UniversitätsSpital Zürich

Klinik für Hämatologie

University of Zurich

3D cell culture through magnetic levitation

21. October 2014

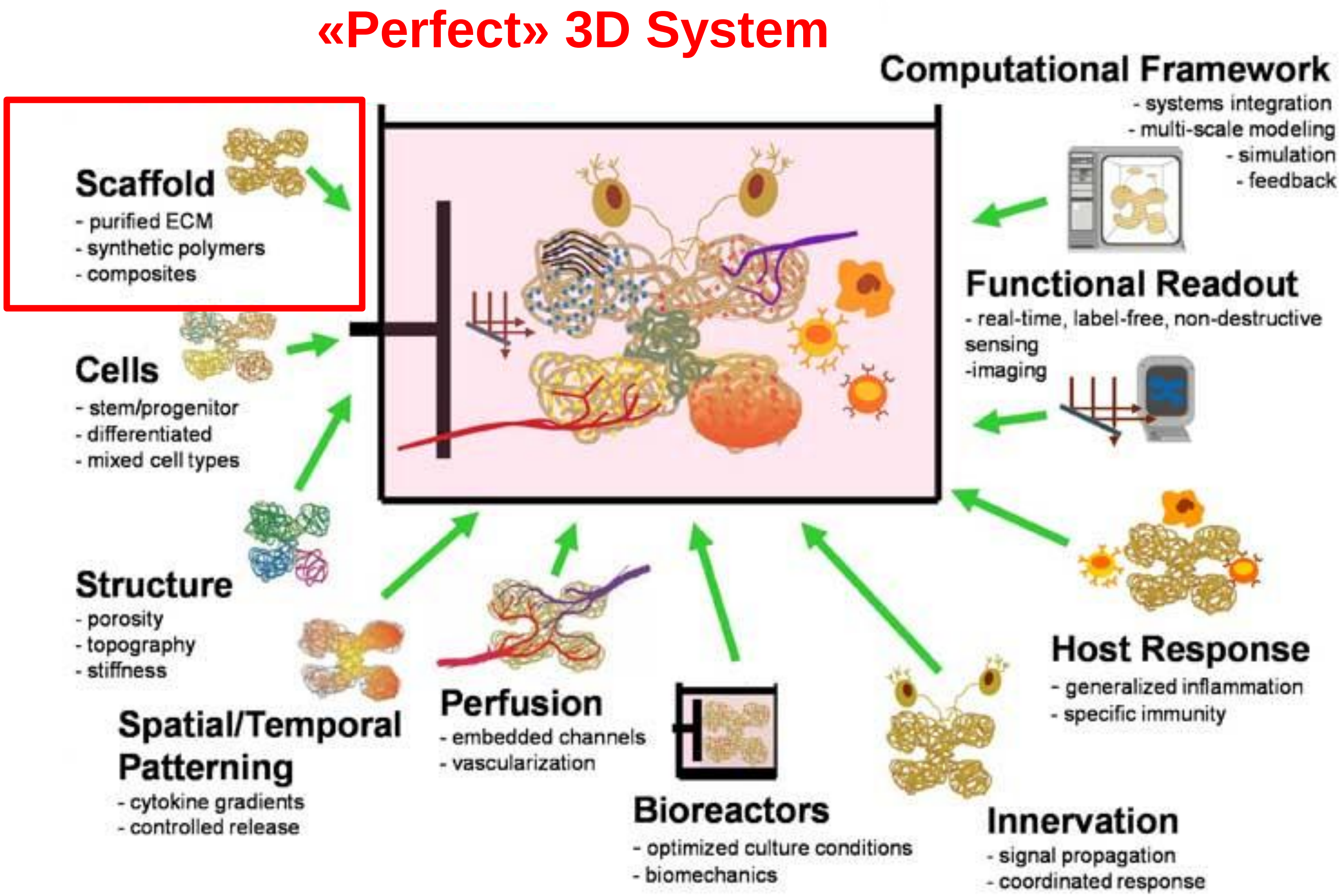
Technical Journal Club

Kristin Fritsch

3D cell culture – Why ?

- Cells in tissues are in a three-dimensional environment having characteristic biophysical and biomechanical signals (e.g. migration, adhesion, proliferation and gene expression)
- 3-D cell culture more accurately simulates normal cell morphology, proliferation, differentiation and migrations, as compared to 2-D cell culture
- 3-D culture systems can be used to study disease models by cellular modeling different disease states
- 3-D culture systems can be used to study the effect of drug dosages, drug screening for toxicity and efficacy
- 3-D cell culture has direct applications in tissue engineering and regenerative medicine

Generalized Components of a 3D Tissue Model



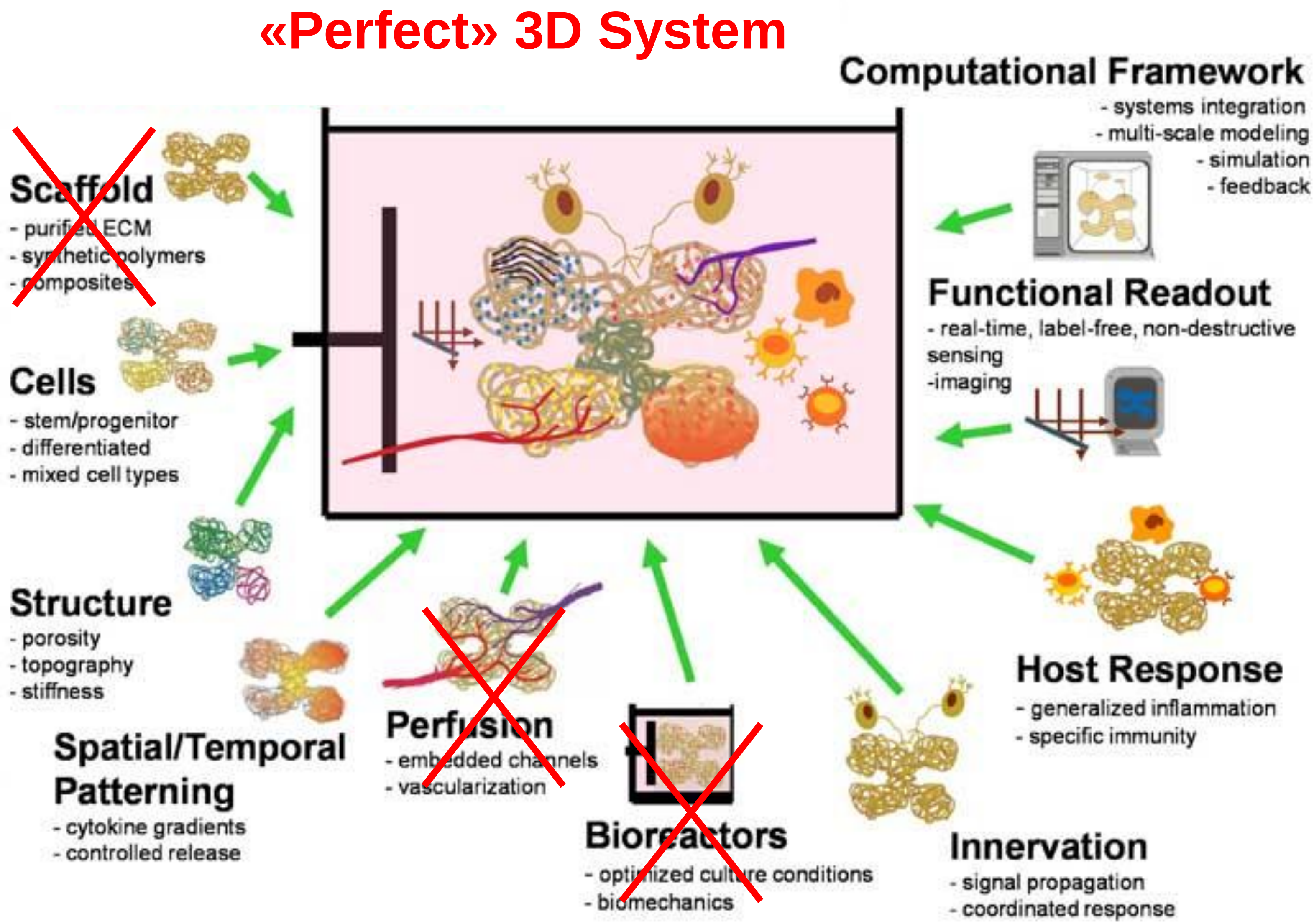
http://www.niehs.nih.gov/news/newsletter/2011/august/science-symposium/

Scaffolds

	Materials	Advantages
Natural	Silk, collagen, gelatin, fibrinogen, hyaluronic acid, alginate	• Biodegradable • Easily available • Bioactive, interact with cells
Synthetic	PEG, PGA, PMMA, PLGA	• Facilitate restoration of structure of damaged tissues • Inert • Long shelf-life • Easily tailored for desired porosity and degradation time • Predictable and reproducible mechanical and physical properties

http://www.labome.com/method/Three-Dimensional-Cell-Culture-A-Review.html

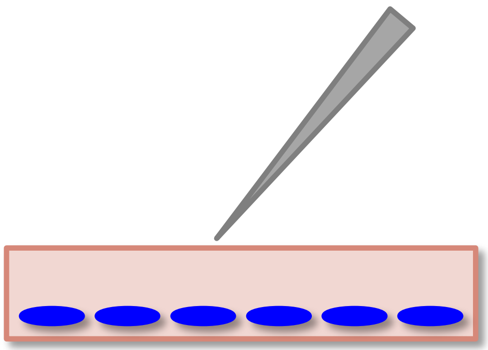
magnetic levitation method (MLM)



