Three-dimensional cellular spheroides in oncology research- a bridge between *in vitro* and *in vivo* studies

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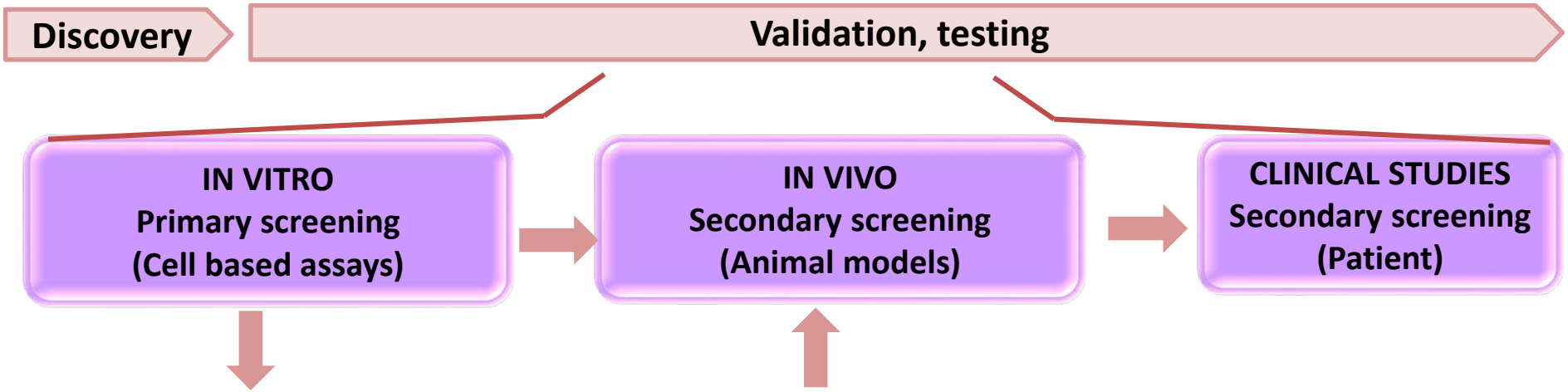
Spheroids



SPHEROID

- 3D spheroid cultures better represent in vivo tissues
- more realistics cell-cell and cell-matrix interactions
- nutrient, oxygen and waste gradients present
- increases confidence in *in vitro* testing results

Targeted research areas:

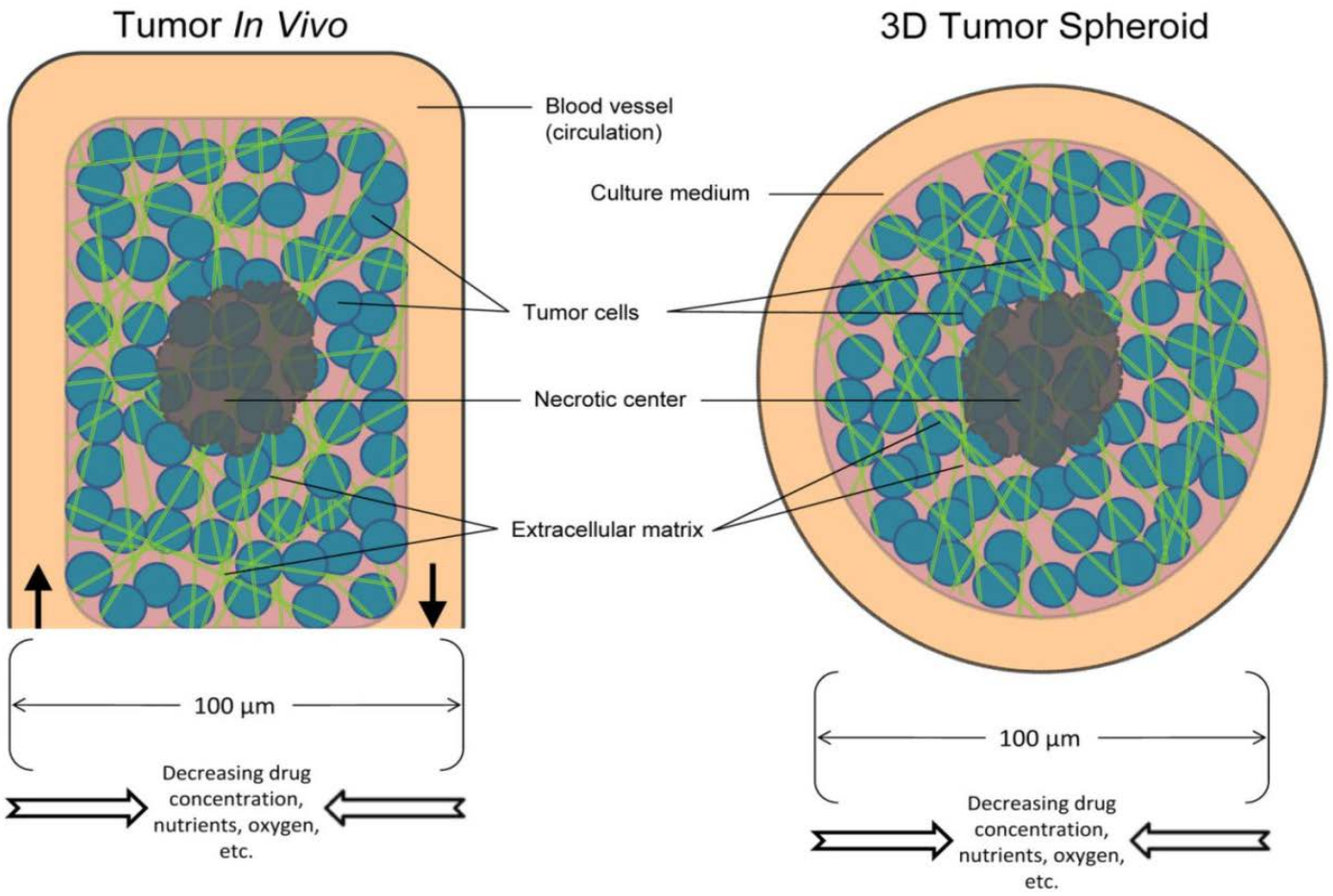
- cancer research (model for the microareas in the tumor, growth, metastasizing, invasion, angiogenesis)
- screening for new drugs (high throughput screening)
- toxicology
- stem cell research
- tissue engineering

