

SPHEROIDS AS 3D SYSTEMS FOR CANCER RESEARCH – COMPARISON OF DIFFERENT LABORATORY APPROACHES

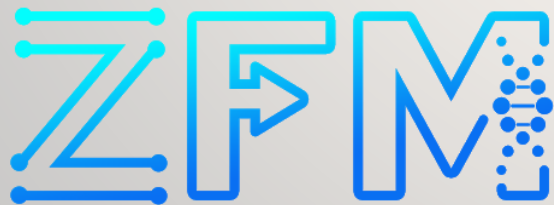
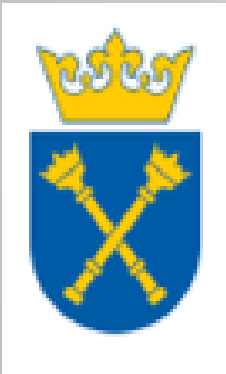
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SPHEROIDS – A NEW MODEL IN CANCER TREATMENT

- Spheroid – 3D cell culture
- Structure, organization, microenvironment and drug resistance → observed in solid tumor and spheroid
- The layered structure, cell signaling, physical contact of cells and the extracellular matrix → protective barrier for the penetration and distribution of anticancer drugs and increase resistance to radiotherapy
- The growth phases, pattern of gene expression similar in spheroids and tumors

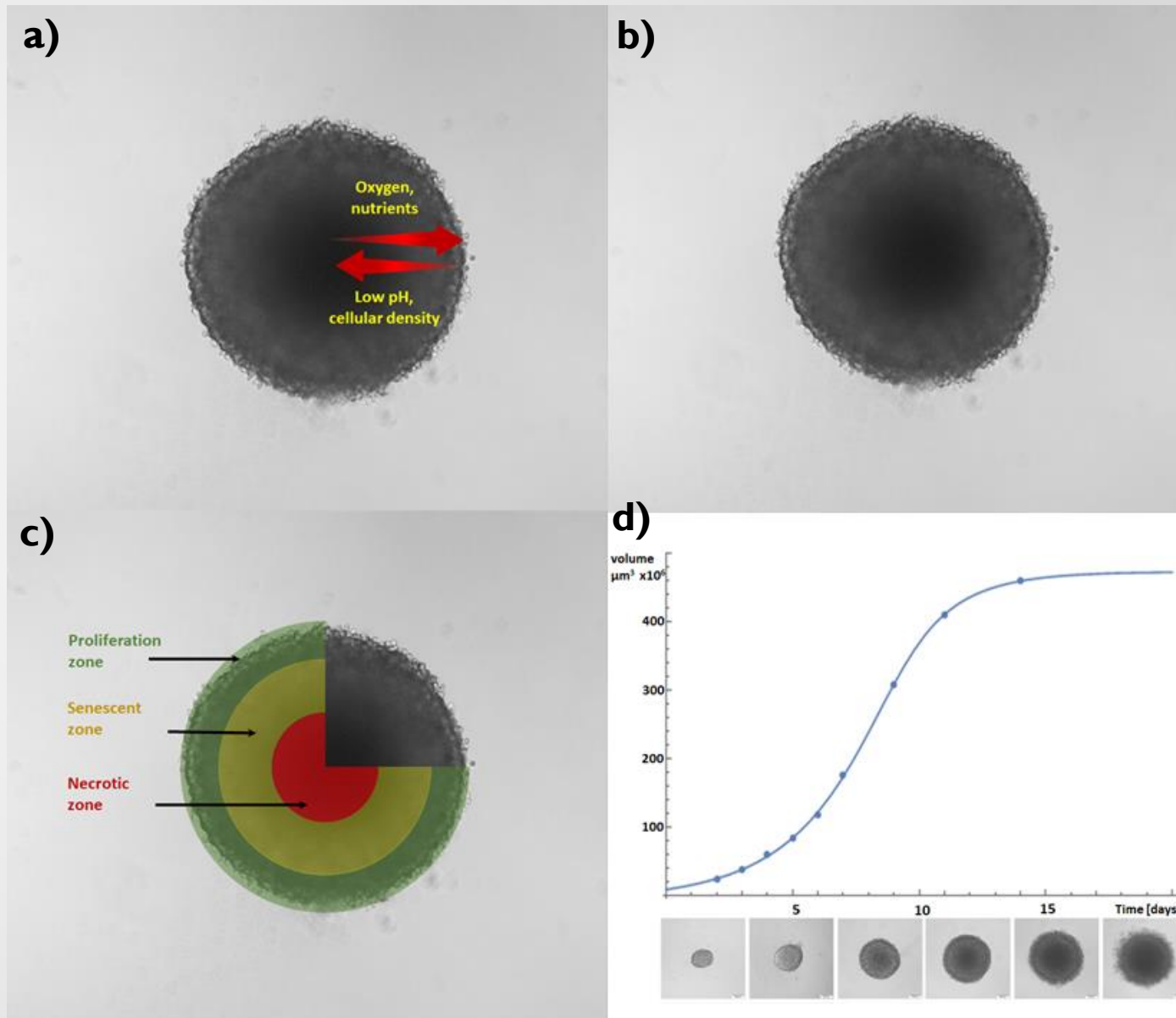
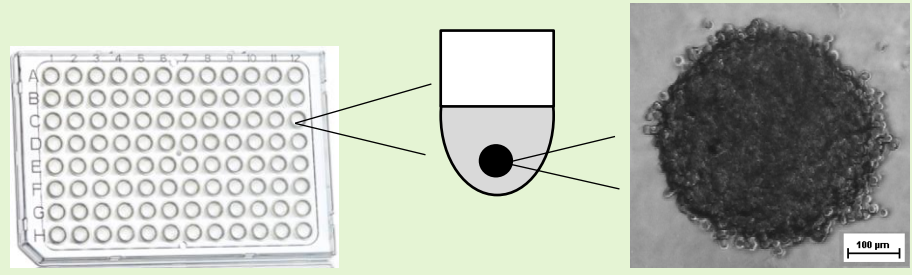