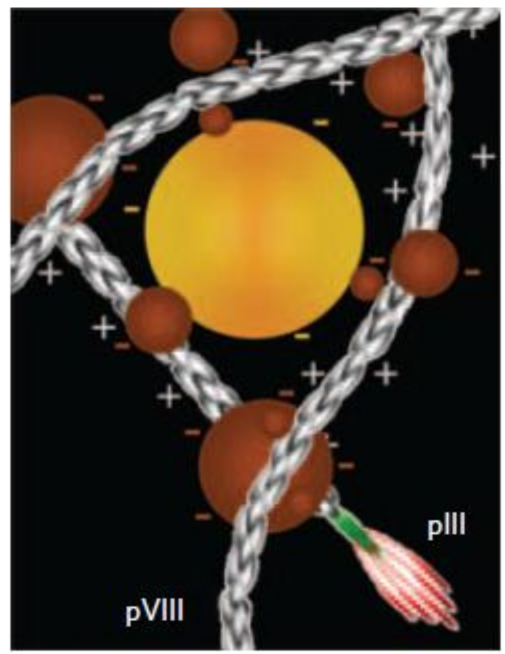
http://www.niehs.nih.gov/news/newsletter/2011/august/science-symposium/

magnetic levitation method (MLM)

magnetic nanoparticle
(Nanoshuttle)



2D-culture



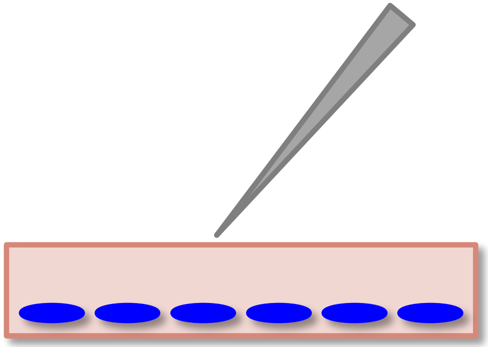
Souza et.al., Nature Nanotech 2010

cell-adhesive
peptide sequences
(bacteriophage or
Polylysine)
+
magnetic iron oxide
+
gold nanoparticles

> self-assemble into
hydrogels

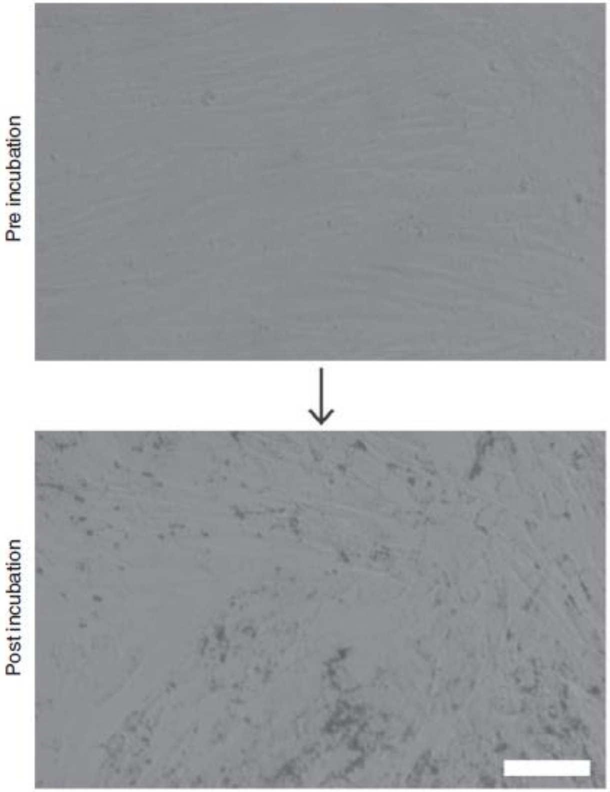
magnetic levitation method (MLM)

magnetic nanoparticle
(Nanoshuttle)



2D-culture

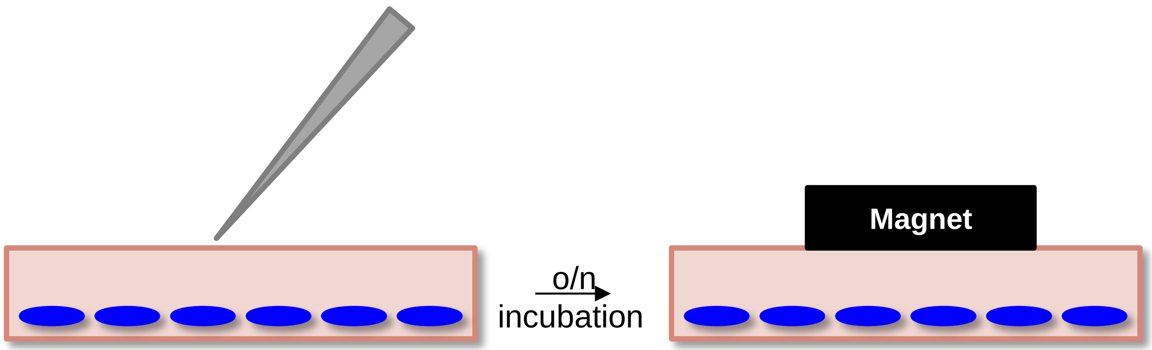
o/n
incubation



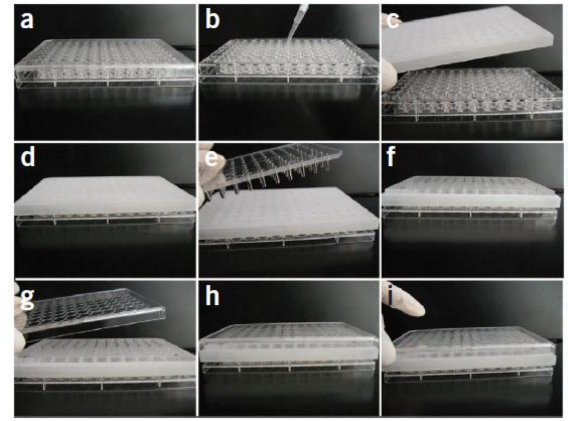
Haisler et.al., Nature 2013

magnetic levitation method (MLM)

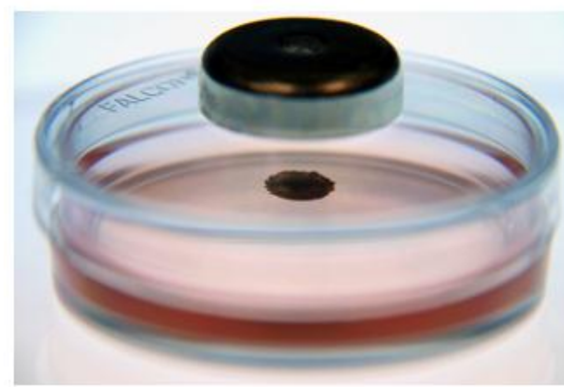
magnetic nanoparticle
(Nanoshuttle)



2D-culture



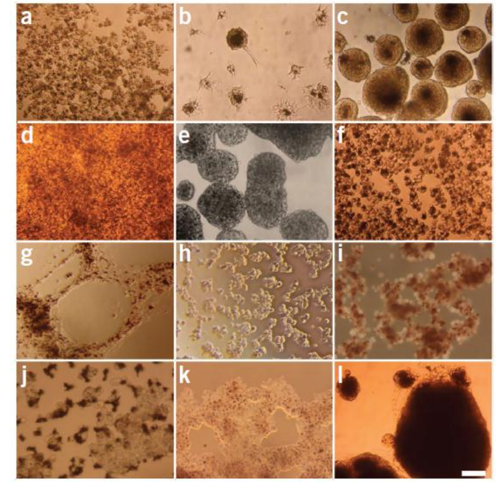
Haisler et.al., Nature 2013



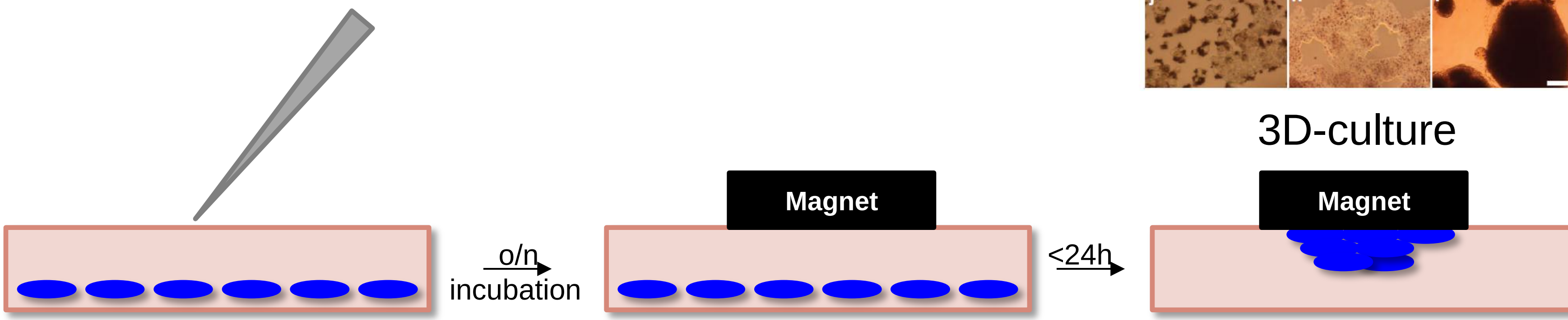
http://www.n3dbio.com/about/our-mission/

magnetic levitation method (MLM)

