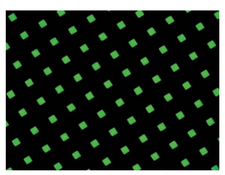
Dynamics of molecular processes in living cells appears to be heterogeneous at the single-cell level. Hence time-lapse microscopy becomes increasingly important as it allows measurement of e.g. cell fate decisions and cellular responses in general. Nanoparticles show cytotoxicity in many circumstances. Yet the signaling pathway is not known. In order to resolve the pathway we developed a high-throughput single cell platform that allows to follow the time course of several markers in thousand of cells in parallel. Events like lysosomal break, loss of mitochondrial outer membrane permeabilization, increase of ROS level, exposure of phosphatidylserine to the outer membrane and loss of membrane integrity can be monitored simultaneously.

Setup

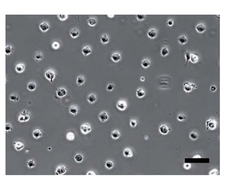


Toxicity of various NP is measured on an microstructured single cell array. 6 different conditions can be measured on an 6 channel slide. Cell are monitored via a auotmated microscope uo to 72 hours. The signal of eache cells can be read out in automated way via image analysis on the computer.

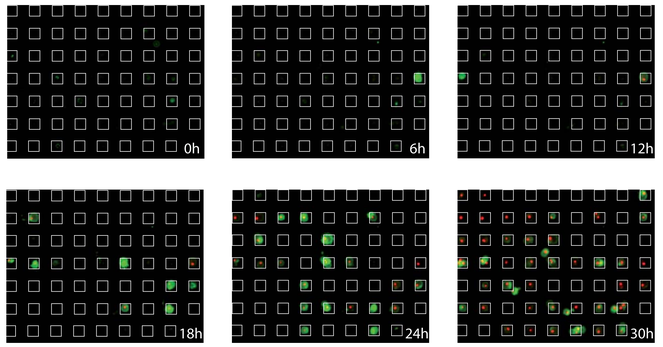




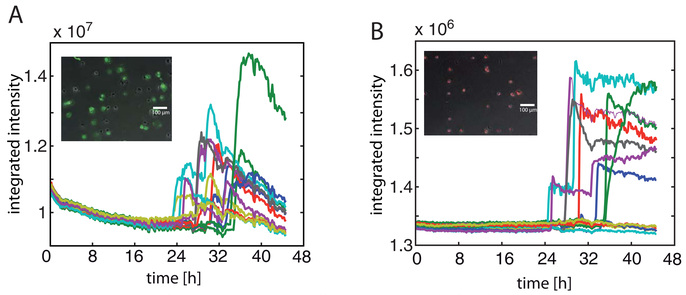
A micro-structured surface is created by plasma-induced patterning with a backfill on a topas surface. Plasma-treated areas are incubated with PLL(20k)-g(3.5)-PEG(2k).PEG-free areas are coated with fibronectin (FN, green). After seeding, cells arrange themselves onto the protein-coated islands, obviating the need for any washing steps. Scale bars: 100 µm [1].



Apoptosis Detection on Single Cell Arrays

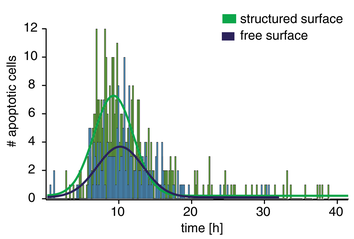


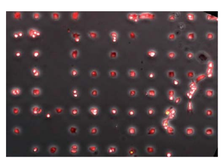
During early Apoptosis, Phosphatidylserine (PS) is exposed to the outer membrane which can be detected with a polarity Sensitive Indicator of Viability & apoptosis (pSIVA). Distinguishing from late apoptosis or necrosis, a second, nucelus staining marker is used (loss of membrane intergrity).