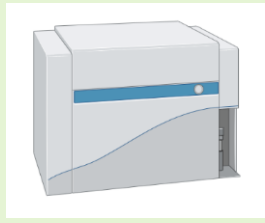
Fig. 1. a) decomposition of nutrients and c) the associated layered structure of spheroids b) image of the spheroid using an optical microscope d) kinetics of spheroid growth

1 Low-adhesive plate

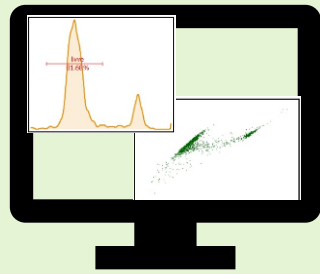


Optical microscope imaging

Image analysis by macro (ImageJ)

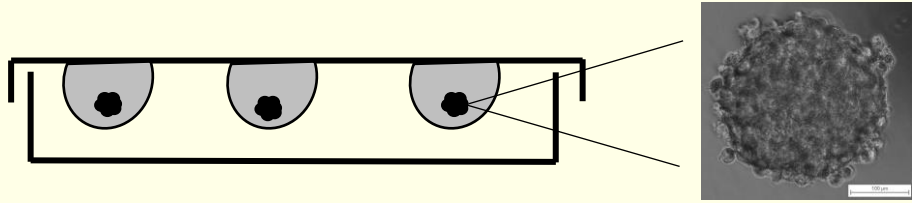


Viability test – flow cytometer



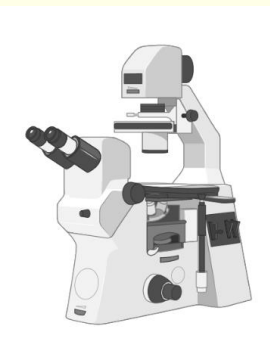
Data analysis

2 Hanging drop method

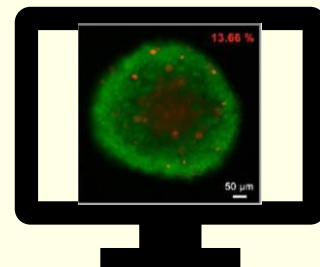


Optical microscope imaging

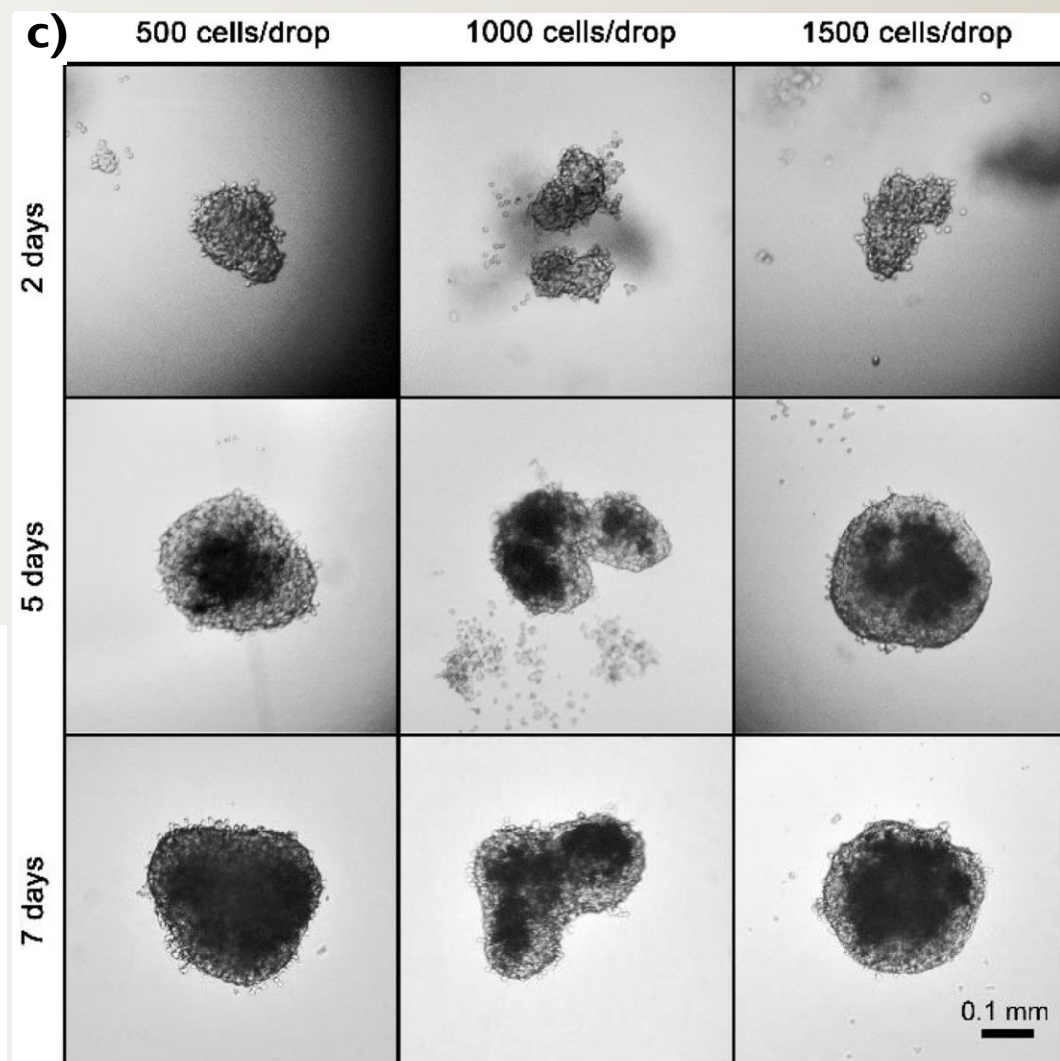
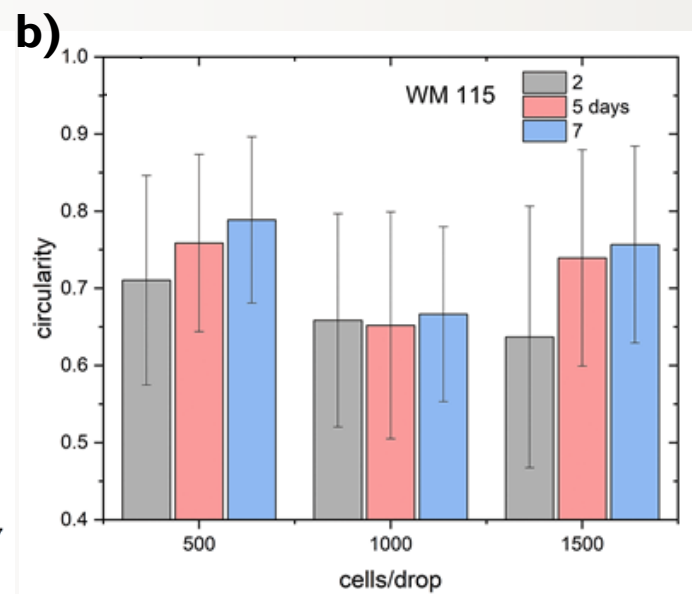
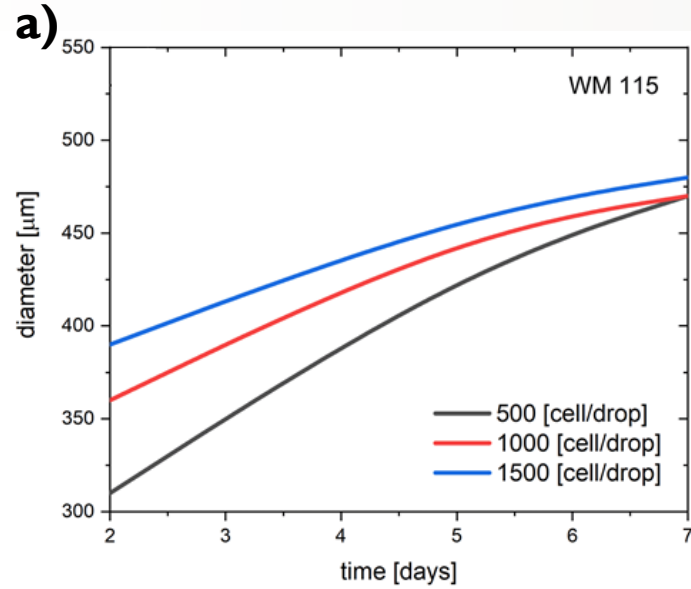
Image analysis (ImageJ)