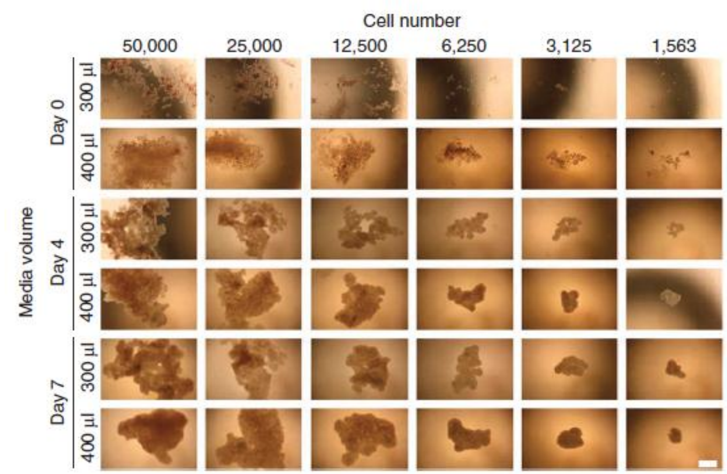
magnetic nanoparticle
(Nanoshuttle)



3D-culture

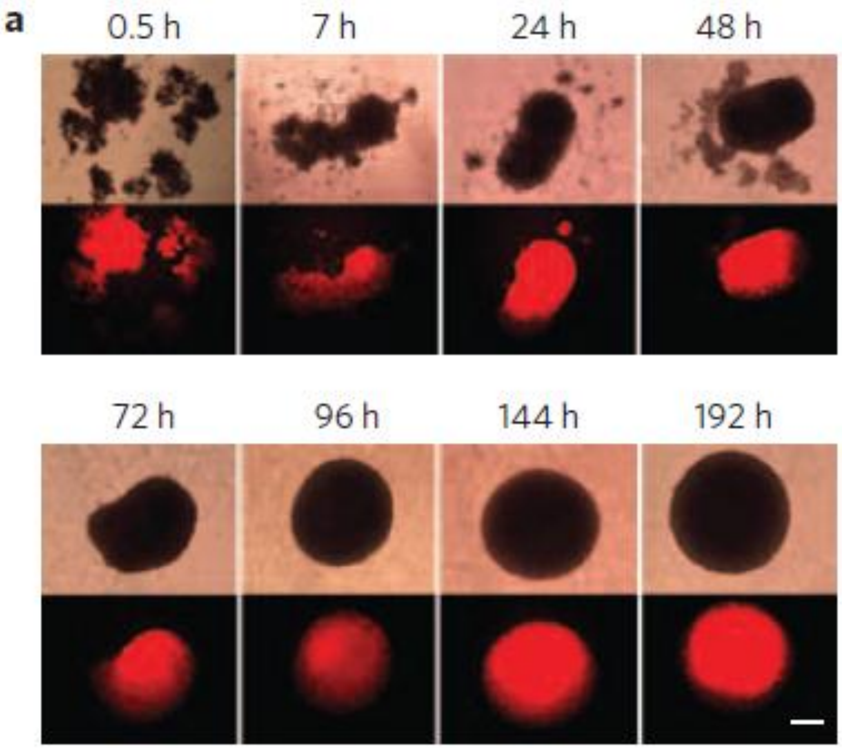


2D-culture

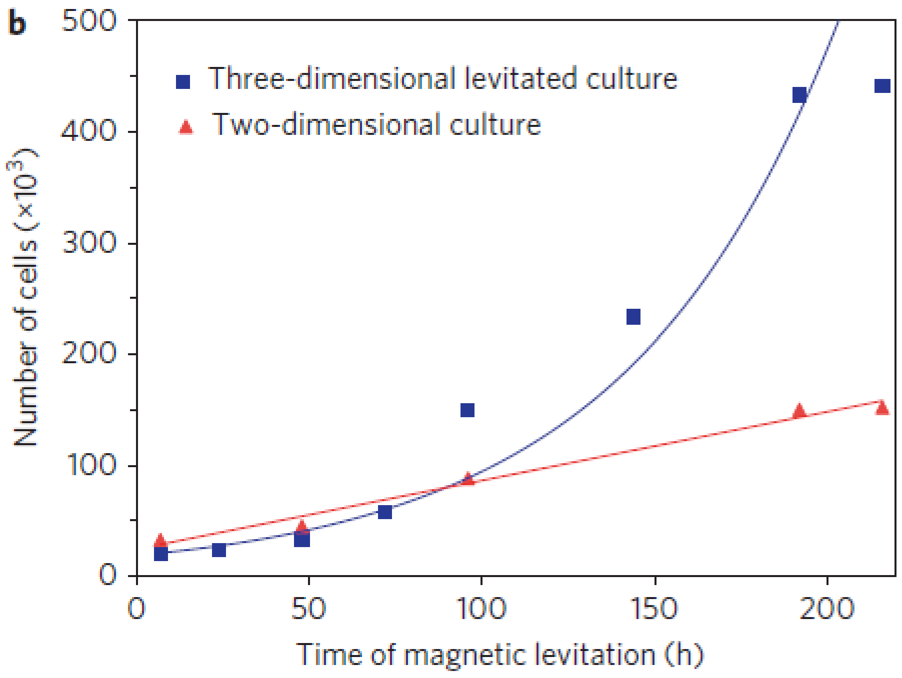


Haisler et.al., Nature 2013

Comparison of three-dimensional cell growth with standard two-dimensional tissue culture

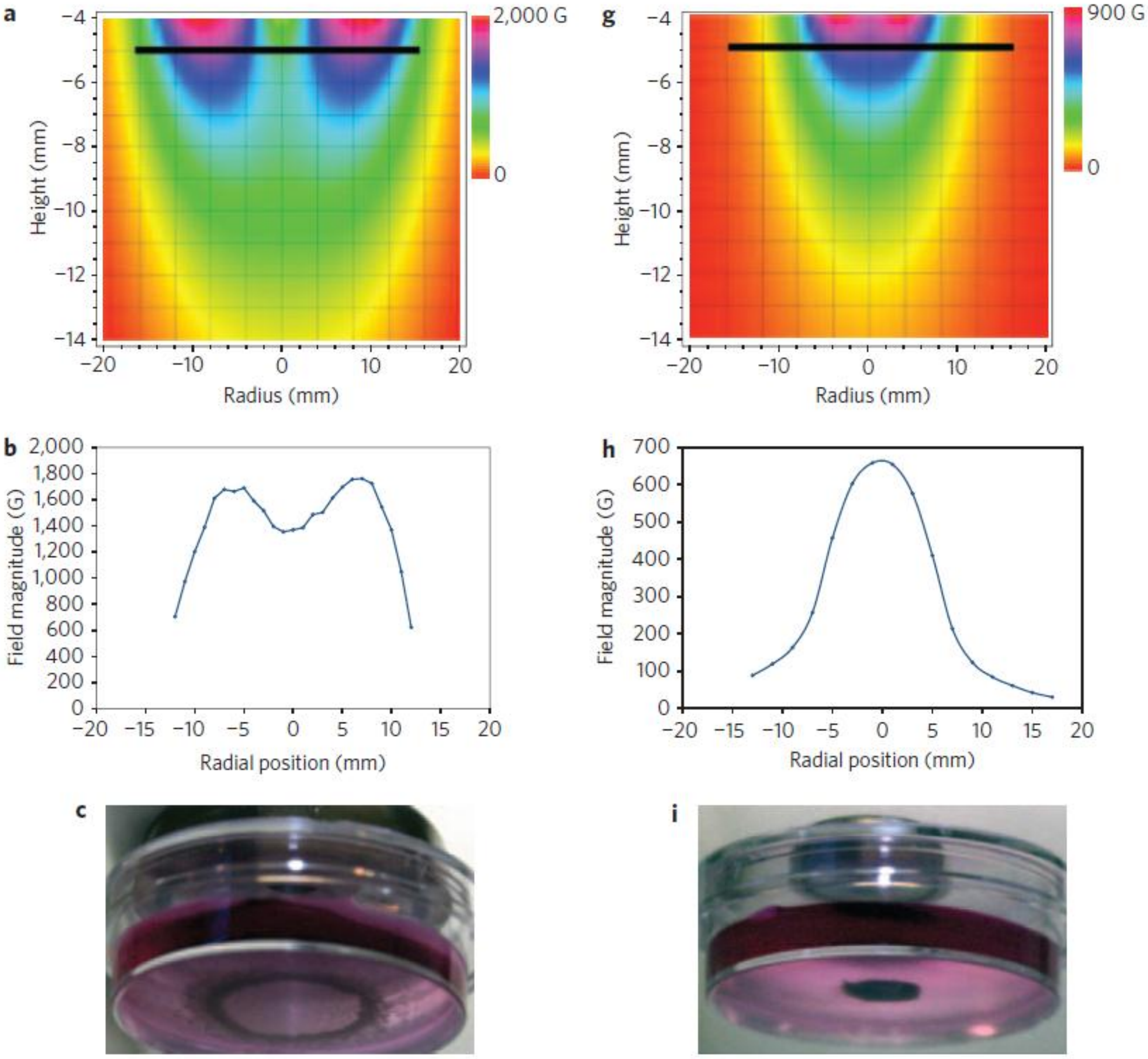


~1 mm



Souza et.al., Nature Nanotech 2010

Shape control of magnetically levitated culture



Calculation of magnetic fields

$$\vec{B}(\vec{r}) = \frac{\mu_0}{4\pi}\int_S \frac{\vec{K}(\vec{r}\,')\times(\vec{r}-\vec{r}\,')}{\left|\vec{r}-\vec{r}\,'\right|^3}da'$$