Characteristics of 2D and 3D cell cultures-spheroids

Characteristic	2D	3D
Secretion of extracellular matrix (ECM)	no	collagens, laminin, fibronectin, glycosaminoglycans; similarly organized as in the tissues
Cell-cell interactions	no	yes (homo and hetero interactions)
Cell- ECM interactions	no	yes (cell-matrix adhesions)
Proliferation and growth rate	activated, fast	regulated; slower
Gene expression and protein secretion profile	qualitatively and quantitatively different	upregulation of the expression of genes: involved in progression and metastatic processes (IL-8, GROα ali MIP-3α), regulation of the ECM components, intercellular junctions secretion of: growth factors, proangiogenic factor VEGF, TNFα
Drug sensitivity	more	less

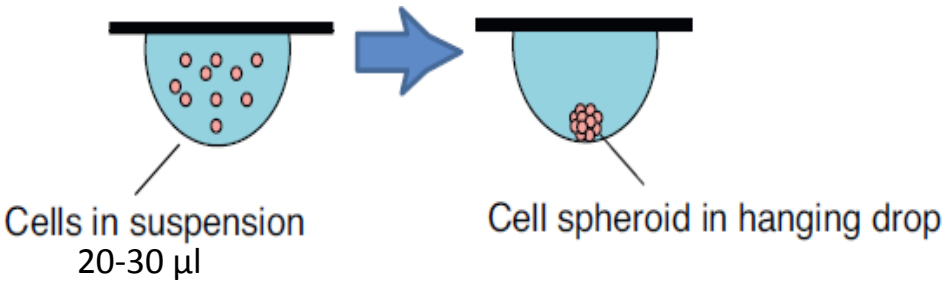
Characteristics of spheroides

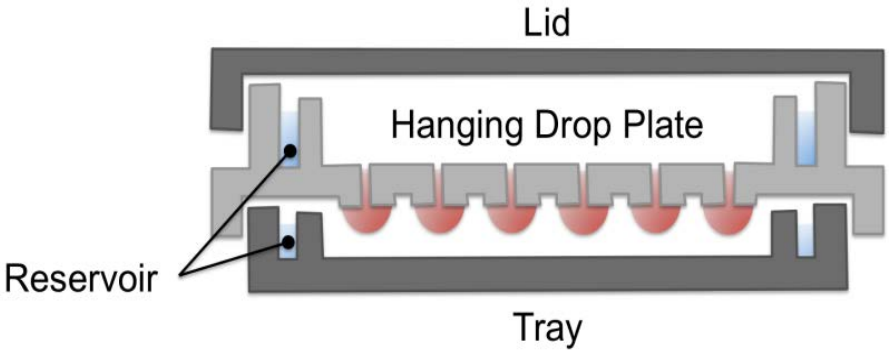
- nutrient, oxygen and waste gradients
- necrosis area 2r›100 µm; 2r›500 µm hipoxia and necrosis in the center
- transplation *in vivo*



3D cell culture-spheroid methods

Method	Advantages	Disadvantages
Hanging drop	-inexpensive (96 well plate, petry dish coated with agar) -homogenous -suitable for high-throughput testing -easily accessible spheroides	-expensive if using specialised plates -difficult exchange of medium due to small culture volume -labour intensive if preparing the plates -diameter up to 600 µm