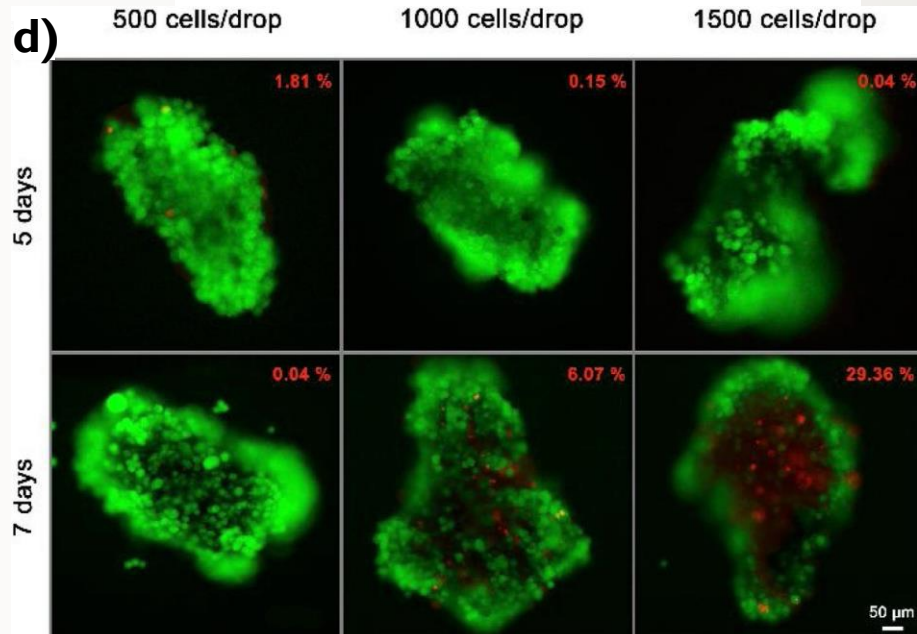
Fluorescent microscope imaging



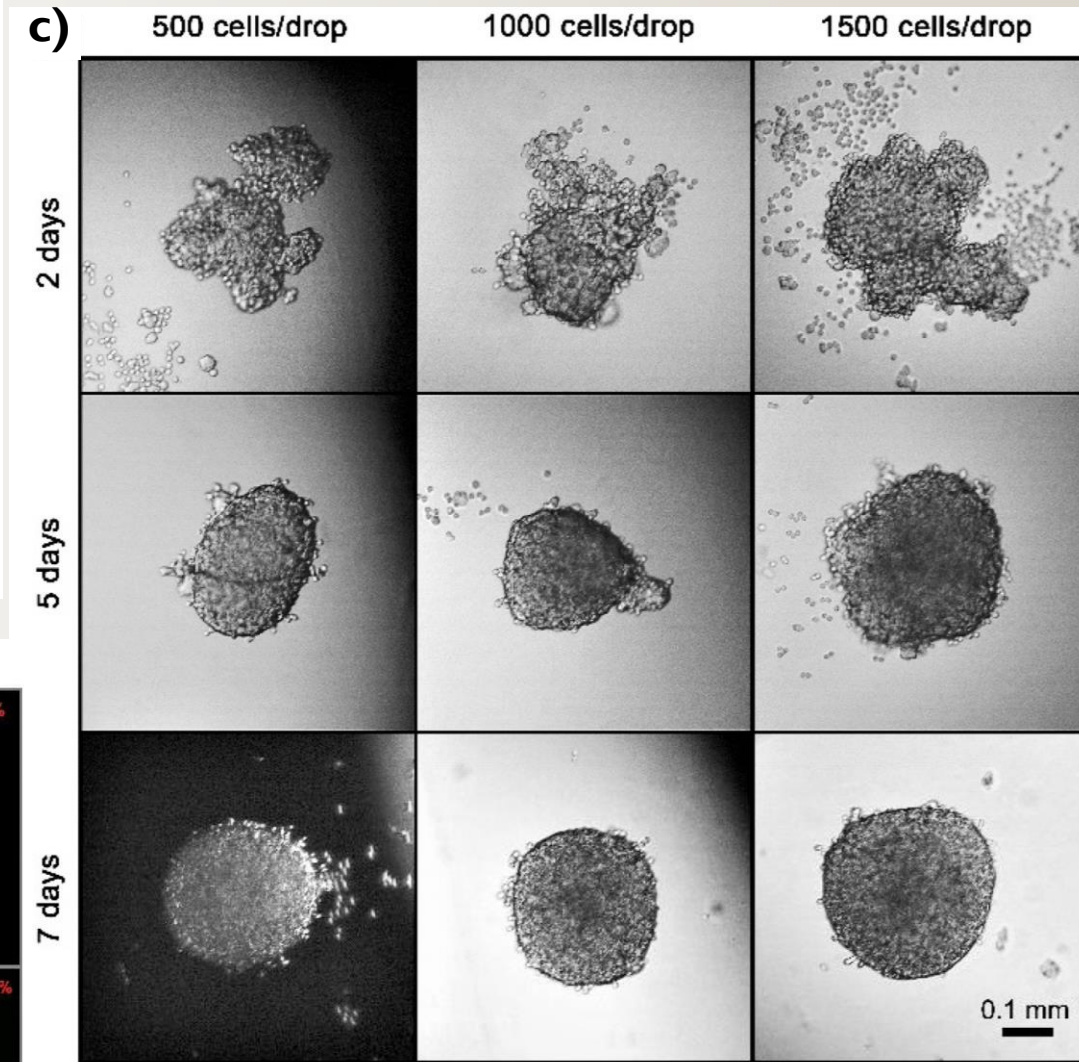
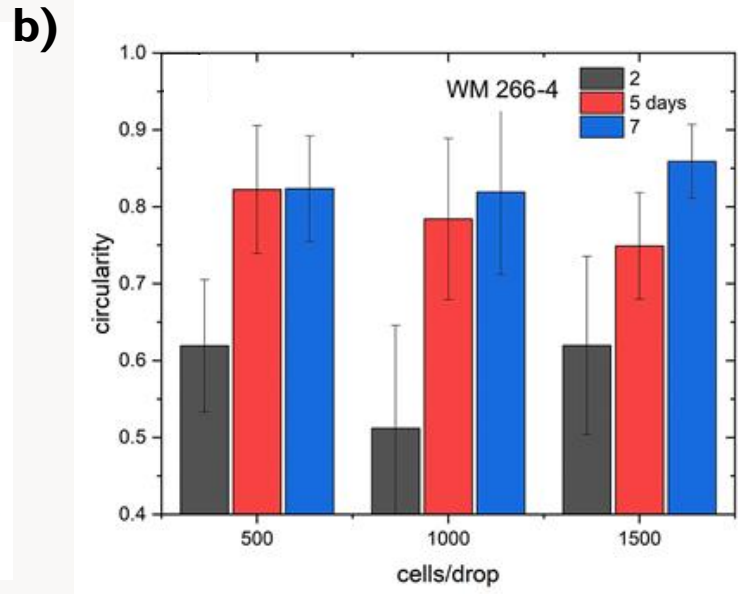
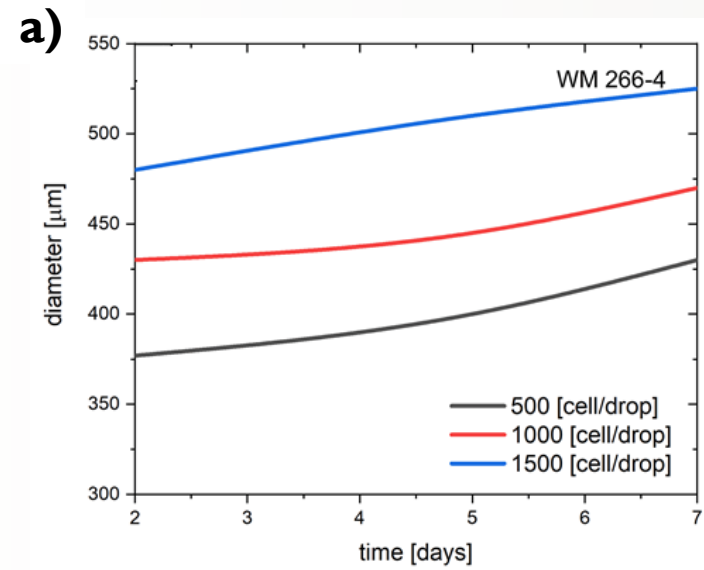
Hanging drop method results – WMI 15