$\vec{r}$ of

Souza et.al., Nature Nanotech 2010

Applications

	Name	Animal	Type
Cell lines	HEK293	Human	Embryonic kidney
	MDA-231	Human	Mammary epithelial
	MCF-10A	Human	Mammary epithelial
	LNCaP	Human	Prostate epithelial
	A549	Human	Alveolar epithelial
	HepG2	Human	Hepatocyte
	3T3-L1	Mouse	Fibroblast[18]
	bEnd.3	Mouse	Brain endothelial[18]
	H-4-II-E	Rat	Hepatoma
	U251-MG	Human	Glioblastoma[13,19]
Primary cells		Human	Astrocyte[13,19]
		Human	Pulmonary endothelial[17]
		Human	Type II alveolar epithelial
		Human	Bronchial epithelial[17]
		Human	Tracheal smooth muscle[17]
		Human	Pulmonary fibroblasts[17]
		Human	Umbilical vein endothelial
		Human	Chondrocytes
		Human	Aortic vascular smooth muscle[22]
		Porcine	Aortic valvular interstitial
		Porcine	Aortic valvular endothelial
Stem cells		Human	Neural stem cells[13]
		Human	Mesenchymal stem cells
		Human	Dental pulp stem cells
		Human	Adipose stem cells[18]

- cell lines, stem cells and primary cells
- different biochemical or environmental conditions
- scalable in size (96-well plates, 6-well plates or Petri dishes)
- Analysis by common biological research techniques (WB, IHC)

Haisler et.al., Nature 2013

Advantages

- Not time consuming (MLM takes about 16 h to form 3D cultures)
- No artificial protein substrate (e.g. synthetic polymer scaffolds) needed
- no specialized media required
- No extensive fabrication
- magnetic nanoparticles have been shown to not affect cell proliferation and metabolism or induce an inflammatory response

➔ **MLM is a simpler tool for creating representative 3D cell culture environments compared with other methods**

TISSUE ENGINEERING: Part C
Volume 19, Number 5, 2013

DOI: 10.1089/ten.tec.2012.0198

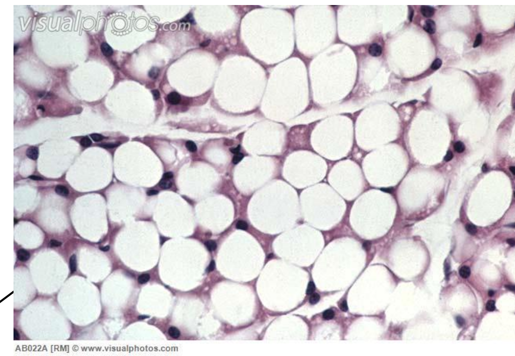
Adipose Tissue Engineering in Three-Dimensional Levitation Tissue Culture System Based on Magnetic Nanoparticles

Alexes C. Daquinag, PhD,[1] Glauco R. Souza, PhD,[2] and Mikhail G. Kolonin, PhD[1]

aim

tissue culture model simulating the complex intercellular interactions of white adipose tissue (WAT) components

white adipose tissue (WAT)



differentiated adipocytes

stromal mesenchymal progenitors = adipose stromal cells (ASC)

endothelial vascular cells

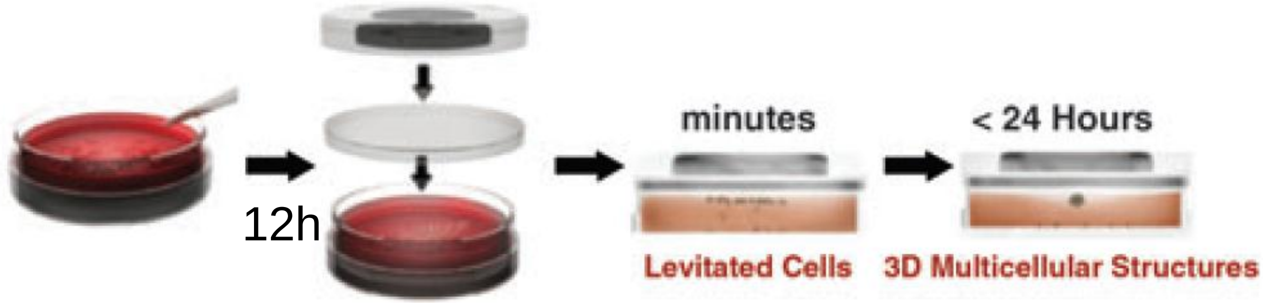
infiltrating leukocytes

Adipocyte-depleted stromal vascular fraction (SVF)

magnetic levitation system for adipocyte culture

primary cells/
cultured cells

magnetic
drive



+
Nanoshuttle
Solution

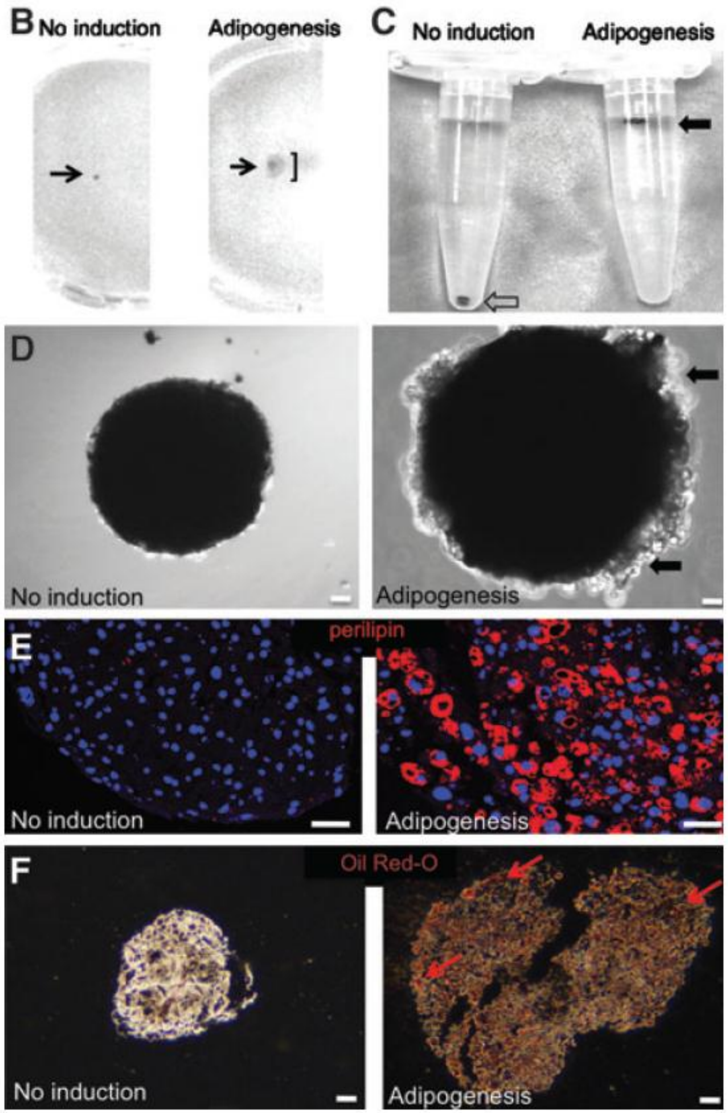
multiwell ultralow-
attachment plate

Self-assemble
into spheroid

Adipogenesis induction

d14

d8



3T3-L1 preadipocytes
(levitated for 1 day)

+

Adipogenic differentiation medium for 72h
(0.5mM isobutylmethyxanthine, 1 mM dexamethasone, 0.2mM indomethacin, and 1.7 mM insulin in DMEM/10% FBS (v/v))

↓

Replacement of medium
(with DMEM/10% FBS containing 1.7 mM insulin)