Single time traces of apoptotic HuH7 cells over a period of 47 h (induced by 2 µM staurosporine). pSIVA-IANBD (annexin XII derivate) detects phosphatidylserine exposure (A) and propidium iodide detects loss of membrane integrity (B) [2]

Comparison: free surface vs. structured surface



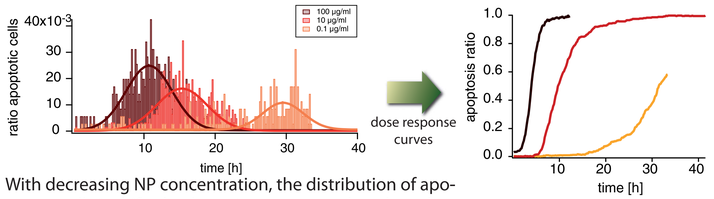


Cell viability after 96h. Reduced C12-resazurin (red) corresponds to healthy metabolism.

Similar apoptotic behaviour on free and microstructured surfaces

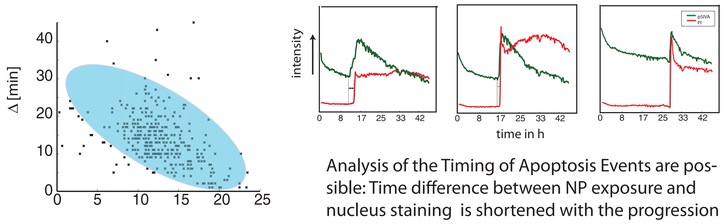
Toxicity of Nanoparticles

Amid funcrionalized Polystyrene Nanoparticles (NP) are used as a first positive control for cell death

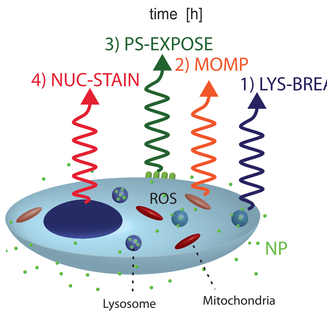


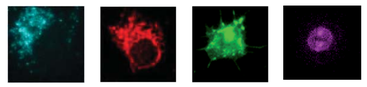
With decreasing NP concentration, the distribution of apoptotic cells is shifted to later time points and get broader.

Correlation btw. time and apoptosis events



Analysis of the Timing of Apoptosis Events are possible: Time difference between NP exposure and nucleus staining is shortened with the progression of the experiment





Lysosomes (LysoTracker), Mitochondria (TMRM), Membrane change (pSIVA-IANBD), ROS level increase (CFCDA) and nucleus staining (PI, Sytox Blue, Toto3 iodide) can be monitored at the same time. For example, testing if PS-NH2 triggers apoptosis via break up of lysosomes

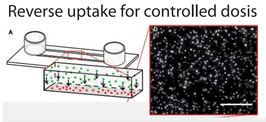
Study different signal pathways of apoptosis

Outlook

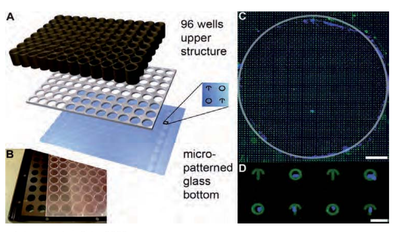
First Results of NP tested for NanoMILE

	Hig dose (50-100µg/ml)	Low Dose (1-10µg/ml)
PS-NH2		
TiO2		
TiO2 F-127		
TiO2 PVP		
ZnO		

Reverse uptake for controlled dosis



Upscaling sample preparation to well plate format



Azioune , Lab Chip, 2009

References:

[1] Röttgermann P. , Piera Alberola A., Rädler J. : Cellular Self-Organization on Microstructured Surfaces, Soft Matter, 2014

[2] Y. E. Kim, J. Chen, J. R. Chan, R. Langen: Engineering a polarity-sensitive biosensor for time-lapse imaging of apoptotic processes and degeneration, Nat. Meth. 7 (2010)