Time Lapse Imaging with zenCELLowl

Protocol

Spheroid Generation and Experimental set-up

Simone Ruckdäschel[1], Stefanie Michaelis[2], Joachim Wegener[1,2]

[1] Institute of Analytical Chemistry, Chemo- & Biosensors, University of Regensburg
[2] Fraunhofer Research Institution for Microsystems and Solid State Technologies EMFT, Munich

We performed live cell imaging of 3D multicellular spheroids in contact to non-adhesive or adhesive surfaces using the zenCELLowl. This allowed us to monitor the differential growth and adhesion behavior of the spheroids in real-time.

MCF-7 cells, cultivation and spheroid generation

MCF-7 cells were kindly provided by the research group of Prof. Göpferich (University of Regensburg). Cells were grown in Minimum Essential Medium Eagle, supplemented with fetal calf serum (10 % (v/v)), penicillin (100 µg/mL), streptomycin (100 µg/mL), L-glutamine (2 mM) and pyruvate (1 mM). Cells were split once per week in a ratio of 1:20 and cultivated at 37 °C with 5 % CO_2. MCF-7 spheroids were prepared by self aggregation of suspended cells in an agarose-coated 96-well plate (6000 cells/well) supported by orbital shaking at 37 °C with 5 % CO_2 over seven days.

Experimental set-up

Live cell imaging of spheroids on adhesive or non-adhesive surfaces was performed using 24-well plates (Eppendorf, Catalog no. 0030722116) in two different experimental set-ups at 37 °C with 5 % CO_2.

Proliferation of Spheroids

A first experimental set-up was designed to monitor the proliferation of spheroids. Spheroids (1 day old, 6000 cells/well) were placed into the wells of a 24-well plate coated with agarose (1.5 % (w/v) in medium, 200 µL/well) in order to prevent the spheroids from adhesion. The spheroids´ growth behavior was observed over four days using the zenCELLowl with the following settings:

- *Total Time Lapse Imaging*: 96 h
- *Interval*: 10 min
- *Focus*: ~500–600
- *Exposure*: -7
- *Illumination*: 30
- *Brightness*: 16

Adhesion and Outgrowth of Spheroids

The second experimental set-up was designed to monitor the adhesion and outgrowth of spheroids. Here, spheroids (7 days old, 6000 cells/well) were placed into the wells of an uncoated, tissue culture treated 24-well plate. The spheroids´ adhesion was observed by the zenCELLowl with the following settings:

- *Total Time Lapse Imaging*: 48 h
- *Interval*: 10 min
- *Focus*: ~400–500
- *Exposure*: -7
- *Illumination*: 30
- *Brightness*: 26

The recorded images of each experiment were processed and analyzed using ImageJ (Wayne Rasband, NIH) and the zenCELLowl software.