↓

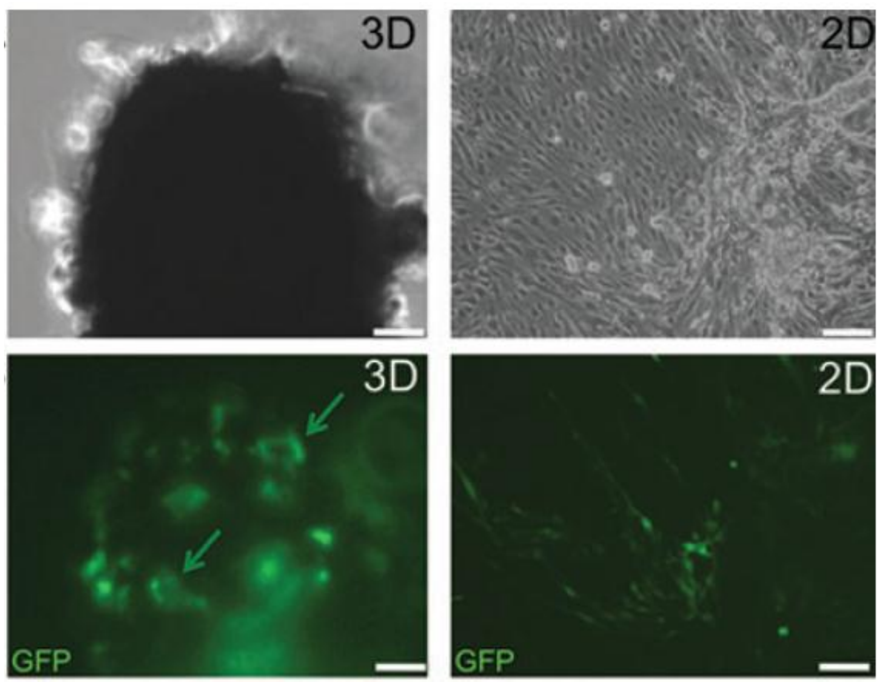
analysis day 45

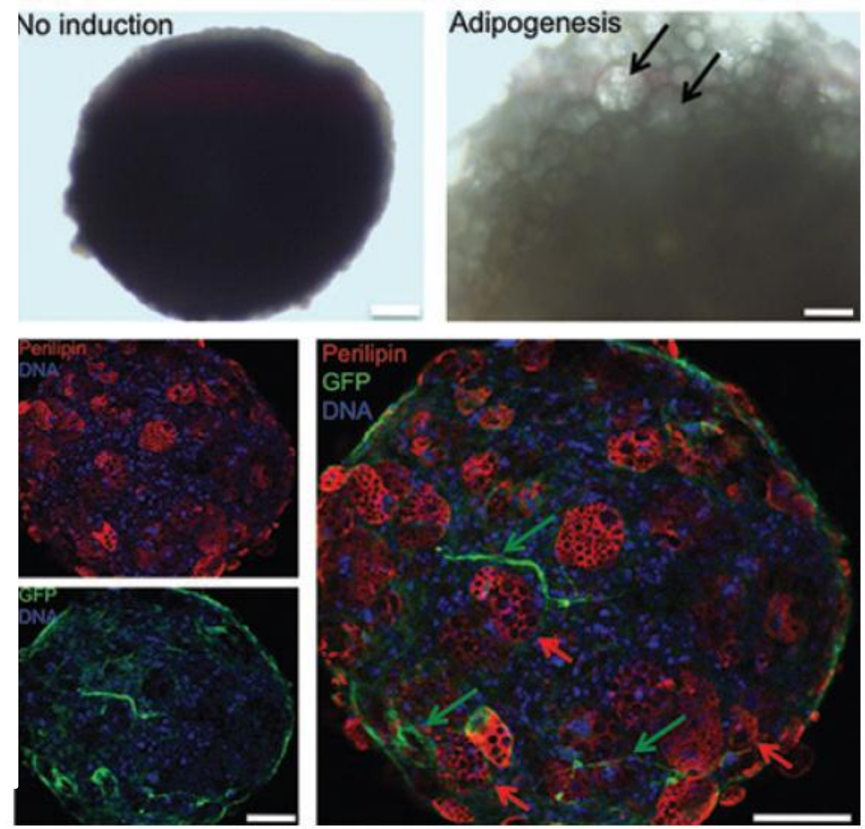
Preadipocytes and endothelial cells cooperate in 3D co-culture

3T3-L1
(Preadipocytes)

bEND.3-GFP
(endothelial cells)

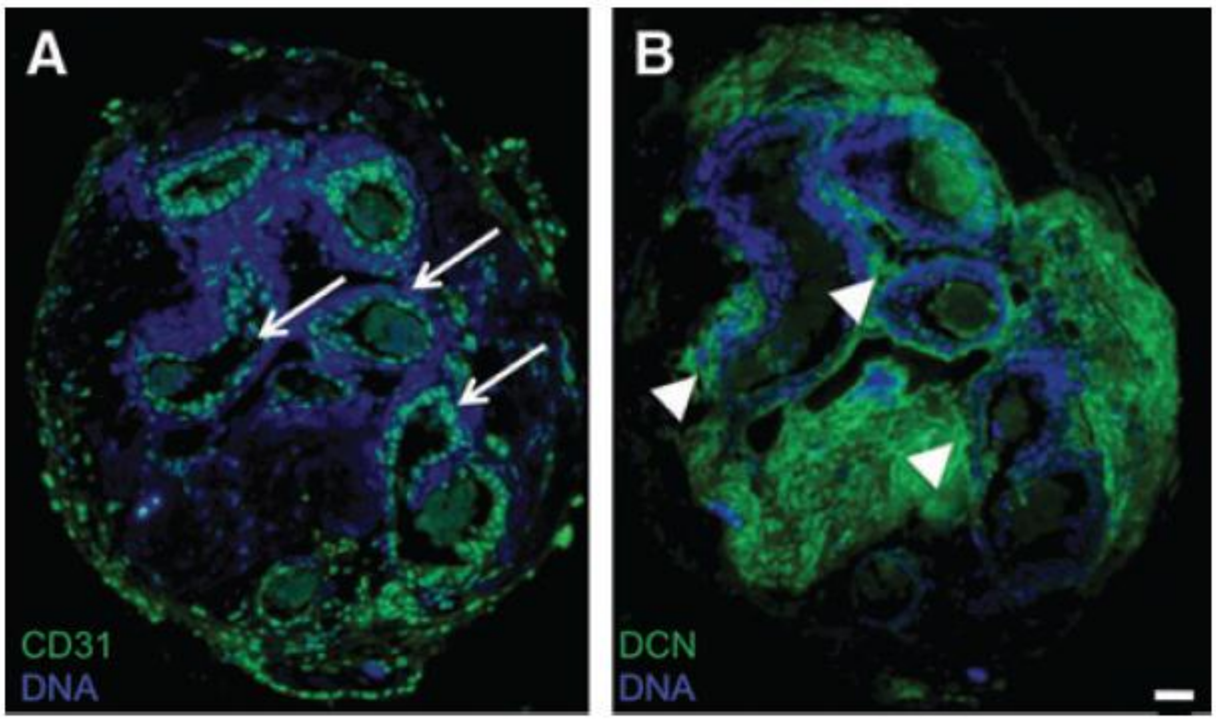
→ Magnetic levitation → Adipiogenesis induction