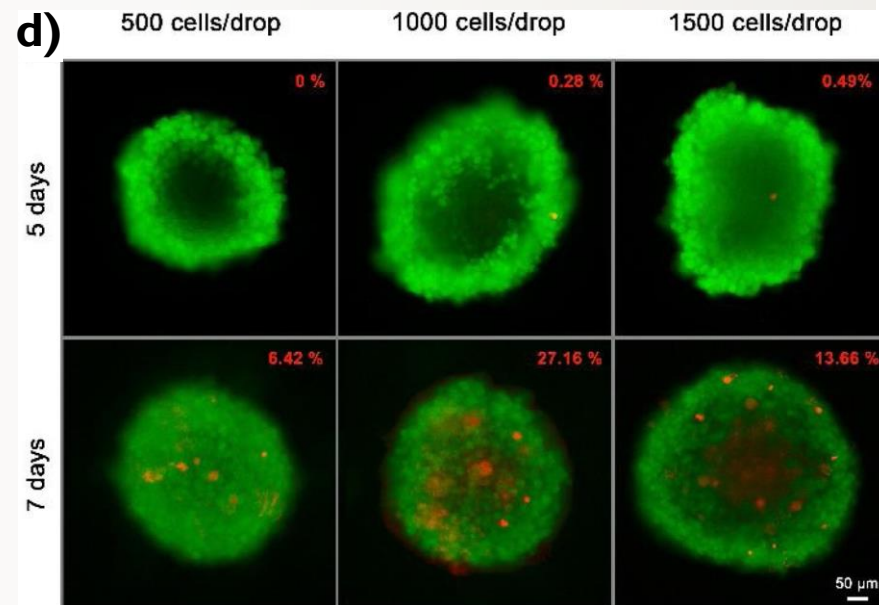
Spheroid growth characterization. (a): Graph showing the increase in spheroid diameter as a function of time for a different initial number of cells in the spheroid (b) Graph indicating changes in spheroid circularity within different day of culture. (c): Representative images of spheroids. (d): representative images from fluorescent microscope – alive cells are green, dead cells are red.



Hanging drop method results – WM266-4

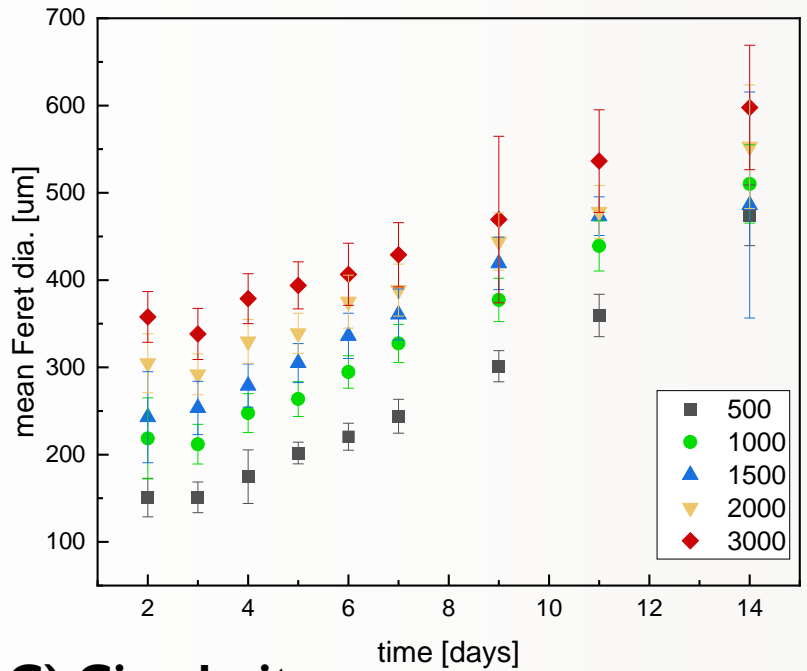


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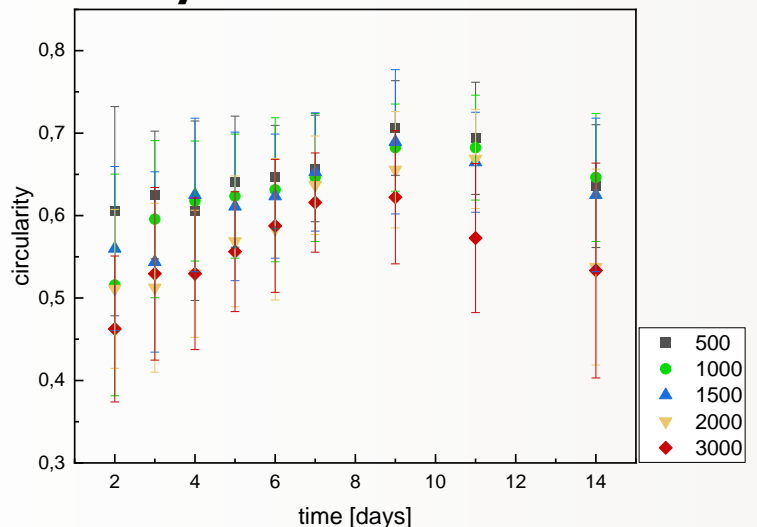


