A new model for spheroid growth

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Introduction

Spheroids are 3D model of cell culture which commonly used in research into new cancer treatments and therapies. They mimic the structure, microenvironment and cells signaling present in solid tumors [1,2]. In their layered composition, three types of cells can be distinguished: dead cells inside the spheroid (necrotic zone); living, non-proliferating cells in the middle (quiescent zone); and living and proliferating cells in the outer layer of the spheroid [3,4]. We propose a new spheroid growth model, based on simulations and experimental data, which reveals the growth dynamics of three spheroid zones. The presented model can potentially provide more information than the standard approach in such studies of the growth dynamics of tumors – the Gompertz curve.

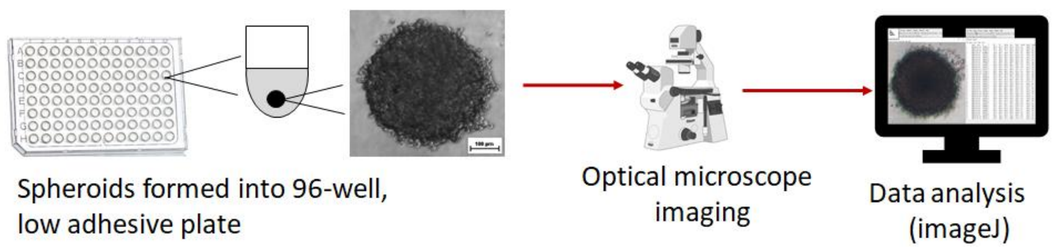
Objectives

- finding a model describing the growth of spheroids (allowing to predict the behavior of the growth curve)
- performing a simulations that could the verify proposed model
- assess numer of proliferative and non-proliferating cells, check how cells in a given state are distributed inside the spheroids, estimation of the fraction of dead cells indirectly, only from the growth curve, estimation of transitions probabilities

Experimental methods

Formation of spheroids from human melanoma cell line WM266-4 on U- shape, low adhesive 96-well plates. Spheroids were cultured for 5 different initial cell numbers.

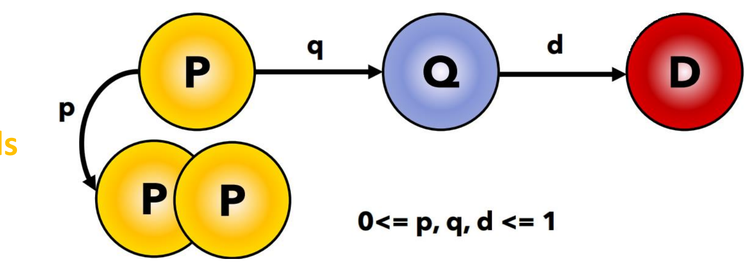
Spheroid culture and image analysis



Theoretical methods

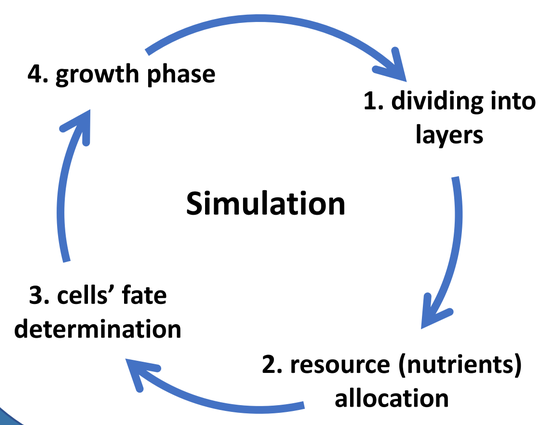
Concept of a new model of spheroid growth and dedicated simulation.
Model and simulation assumption:

- Cells could be in one of three states
- States are defined by transition and division parameters