Vascularization in adipospheres formed by primary WAT cells

SVF of mouse visceral WAT → Magnetic levitation → Angiogenesis induction → WAT

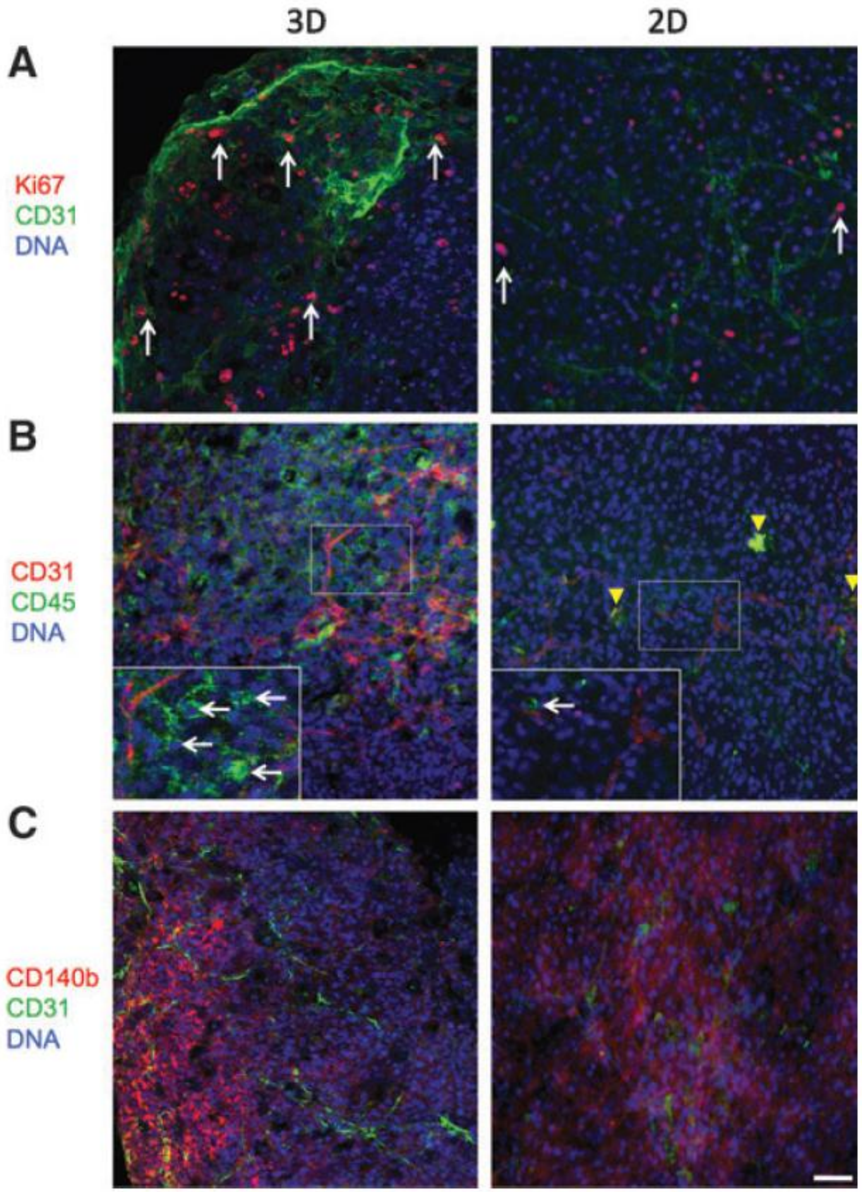


lumen formation by endothelial (CD31 +) cells

DCN+ stroma
perivascular localization of ASC

➡ **perivascular localization of ASC**

Cell composition, organization, and proliferation in primary adipospheres

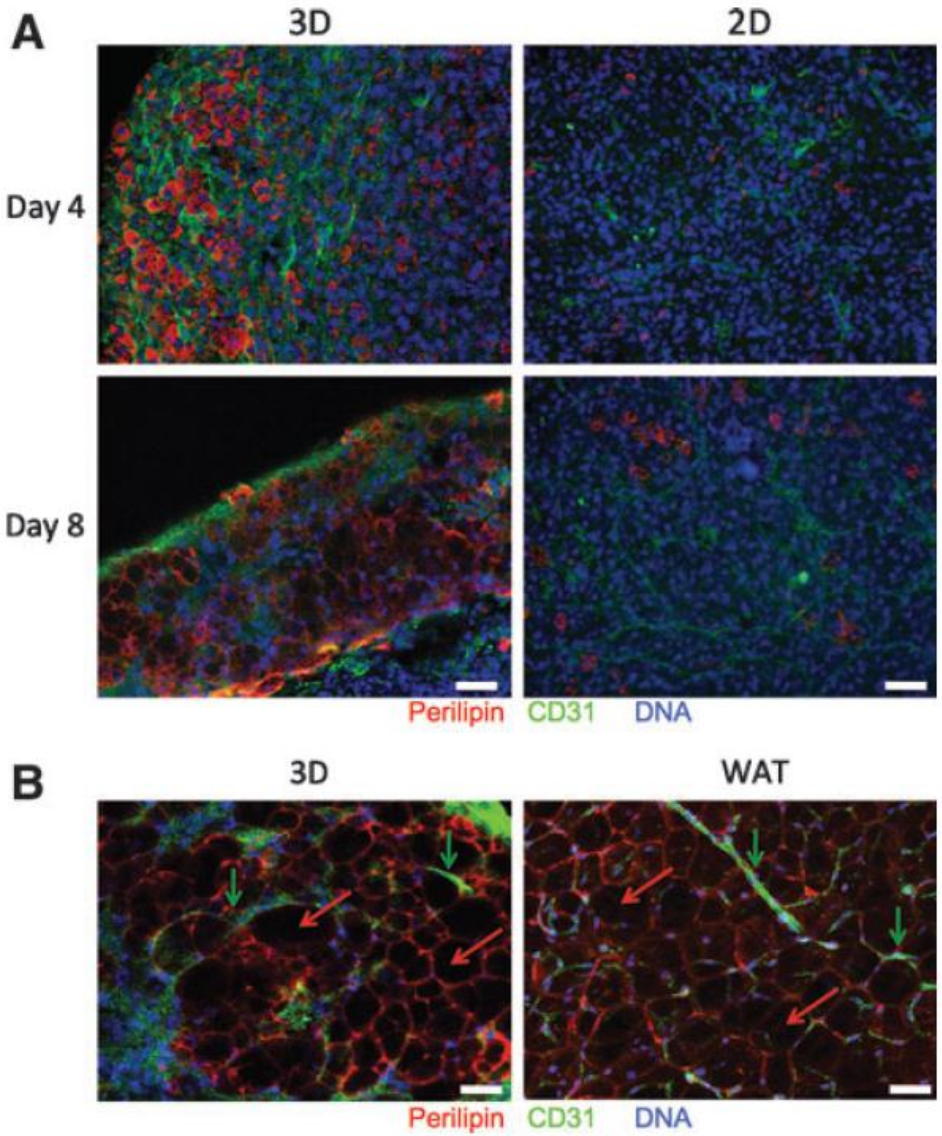


→ increased frequency of proliferating Ki67 + cells

→ increased frequency of hematopoietic (CD45+) cells

→ Formation of stromal/vascular network

Adipogenesis in adipospheres made from primary WAT cells



→ Larger and more numerous perilipin + lipid droplets

→ similar sizes of adipocytes and comparable appearance of CD31 + vessels

summary

- 3T3-L1 preadipocytes remain viable in spheroids for a long period of time, while in 2D culture, they lose adherence and die after reaching confluence

- adipogenesis induction in efficiently formed large lipid droplets

- Adipocyte-depleted stromal vascular fraction (SVF) of mouse WAT cultured in 3D underwent assembly into organoids with vascular-like structures containing luminal endothelial and perivascular stromal cell layers

- Adipospheres made from primary WAT cells displayed robust proliferation and complex hierarchical organization

- Adiposphere-based coculture of preadipocytes with murine endothelial cells led to a vascular-like network assembly

➢ **Method provides WAT modeling ex vivo and new platform for functional screens to identify molecules bioactive toward individual adipose cell populations**
➢ **can be adopted for WAT transplantation applications and aid other approaches to WAT-based cell therapy**

SCIENTIFIC REPORTS

Three-Dimensional *In Vitro* Co-Culture Model of Breast Tumor using Magnetic Levitation

Hamsa Jaganathan[1]*, Jacob Gage[2]*, Fransisca Leonard[1]*, Srimeenakshi Srinivasan[1], Glauco R. Souza[2], Bhuvanesh Dave[3] & Biana Godin[1]

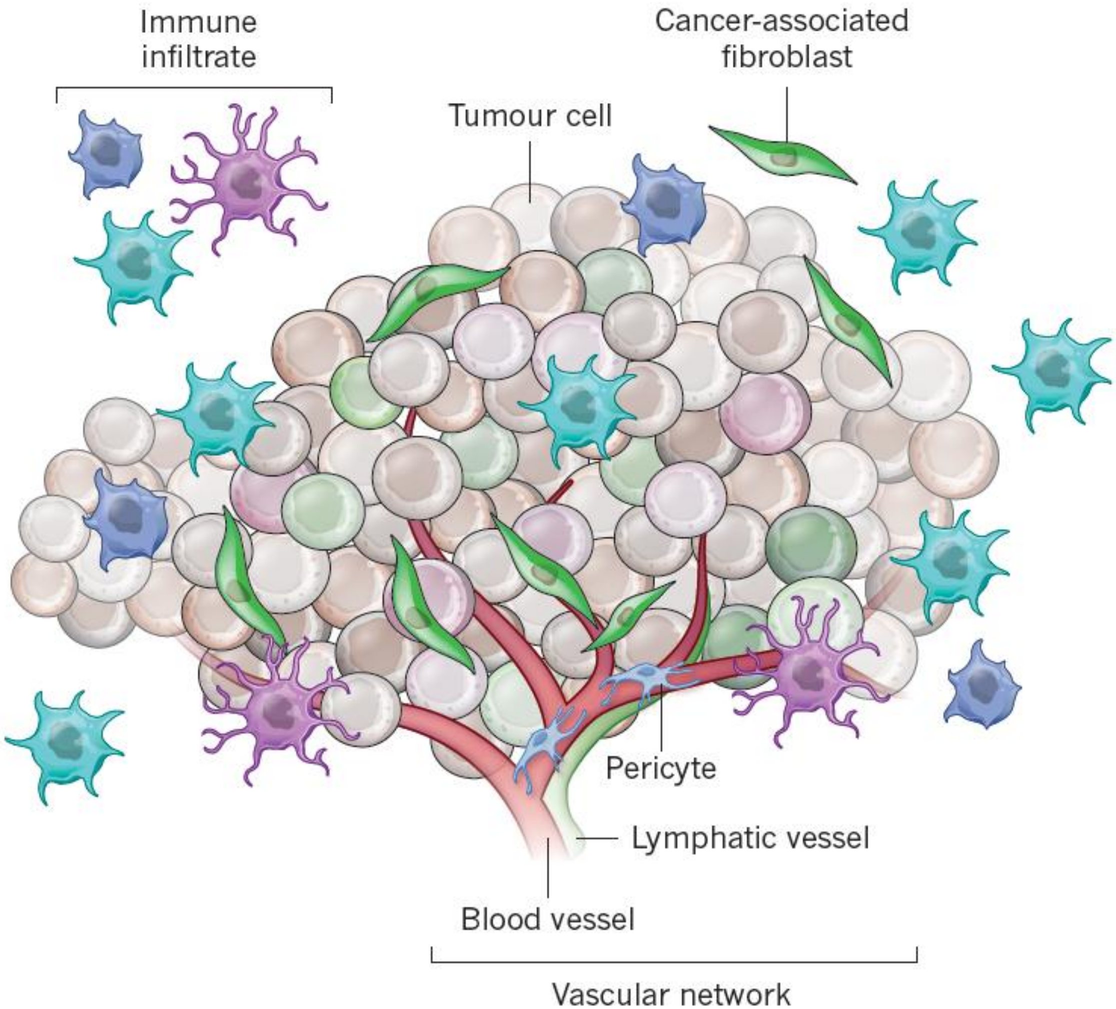
[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030 USA, [2]n3D Biosiences Inc, Houston, TX, 77030 USA, [3]Cancer Center of Excellence, Houston Methodist Research Institute, Houston, TX 77030 USA.