Low-adhesive plate results – WMI 15

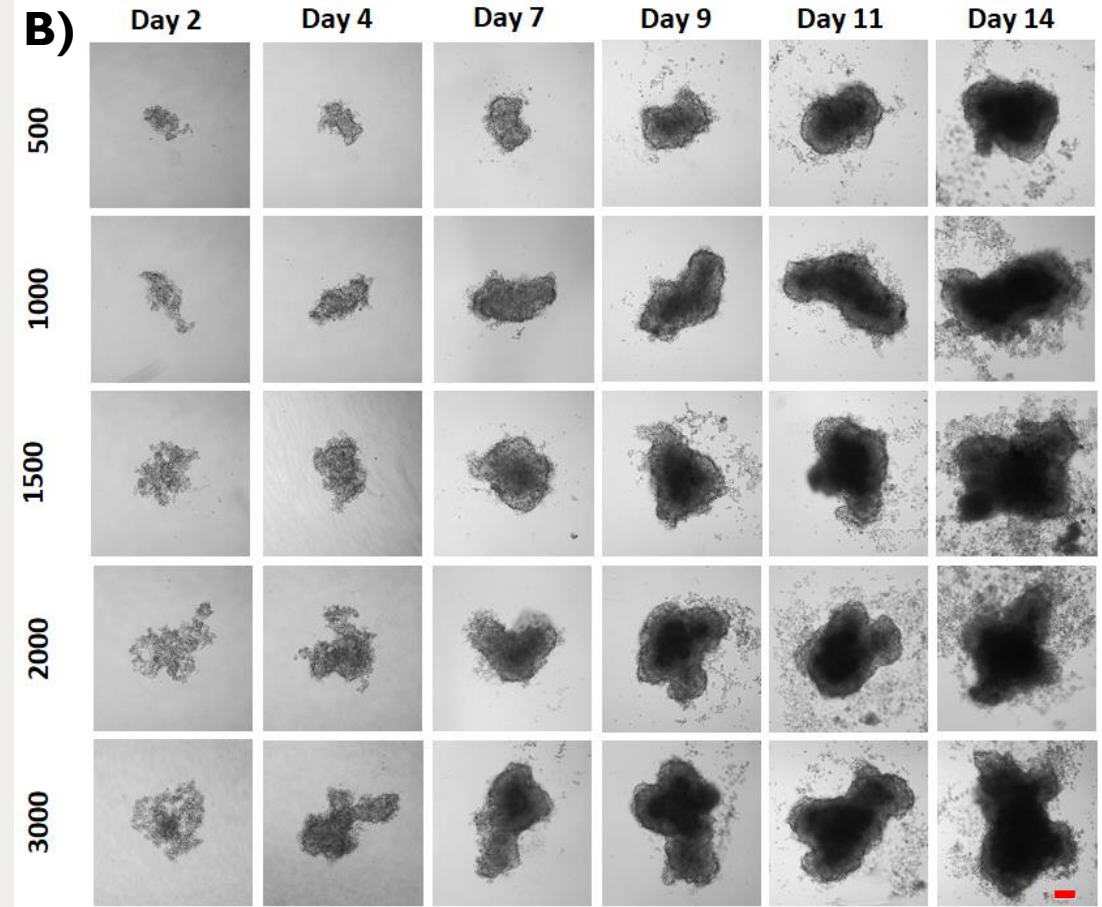
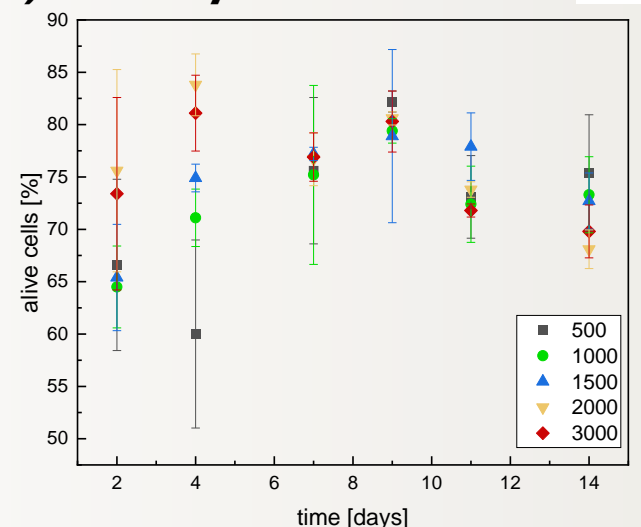
A) Mean Feret diameter



