zenCELL owl

Application Note - Time lapse imaging of spheroids

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* This application note was a joint project of the authors and Omni Life Science (www.ols-bio.com), the distibutional partner of zenCELL

Introduction

Experimental assays based on living cells have emerged to be an established compromise between animal experiments and purely molecular interaction analysis in the life sciences. However, in monolayer or single cell assays the responses to e.g. anti-cancer drugs do not adequately represent the *in vivo* situation. Lately, three-dimensional multicellular aggregates, called spheroids, have been established as tissue models in health and disease. These tissue models mimicking *in vivo* like conditions have the advantage to better simulate cell-cell, cell-matrix and cell-drug interactions (1). Recent advancements in the 3D cell culture systems have fueled its implementation in early drug discovery and development, enabling drug safety and efficacy assessment (2). The zenCELL owl is a 24-channel microscope for fast and automated time lapse imaging. Its small foot-print makes it ideal for the use as an incubator microscope. With its intuitive software and integrated algorithm, the zenCELL owl allows documentation and quantification of cell growth, cell confluence, cytotoxicity tests, migration assays as well as observation of stem cells. We performed time lapse imaging of 3D multicellular spheroids in contact to non-adhesive or adhesive surfaces using the zenCELL owl. This allowed us to monitor the differential growth and adhesion behavior of the spheroids in real-time.

The cells are 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

Timelapse imaging of spheroids on adhesive or nonadhesive surfaces was perfommed using 24 -well plates (Eppendorf, Catalog no. 0030722116) in two different experimental set ups at 37°C with 5% CO_2. 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 zenCELL owl with the following settings:

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

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 zenCELL owl 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 zenCELL owl software.

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Results

Experiment 1: Proliferation of Spheroids

We observed the proliferation of spheroids in agarose coated, non adhesive wells over time using the zenCELL owl automated microscope. The non adhesive surface prevents establishment of cell surface contacts, whereas cell-cell interactions are promoted and three dimensional aggregates formed with a smooth and round shape due to surface tension and optimization. Due to cell proliferating in the outer shell of the spheroid, the overall diameter of the MCF 7 spheroid increases over four days as reported by zenCELL owl imaging (Fig 1.)

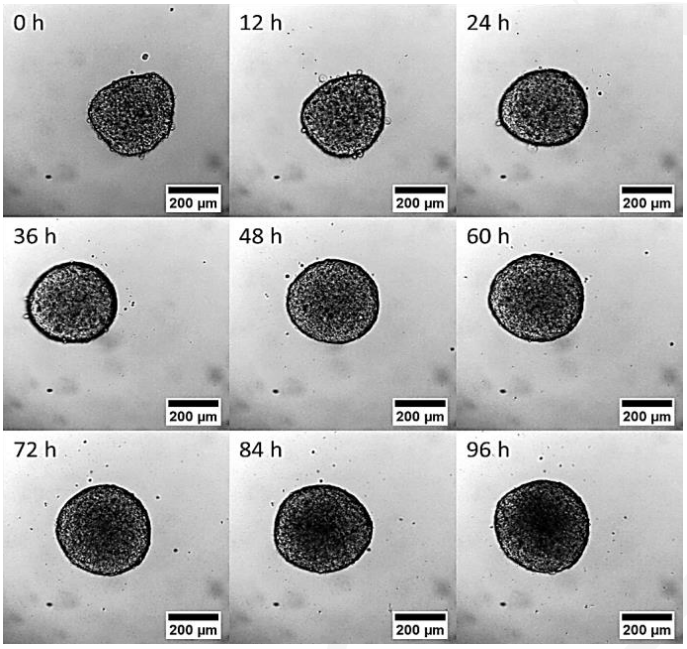
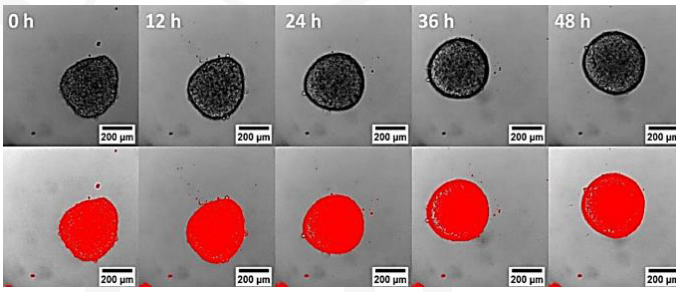


Figure 1: Growth of an MCF 7 spheroid 1 day old, 6000 cells/well) over 96 h in a non adhesive well, monitored with the zenCELL owl. The spheroid becomes round and smooth the diameter increases during time lapse imaging.