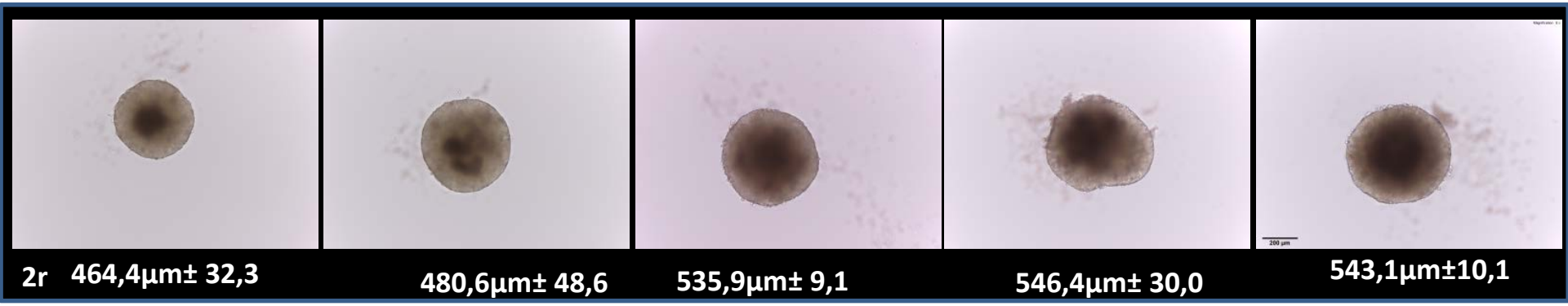




Hanging drop: Human adenocarcinoma colon cancer HT29 spheroides

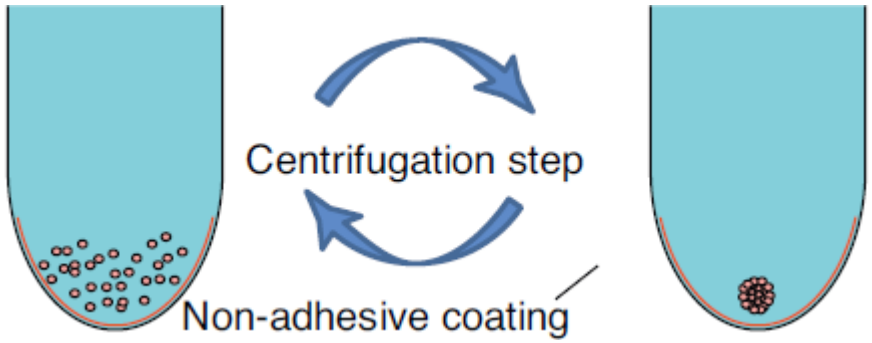
3 days after seeding cells

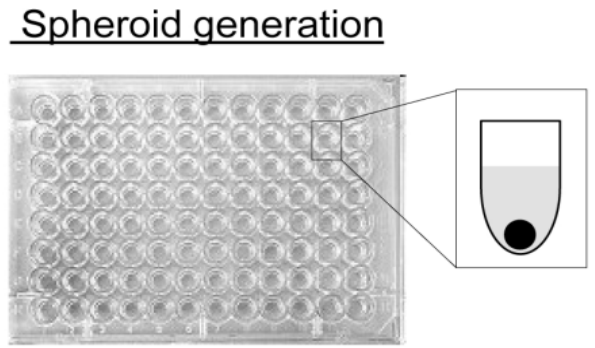
10 days after seeding cells



3D cell culture-spheroid methods

Method	Advantages	Disadvantages
Forced floating	-simple, fast (96 well plate ULA or normal plate coated with agar /diffrent biopolymer; round or conical bottom) -inexpensive -suitable for high-throughput testing -easily accessible spheroides -more culture medium -diameter up to 1500 µm	-variability in size and shape (fixed cell no./well!) -plate coating is relatively labour intensive

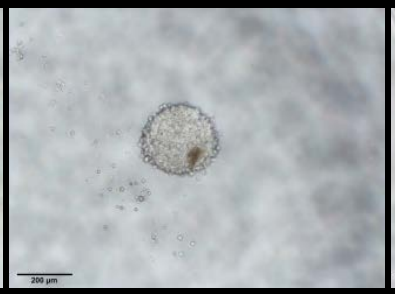
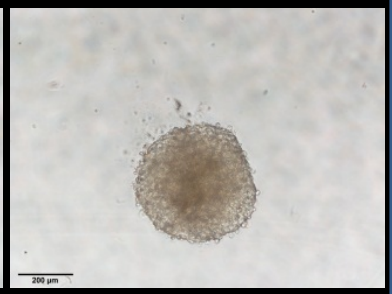




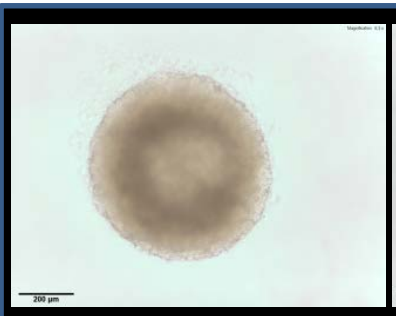
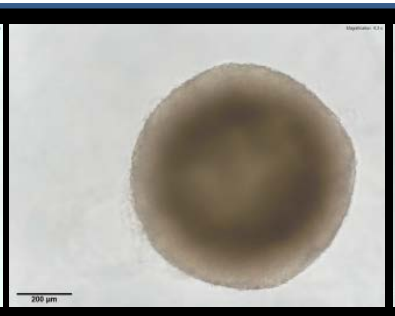
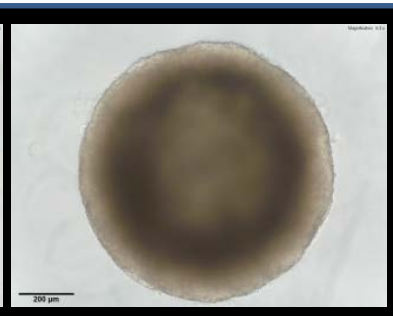
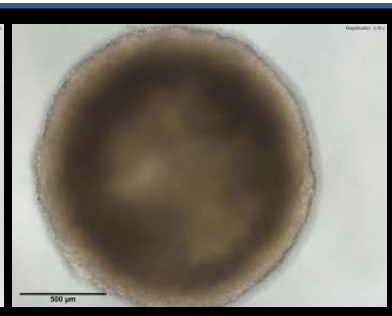
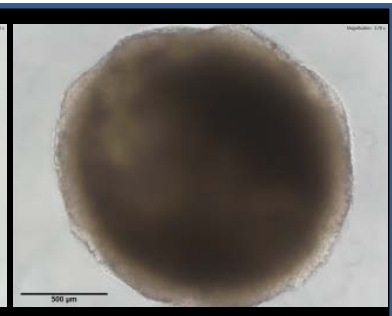
ULA 96-well round-bottom plate

Forced floating: Human squamous cell carcinoma FaDu spheroides

3 days after seeding cells on 96 well plate coated with agar

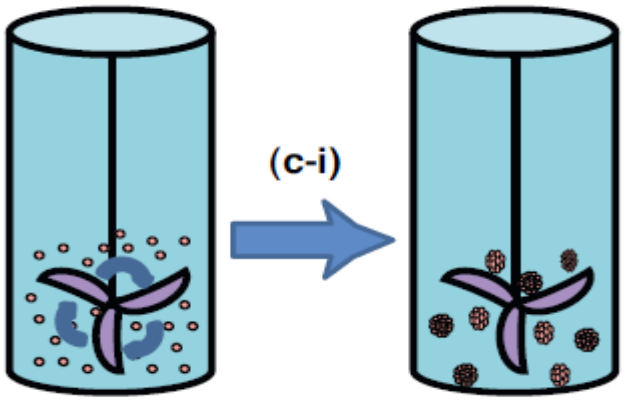
	250 cells	500 cells	1000 cells	2000 cells	3000 cells
					