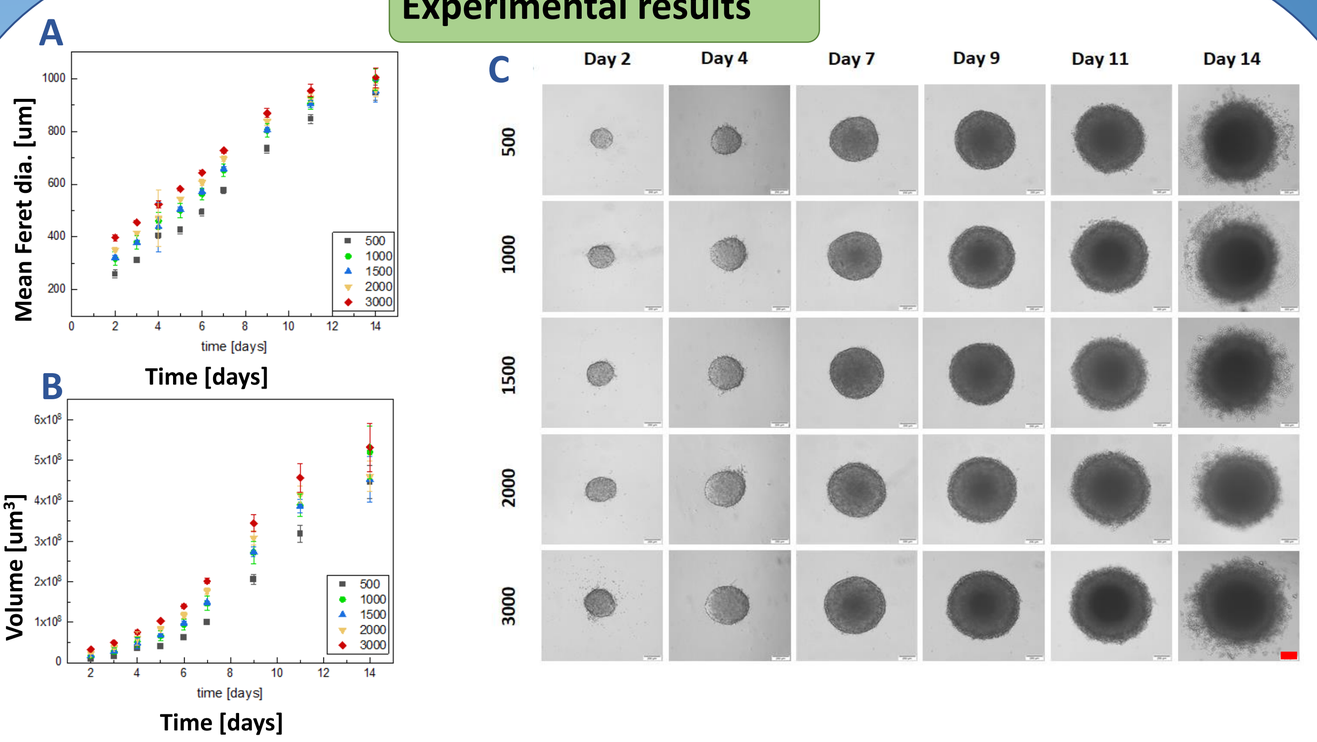
P – proliferating cells
Q – quiescent cells
D – dead cells

1. **Cells – hard balls** : spheroid divided into layers. Layer – rim of sphere with thickness equal to 2x diameter of cell.
2. **Resources:** the most outer layer – maximum resources. Subsequent layers – remnants. Resources are divided equally in a layer.
3. **Checking state** of cells after „feeding" – cells are in one of three states.
4. **Harvest** - new cells = numer of proliferating cells x the probability of division. Removing resources needed for survival from each cell.



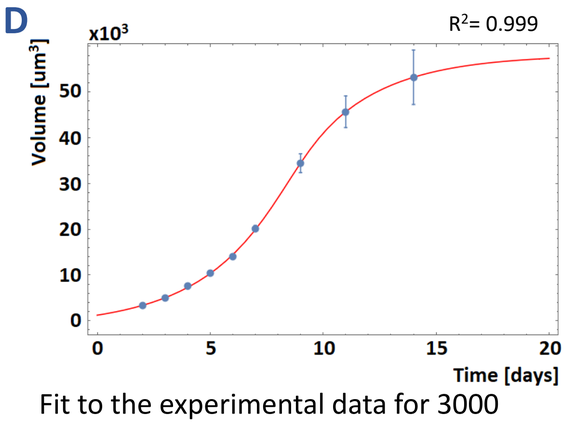
Constant parameters :
Transition and division probabilities, mean volume and shape of cells, amount of resources.
Variables:
Resources allocation, states of cells.