C) Circularity



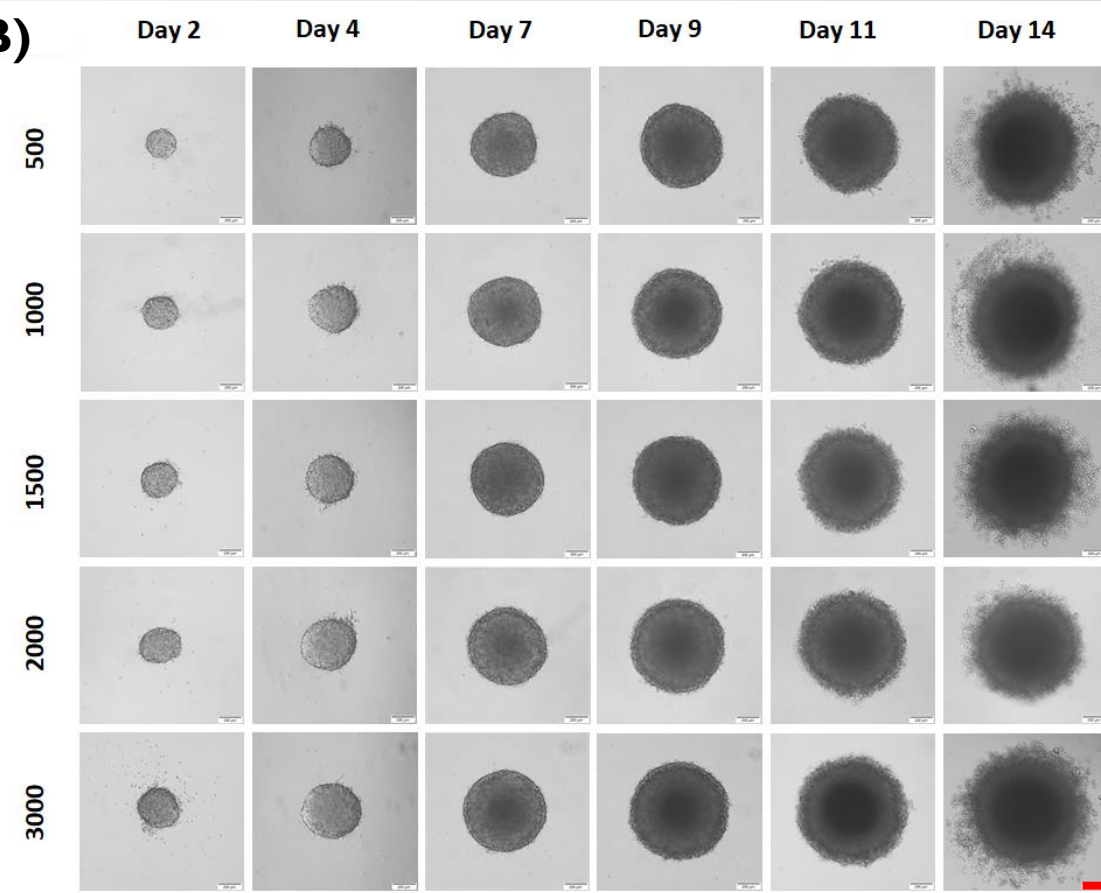
D) Viability



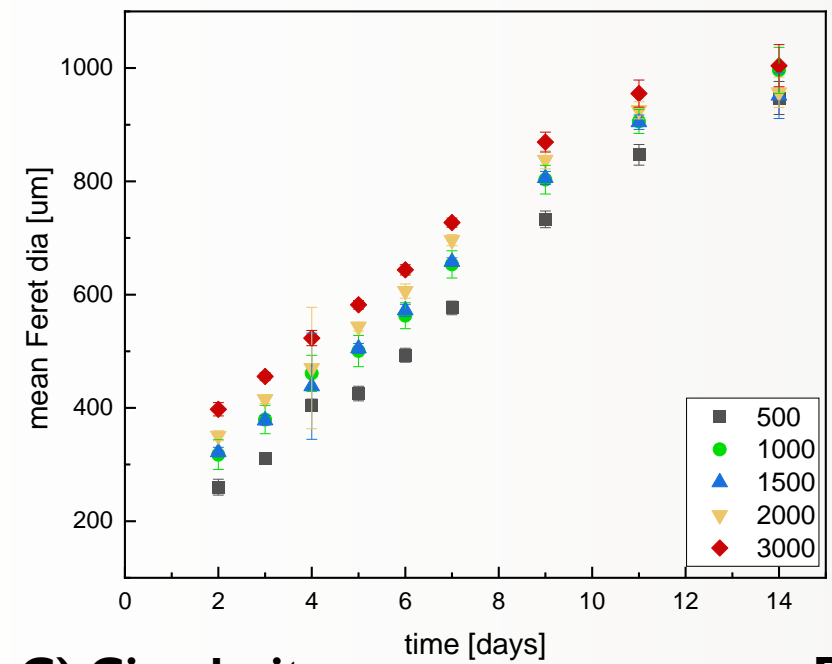
Spheroid growth characterization. (A): Graph showing the increase in spheroid diameter as a function of time for a different initial number of cells in the spheroid (from 500 cells to 3000 cells per well). Data represent the mean \pm standard deviation (SD). (B): Representative images of spheroids of five different sizes. Scale bars, 200 μ m. (C): Graph indicating changes in spheroid circularity within different of culture. (D): Changes in the viability of cells in the spheroid during culture

Low-adhesive plate results – WM266-4

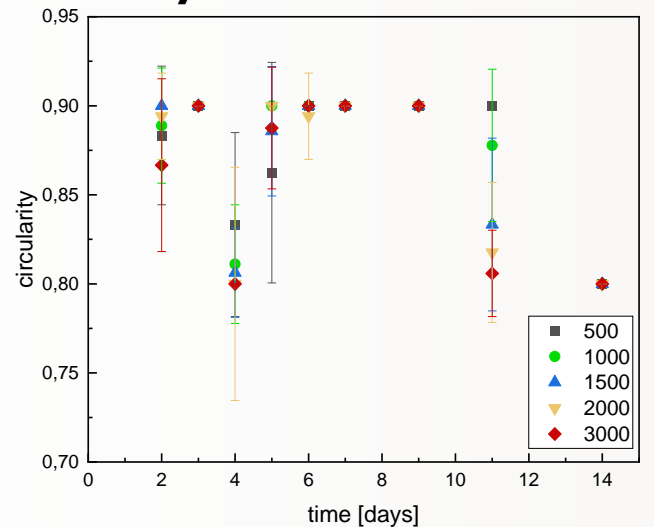
B)



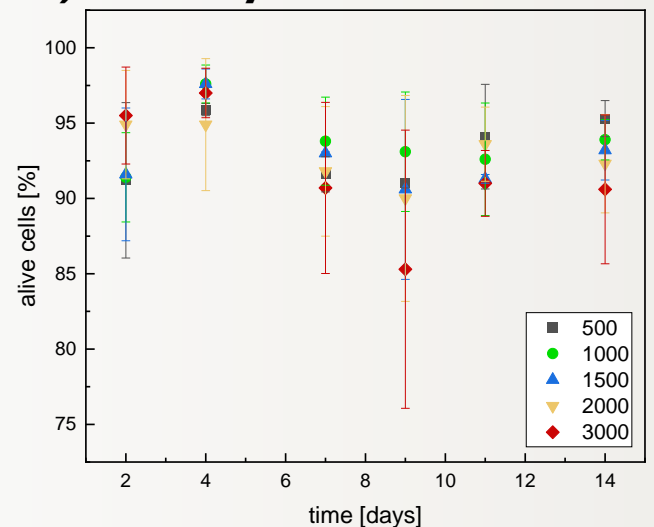
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Advantages