Nature, October 2014

Aim

in vitro model to mimic heterogeneous breast tumors without the use of a scaffold while allowing for cell-cell and tumor-fibroblast interactions

tumour heterogeneity

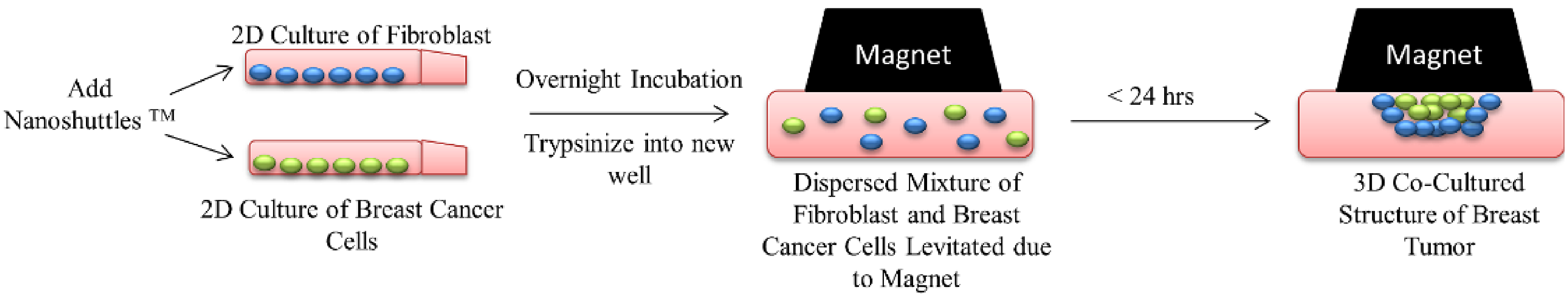


breast tumor stroma consists of:

- fibroblasts
- adipocytes
- endothelial cells
- inflammatory cells with different enzymes and growth factors

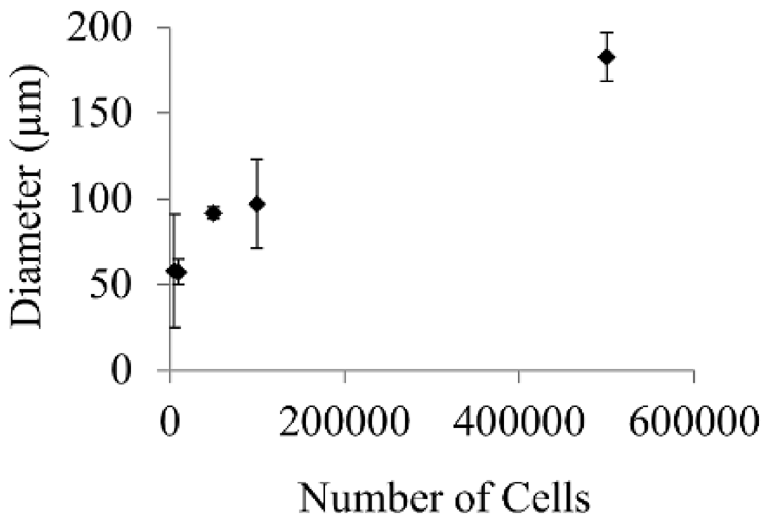
Junttila & Sauvage, Nature Review 2013

formation of 3D in vitro breast tumors using a co-culture of breast cancer and fibroblast cells

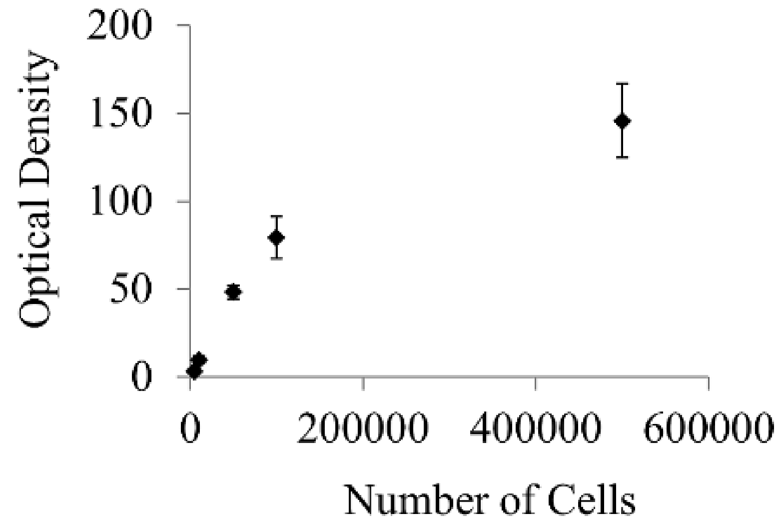


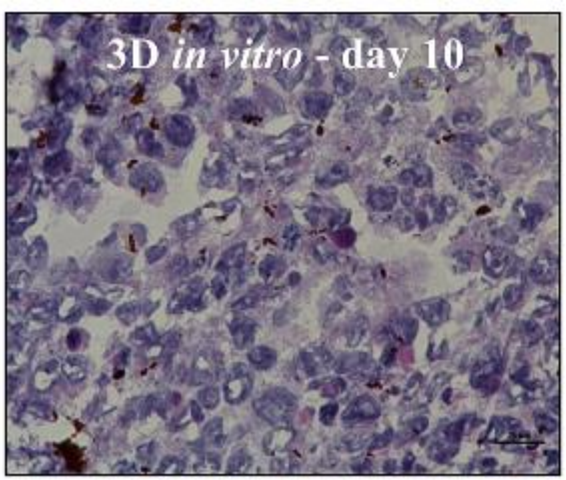
formation of 3D in vitro breast tumors using a co-culture of breast cancer and fibroblast cells