The number of proliferating cells in the outer shell depends on the initial cell number and the growth time after spheroid formation. For different initial cell numbers and growth times, spheroids with individual diameters are formed. Accordingly, the size of spheroids can be controlled by i adjusting the initial cell number and growth time to the needs of the experiment It is noteworthy that different cell types form spheroids of individual shape and size even if the same number of cells were initially allowed to aggregate. This phenomenon indicates an individual architecture of the resulting spheroids with individual degrees of compaction, cell-cell interactions and amount of extracellular matrix deposited between individual cells.

The zenCELL owl allows analyzing the growth and shape of spheroids by automated time lapse microscopy as a function of initial cell number, cell type, or spheroid age over days. Figure 2 illustrates how different modes of image analysis provide a quantitative description of spheroids studied by the zenCELL owl. Different parameters such as the projected area of the spheroid, its width, height and roundness and how these parameters evolve with time were assessed using ImageJ software.



	Area [µm²]	Width [µ]	Height [µm]	Roundness
0 h	125.000	404	428	0.907
12 h	124.000	413	432	0.893
24 h	122.000	417	402	0.921
36 h	129.000	419	396	0.952
48 h	136.000	421	419	0.959

Figure 2: Growth of an MCF 7 spheroid 1 day old, 6000 cells/well) over 48 h in a non adhesive well, monitored with the zenCELowl processed and analyzed with ImgeJ. The spheroid's projected area, width, height and roundness change over time due to cell proliferation and spheroid compaction

Results

Experiment 2: Adhesion and Outgrowth of Spheroids

In a second experiment, we observed the adhesion and outgrowth of spheroids over time upon a tissue culture treated surface by recording time lapse images with the zenCELL owl. When spheroids are allowed to settle in tissue culture treated wells, the cells adhere to the surface and they start to grow out (Fig.3). First, cells from the outer shell attach to the surface and form cell surface contacts mediated by the ECM. The spheroid flattens and more cells migrate out of the initial contact zone within 12 h after loading the spheroid into the well. This outgrowth of the spheroid starts initially in one direction, increases gradually all around the spheroid and finally leads to a centrosymmetric seam of spread cells After 48h the adhered spheroid consists of a semispherical three dimensional part and a surrounding cell monolayer.

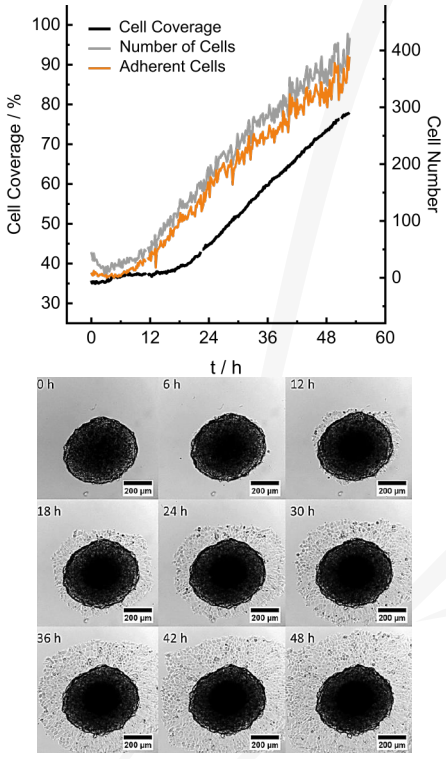


Figure 3: Adhesion and outgrowth of an MCF 7 spheroid (7 days old, 6000 cells/well) over 48 h in a tissue culture treated well, monitored and analyzed with the zenCELL owl equipment Time lapse imaging and subsequent image analysis show the spheroids´ attachment and outgrowth of a cell monolayer

The time dependent attachment and outgrowth of spheroids is analyzed by processing the time lapse images using the zenCELL owl software. Parameters like *Cell Coverage* across the whole field of view, the total *Number of Cells* and the *Number of Adherent Cells* quantitatively describe the spheroid's adhesion and outgrowth over time (Fig.3). The time course is dependent on various factors and may differ significantly for individual cell types, cell numbers, spheroid age and culture conditions. The individual impact of these factors is assessable using the zenCELL owl real time monitoring.

Conclusion

Based on the time resolved and non invasive monitoring capabilities of the zenCELL owl it is possible to study size, growth, shape and morphological changes of multicellular spheroids under regular cell culture conditions without any interference by the operator. Data acquisition is completely automated, software controlled and does not require manual handling. By using the zenCELL owl it is posible to study the impact of chemical, biological or physical stimuli on 3D tissue models with low to intermediate throughput. The proprietary software turns stacks of time resolved images into time course data of quantitative parameters describing the biological specimen. Time resolution is easily adjusted to a few minutes providing a detailed perspective on spheroid dynamics upon exposure to any kind of stimulus The zenCELL owl comes as a perfect solution in terms of time saving and efficient real time analysis of these various effects on spheroids.

References

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