2r	167,4µm± 4,0	249,9µm± 16,1	279,6µm± 23,8	369,1µm± 13,4	416,6µm ± 9,7

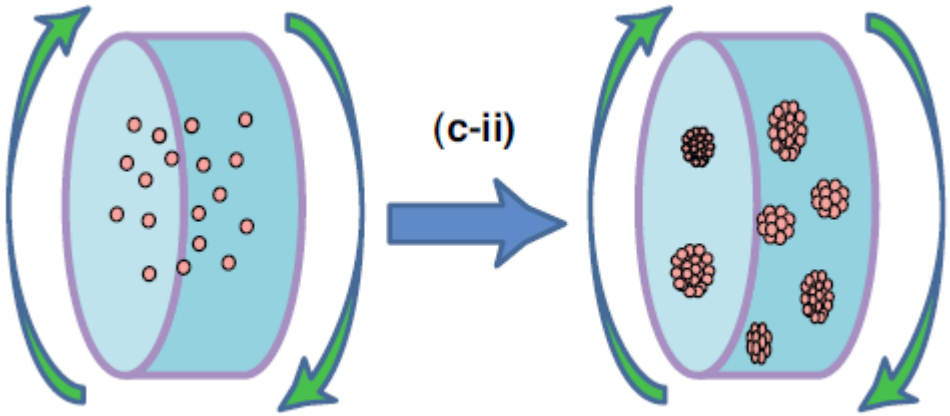
13 days after seeding cells on 96 well plate coated with agar

	250 cells	500 cells	1000 cells	2000 cells	3000 cells
					
2r	667,6µm± 10,3	846,8µm± 17,5	989,9µm± 32,1	1085,4µm ± 120,5	1073,8µm ± 47,9

3D cell culture-spheroid methods

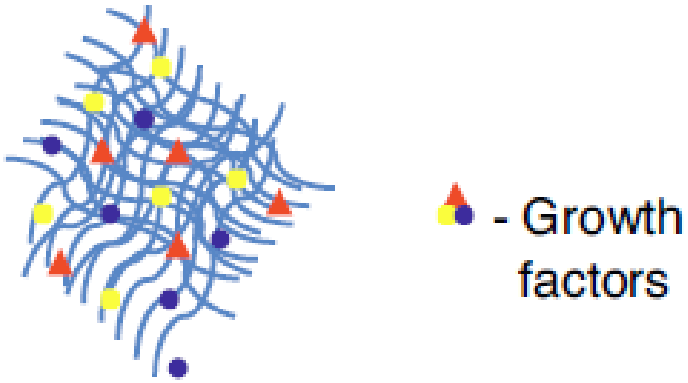
Method	Advantages	Disadvantages
Agitation-based approaches: -spinner flask bioreactors -rotational culture system	-simple to culture cells -large scale production relatively easily achievable -motion of culture assists nutrient transport -easily accesible spheroides -diameter up to 1 cm	-specialised equipement -no control over size; additional culture step needed to uniform the size -time consuming due to extra step required for homogenous spheroid -cells exposed possibly to shear force in spinner flask (? sensitive cells)





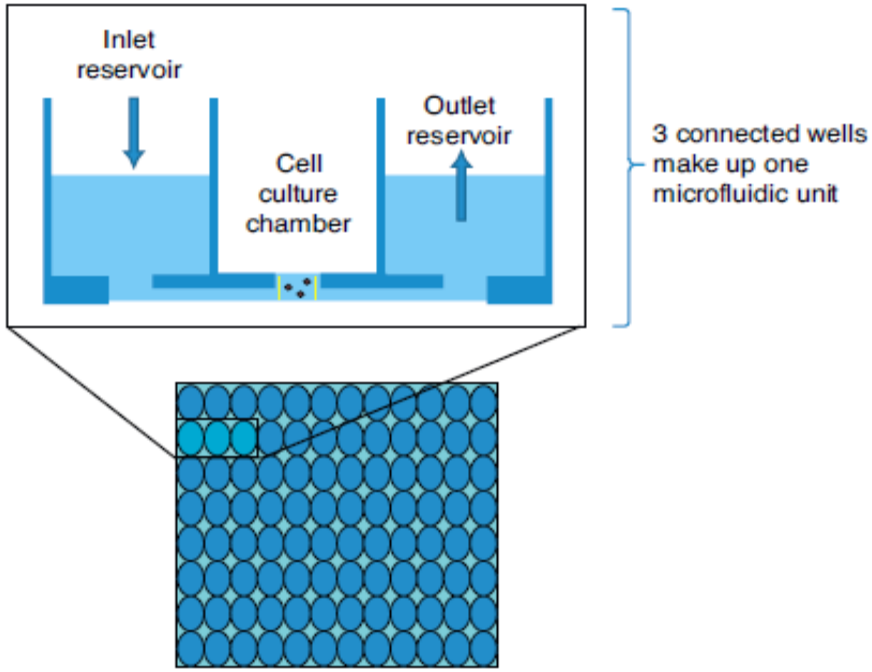
3D cell culture-spheroid methods

Method	Advantages	Disadvantages
Matrices (matrigel) and scaffolds (collagen, laminin, alginate and other biodegradable materials, which form hydrogels)	-provide 3D support that mimics in vivo -some incorporate growth factors	-expensive for large scale production -difficulty in retrieving cells following 3D culture formation -nonhomogenous size