Experimental results

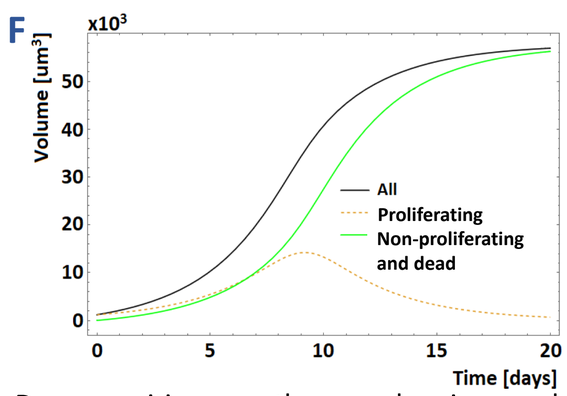


Spheroid growth characterization. (A): Graph showing the increase in spheroid diameter as a function of time for a different initial number of cells in the spheroid. Data represent the mean ± standard deviation (SD). (B): Graph showing the increase volume of spheroids during culture. Volume was calculated assuming sphericity of spheroids. Data represent the mean ± standard deviation (SD). (C): Representative images of spheroids of five different sizes. Scale bars, 200 µm.

Fitting results

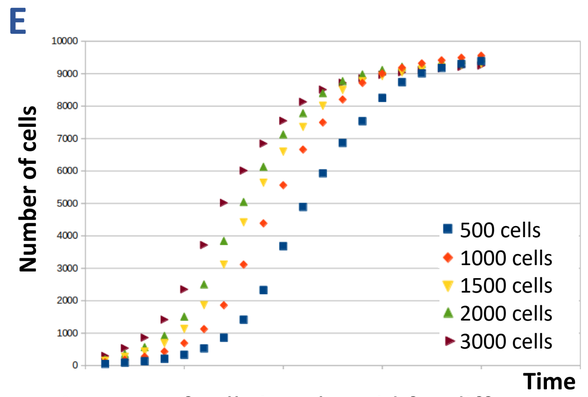


Fit to the experimental data for 3000 initial cells in spheroid

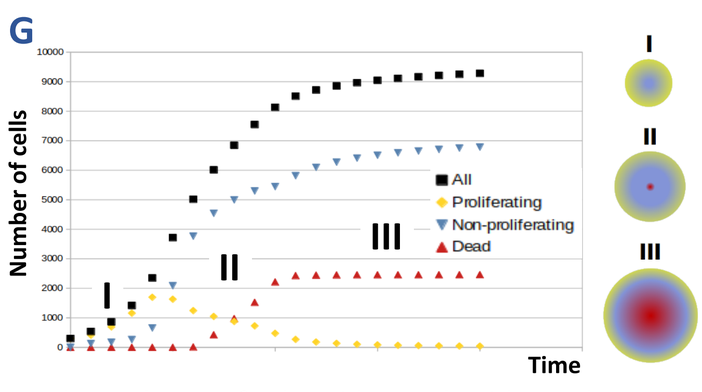


Decomposition growth curve showing number of proliferating, non-proliferafing and dead cells on example 3000 initial cells in spheroid

Simulation results



Amount of cells in spheroid for different initial numer of cells



Three phases of spheroid growth with number and distribution of cells in each of them

Conclusions

- A model was developed to characterize the growth of spheroids by distinguishing between 3 separate cell states
- It was possible to develop a model that reflects the behavior of the experimental spheroid growth curve with a very good agreement with the experimental data
- The developed simulations made it possible to determine the number of cells in each state during the spheroid growth

References:
[1] E.Ł. Stępień et al., Acta Phys. Pol. B 51, (2020).
[2] E. Axpe et al., PLoS One 9, (2014).
[3] H. Karimi et al, Micron 137, (2020).
[4] M. Szczepanek, Acta Phys. Pol. B 51, (2020).

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