Disadvantages

Hanging drops

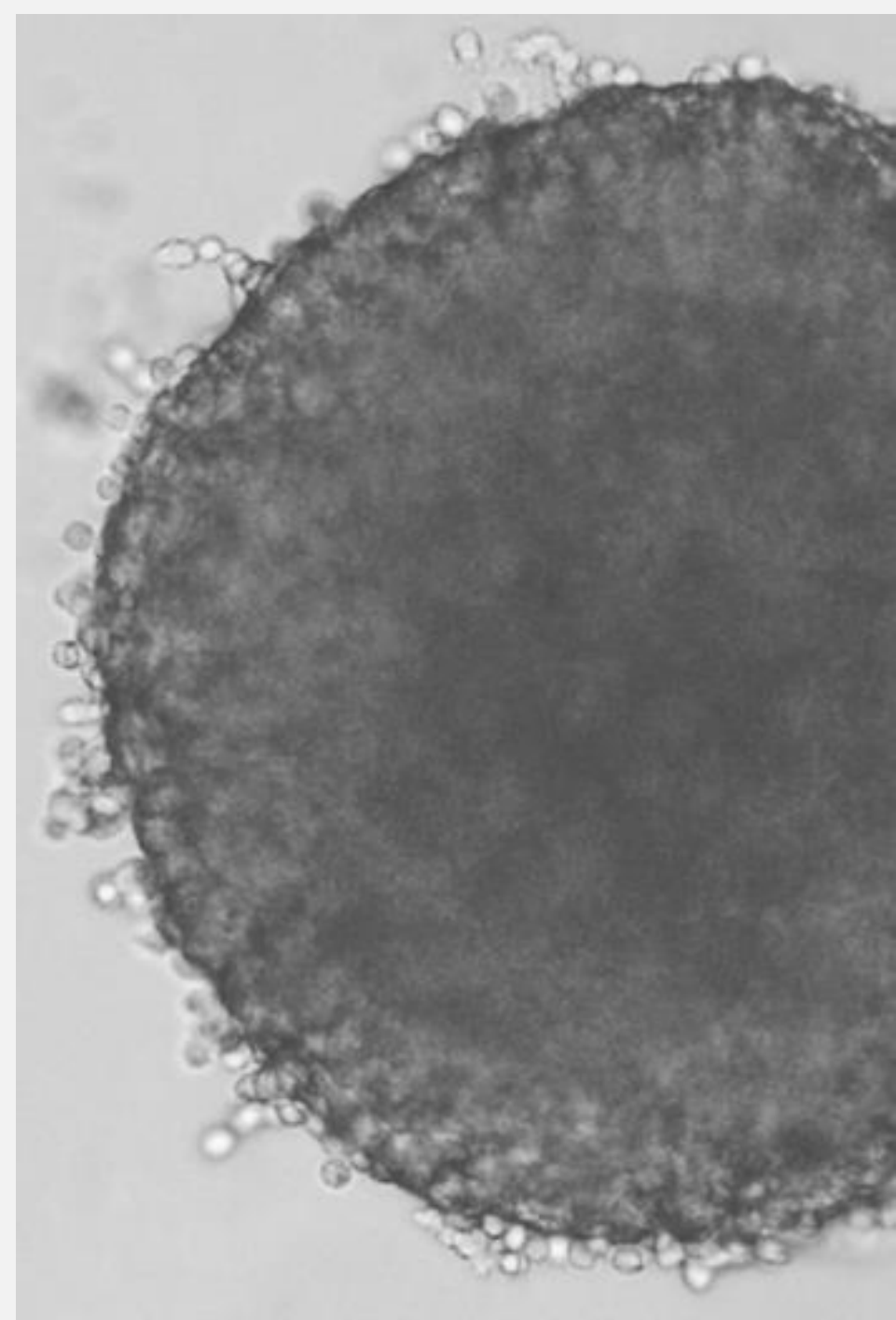
Simple
No specialized equipment required
Uniform spheroid size

Tedious handling
Time consuming
Long-term culture difficult
Not efficient

Low-adhesive plate

Easy handling
Simple
Fast

Expensive
Relatively low efficiency



SUMMARY

- Microscope images and growth curves show a few stages of spheroid growth. Initial – spontaneously self-assembled cell aggregates; than fusing cell aggregates; forming solid spheroids with visible border; final – ruffling spheroid boundary.
- WM266 line shows faster spheroids formation and regularity in size and shape whereas
- WM115 line is characterized by delayed spheroid generation and a lack of regularity in shape
- These studies of spheroids creation and spheroid characteristics evaluation can help us for our future goals, as a model for research into the effectiveness of boron neutron capture therapy and application of positronium as a new biomarker for the in-vivo determination of the cancer malignancy level with total-body PET scanners

Acknowledgments

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[1] Moskal, Paweł, and Ł. Stępień, Ewa. "Prospects and clinical perspectives of total-body PET imaging using plastic scintillators." PET clinics 15.4 (2020): 439-452.

[2] Moskal, Paweł, et al. "Positronium in medicine and biology." Nature Reviews Physics 1.9 (2019):527-529.

[3] Szczepanek Monika „Application of 3D Model of Cancer Cells in Research on the Effectiveness of BNCT in the Treatment of Melanoma” Acta Physica Polonica B 51 (2020):413-419.

THANK YOU FOR YOUR ATTENTION !