3D cell culture-spheroid methods

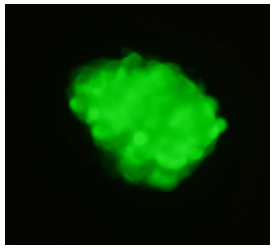
Method	Advantages	Disadvantages
Microfluidic cell culture platforms	-suitable for high throughput testing	-require specialised equipment -further analysis of spheroides are difficult



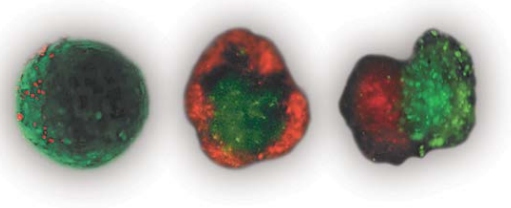
Spheroides-cocultures

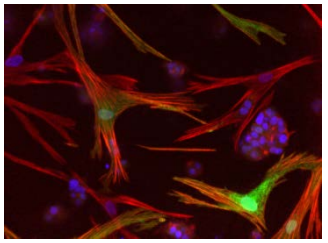
- stable transfected cells with reporter gene (GFP, Luc, dsRed, β-gal) or labeled with fluorescent dye enable easy monitoring and analysing

HepG2-GFP, 10 days old



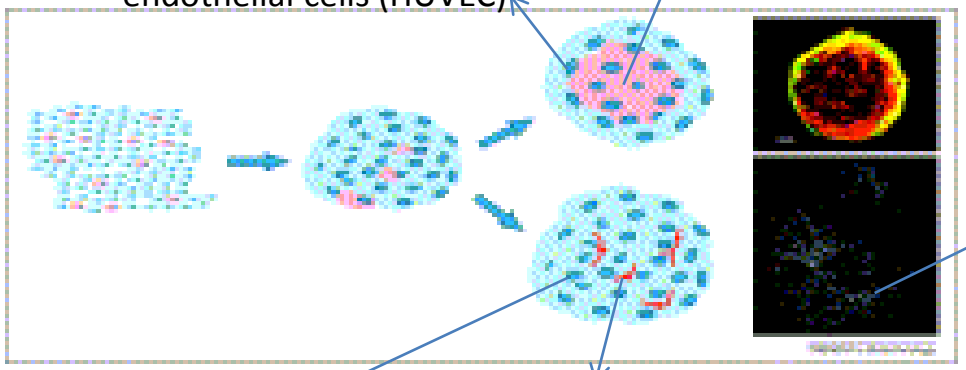
- fibroblasts with tumor cells (epithelial mesenchimal transition-EMT)





Model system for vascularization

- normal endothelial cells with fibroblasts or stem cells (vascularization)



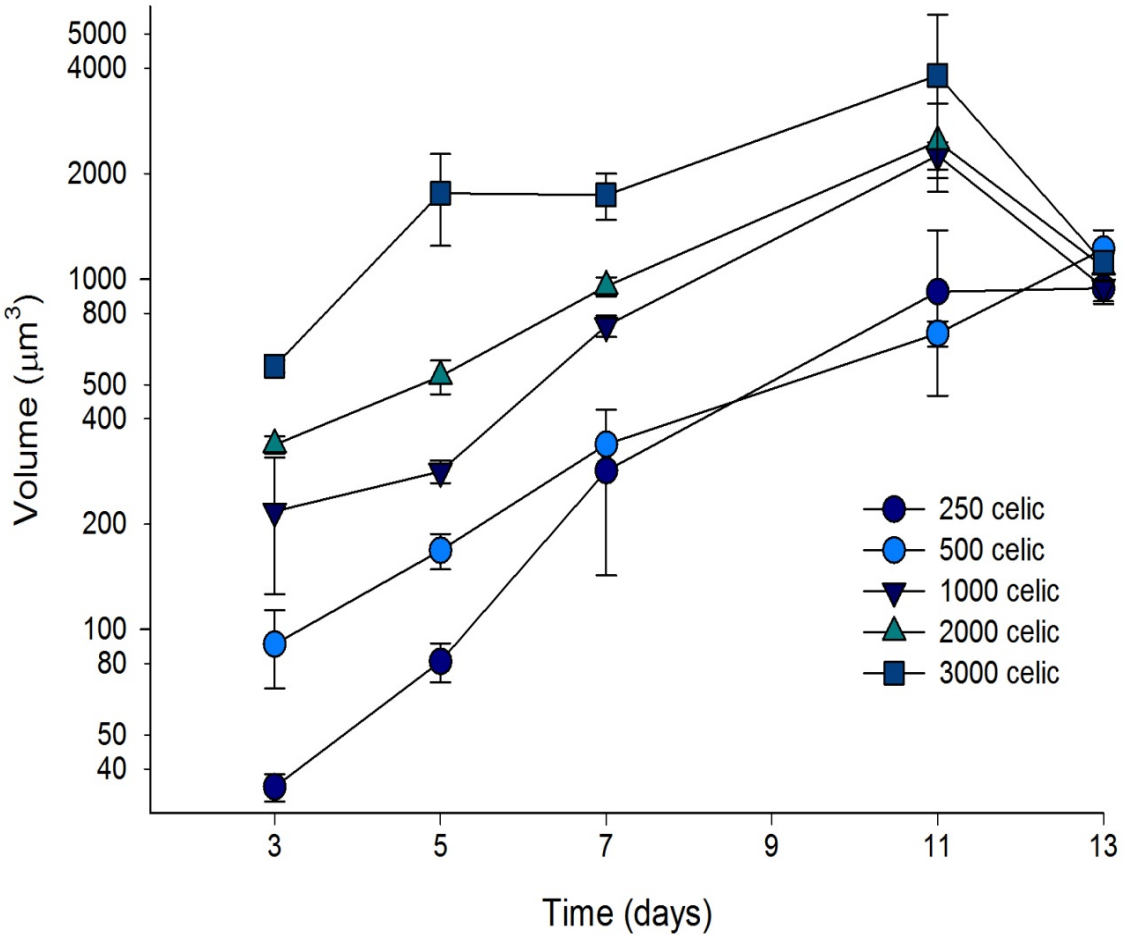
Fennema, Trends in Biotechnology, 2013, 31-2

Analysing and evaluating of spheroides

Growth analysis:

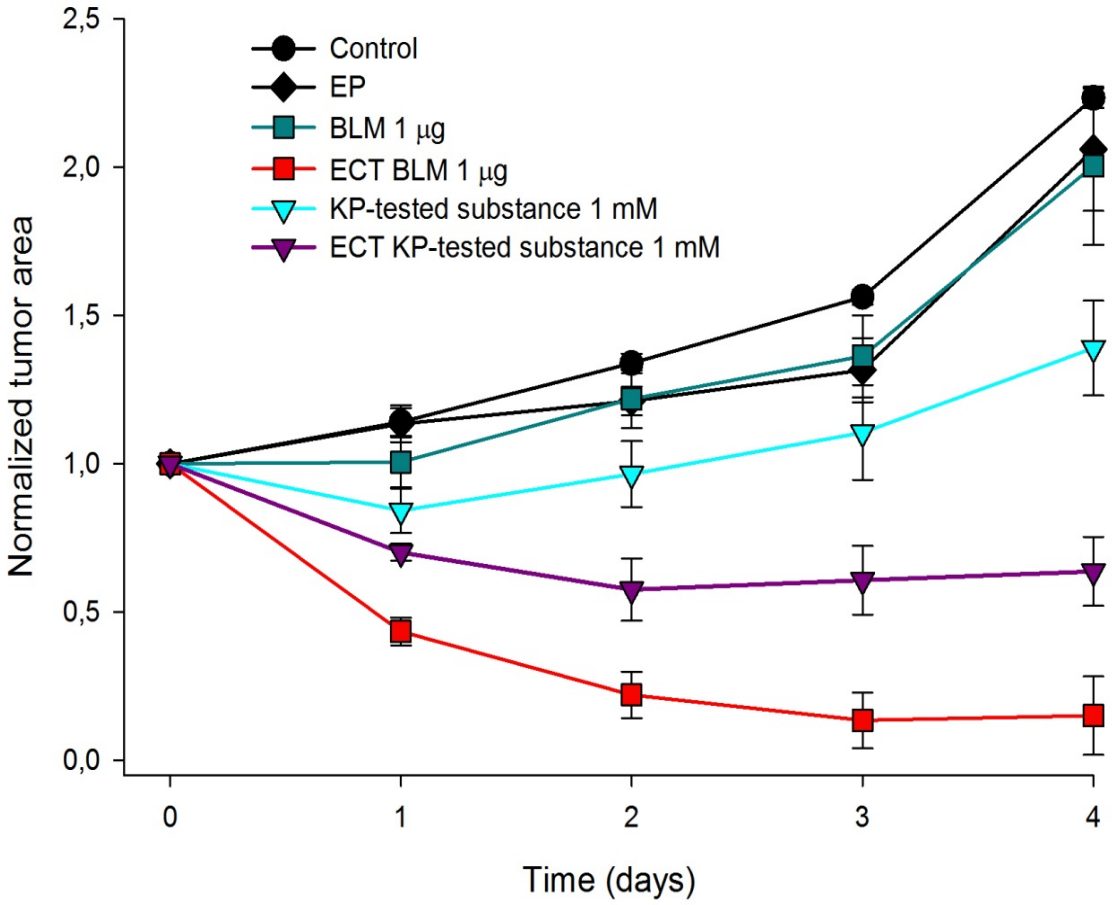
- inverted light or fluorescent microscopy (software for measurement)
- microplate reader (Tecan; Presto Blue, Alarmar Blue, MTS) for evaluating viability, proliferation, migration
- cytometry (Celigo cytometer, fast& automated analysing)
- confocal mycroscopy

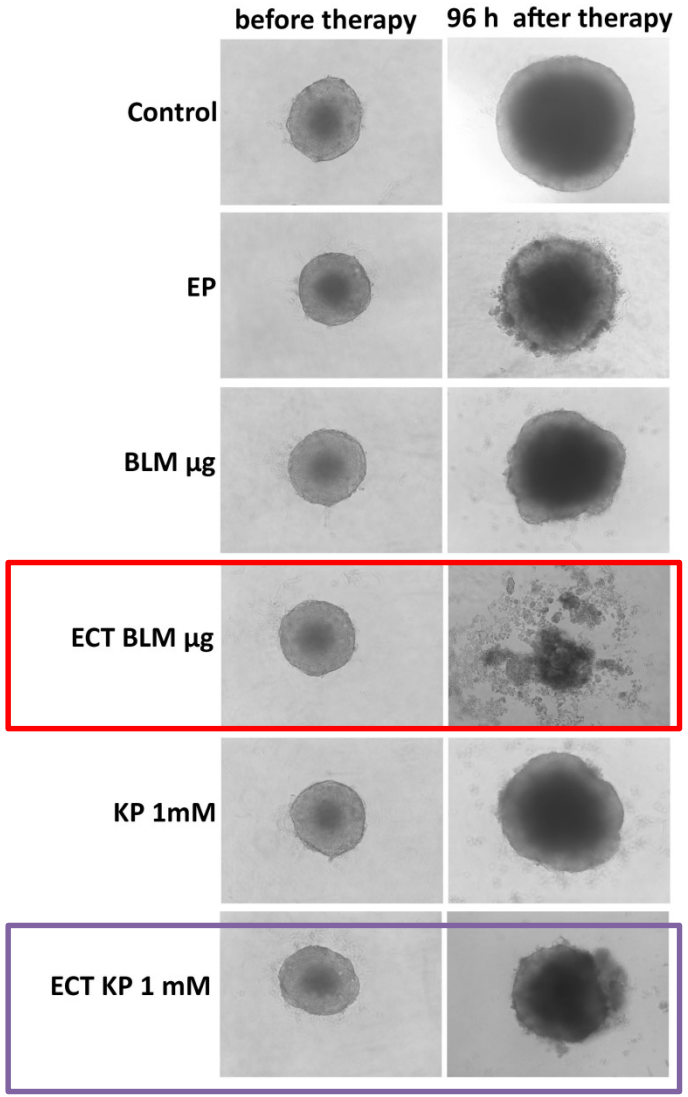
Growth of human squamous cell carcinoma FaDu spheroides



Analysing and evaluating of spheroides after electrochemotherapy

- electrochemoterapy and testing of new drugs (colon cancer carcinoma HT 29spheroides)

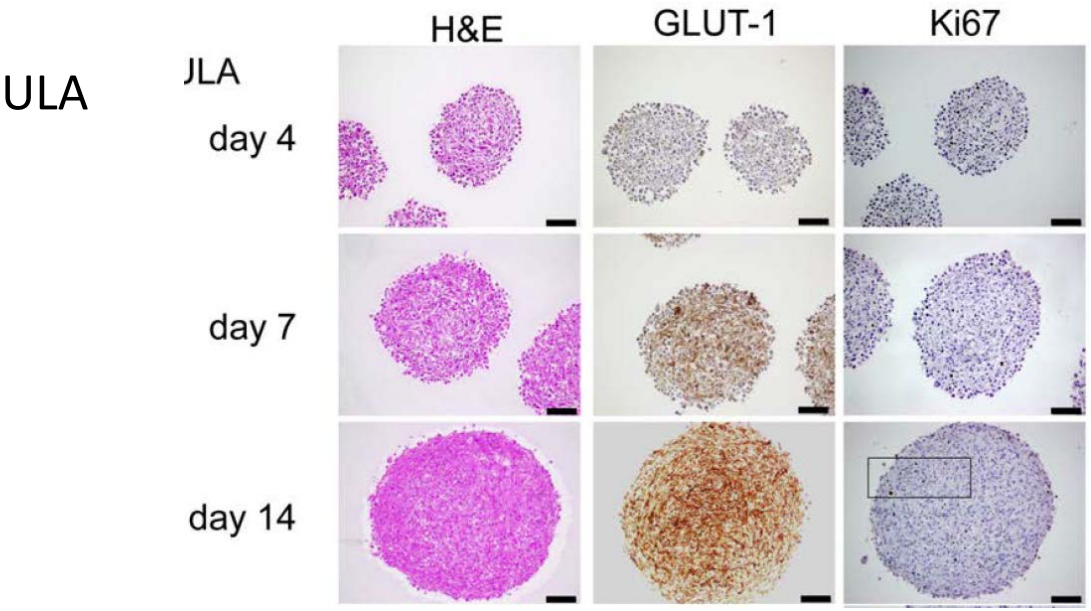




Analysing and evaluating of spheroides

Hystologycal analysis of spheroides:

- embeeding in parafin, Tissue Tek
- immunohistochemistry analysis: hematoxylin and eosin staining, gradient of proliferation (Ki67), hypoxia (glucose transporter 1; GLUT-1)



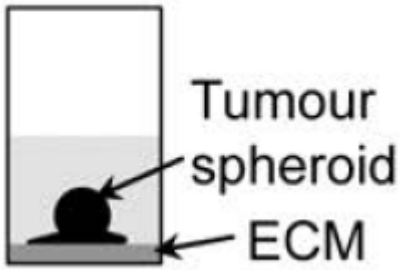
Vinci et al., 2012, BMC Biology, 10:29

Standard analysis on molecular and protein level

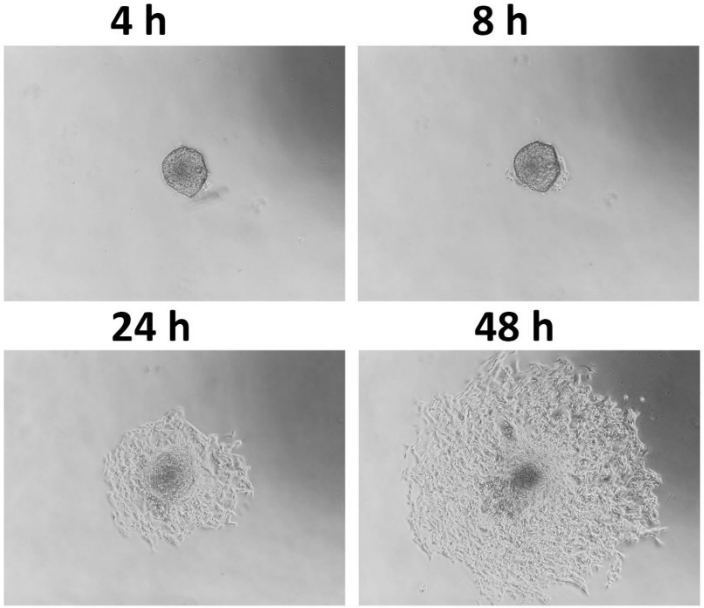
Analysing and evaluating of spheroides

Migration assay on matrix protein:

- migration of cells from spheroid on matrix proteins (fibronectin)

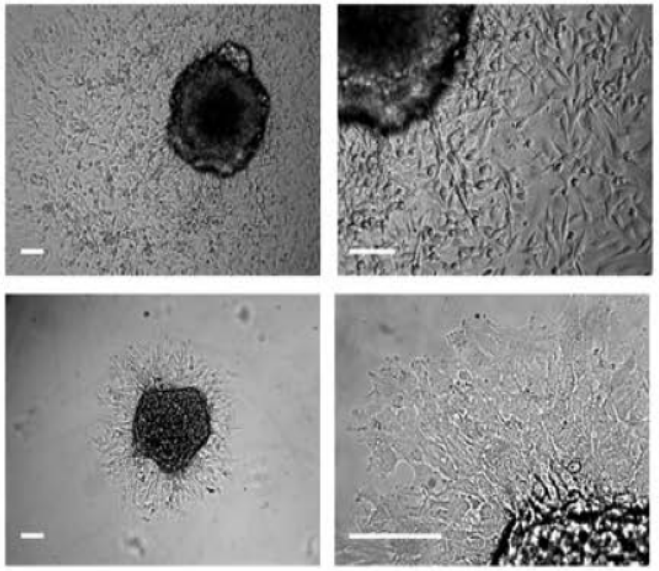


Inverted microscopy



Mouse mamary carcinoma TSA spheroides

Celigo™ cytometer



U-87 MG
dispersed migration

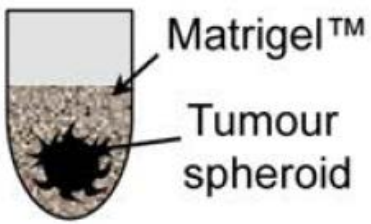
KNS42
radial migration

Vinci et al., 2012, BMC Biology, 10:29

Analysing and evaluating of spheroides

Matrigel invasion assay :

- tumor spheroid embeeded in Matrigel



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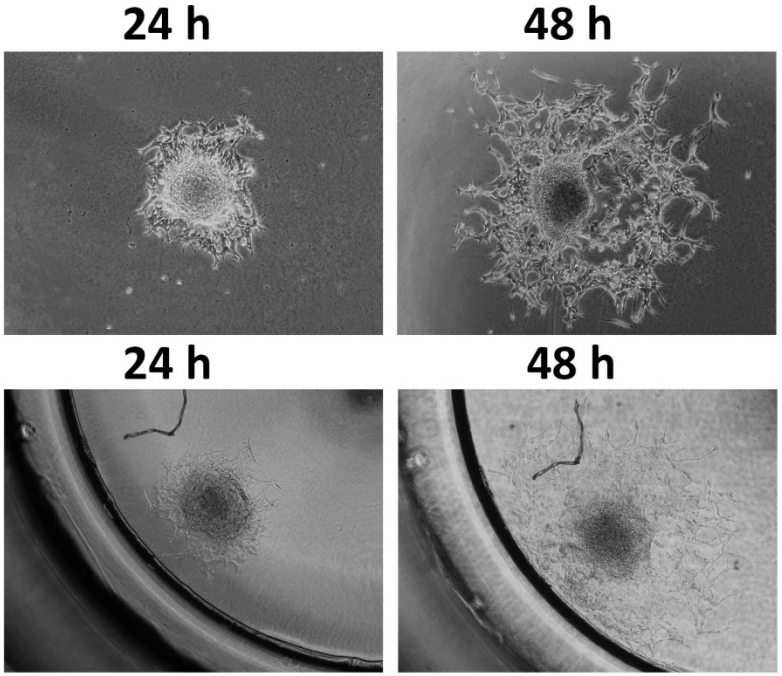

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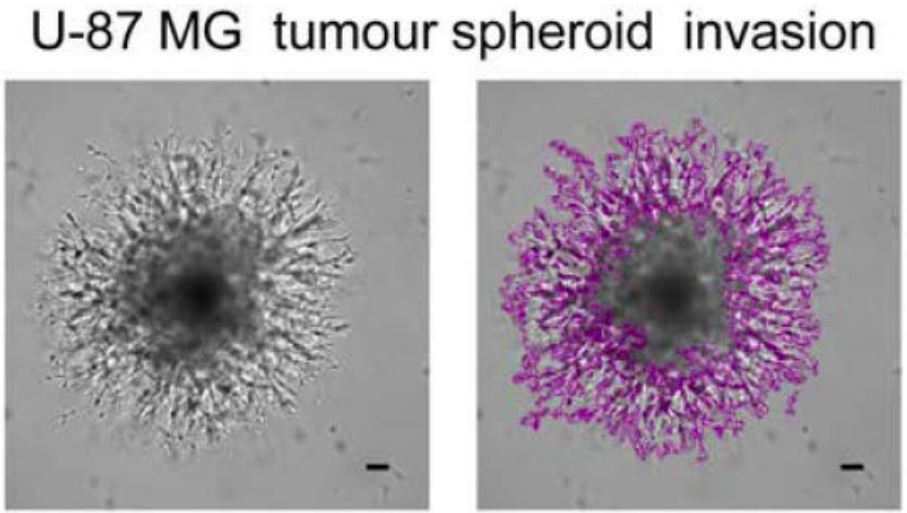

Vinci et al., 2012, BMC Biology, 10:29

Conclusions

- simple and quite unexpensive to grow the spheroides
- the interactions and comunications between the cells in spheroides, secretion of extracelullar matrix - in vivo phenotype of cells is retained
- in spheroides nutrient, oxygen and waste gradients are present
- studies performed on spheroides gives us more **reliable** results *in vitro*
- **importantly complies with the ethical principles of animal research (3 R's: Reduction, Refinement, and Replacement).**

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