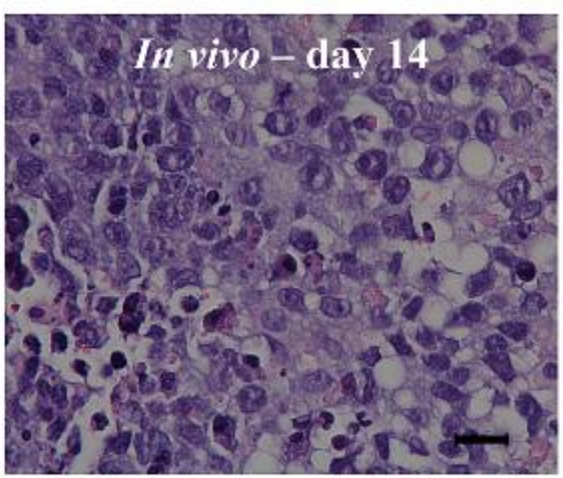
Day 1



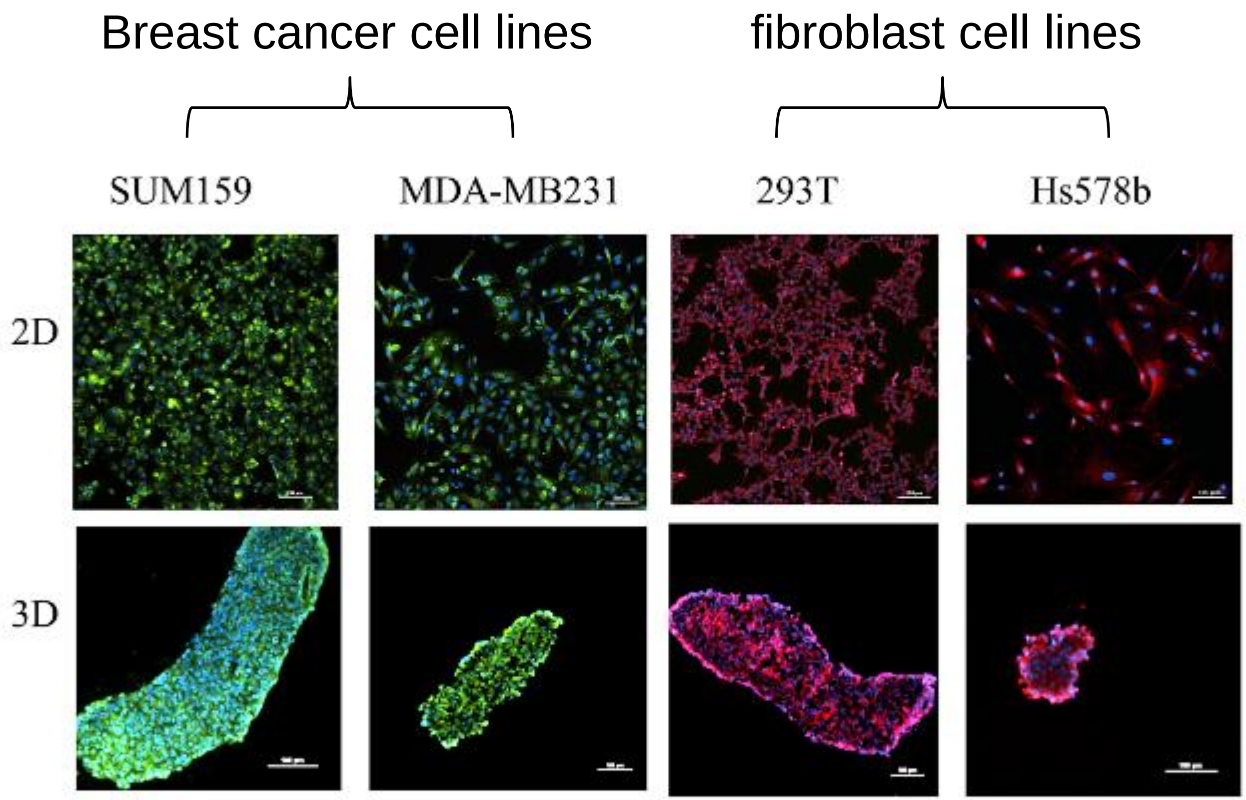
Day 1





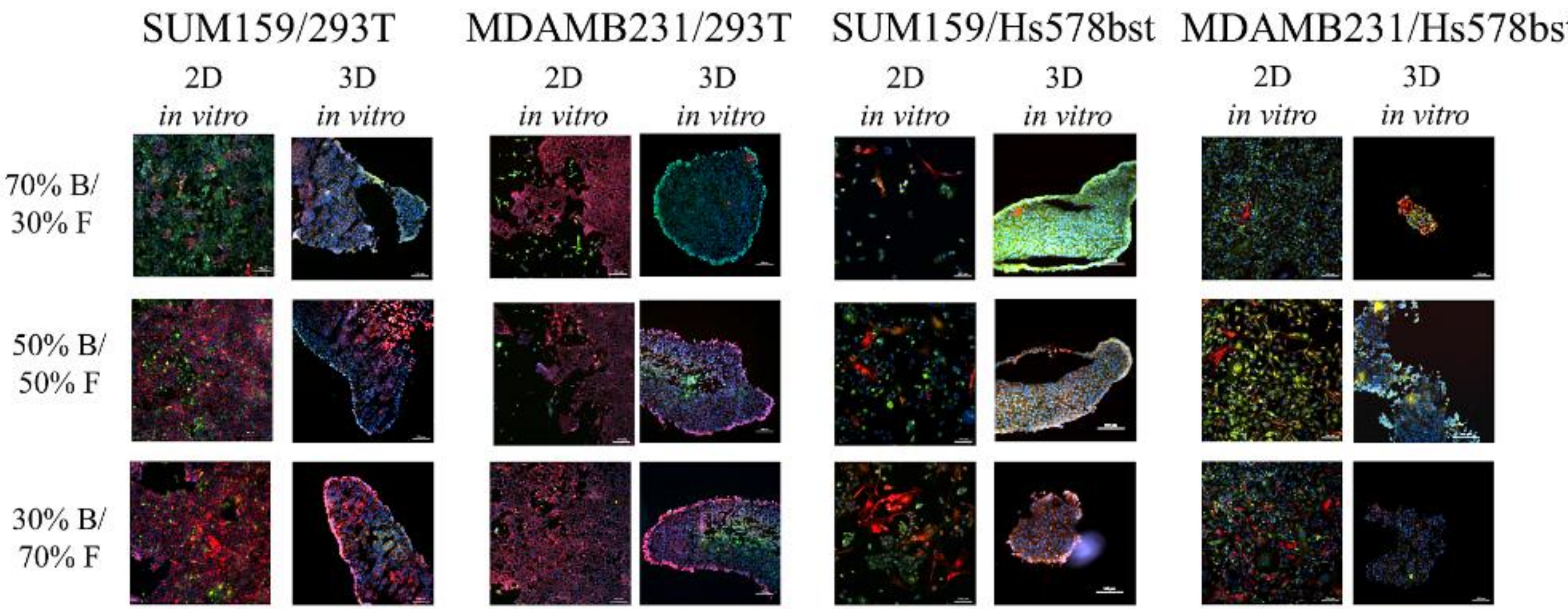


Comparison of 2D co-culture with the 3D in vitro breast tumor model



breast cancer cells
fibroblasts
DNA

Comparison of 2D co-culture with the 3D in vitro breast tumor model



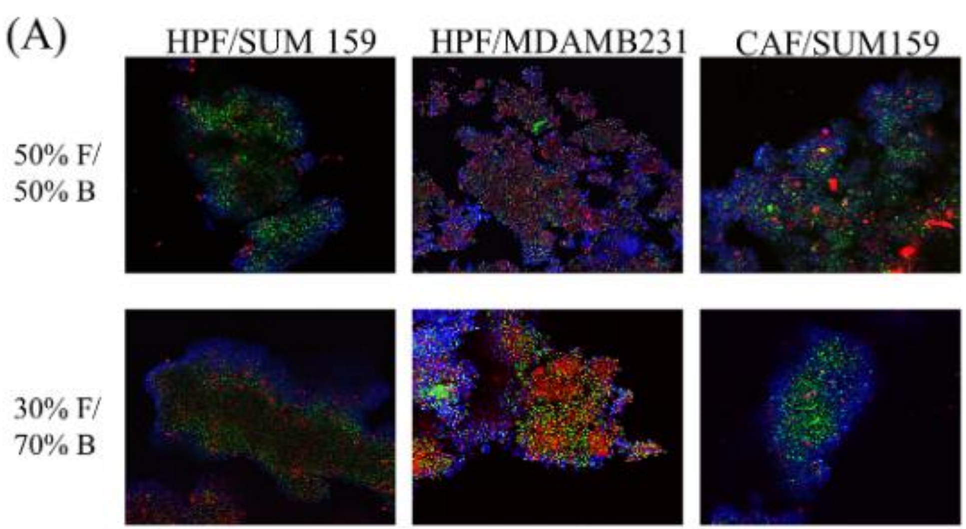
breast cancer cells
fibroblasts
DNA

➜ **3D in vitro culture shows clear tumor tissue-like organization**

3D in vitro tumors grown with primary fibroblasts

CAF = primary cancer breast tumor associated fibroblasts

Day 3

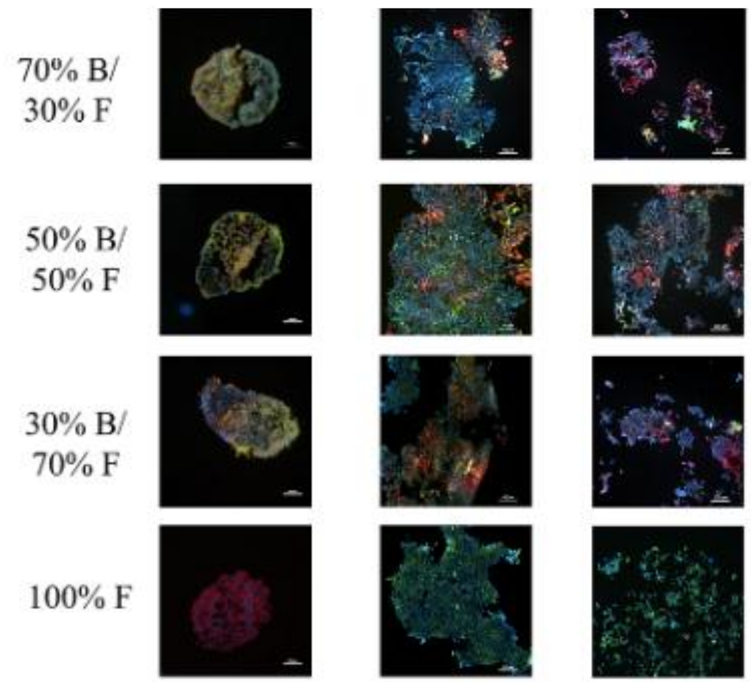


Day 3

MDA-MB-231/
Hs578bs

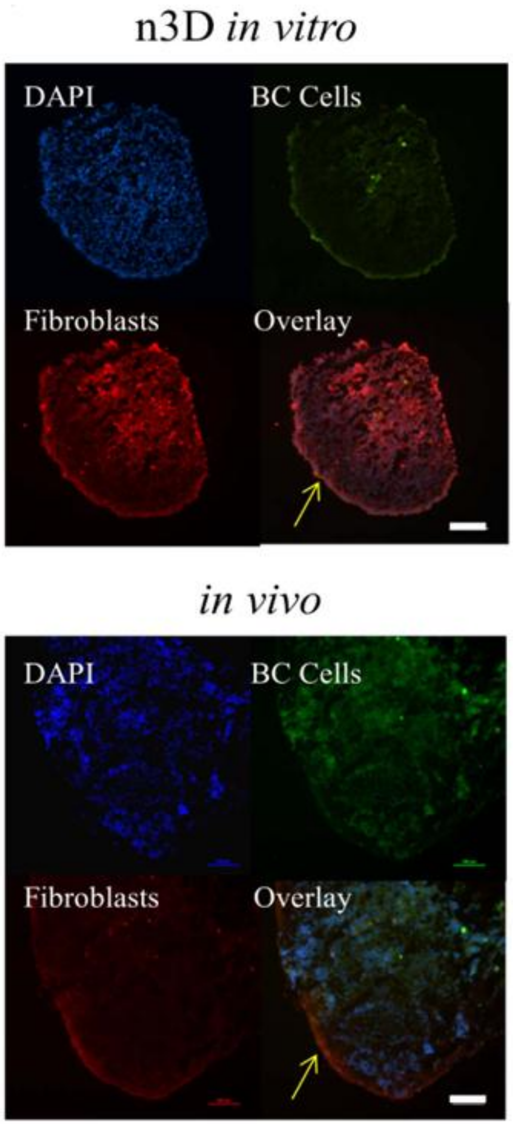
MDA-MB-231

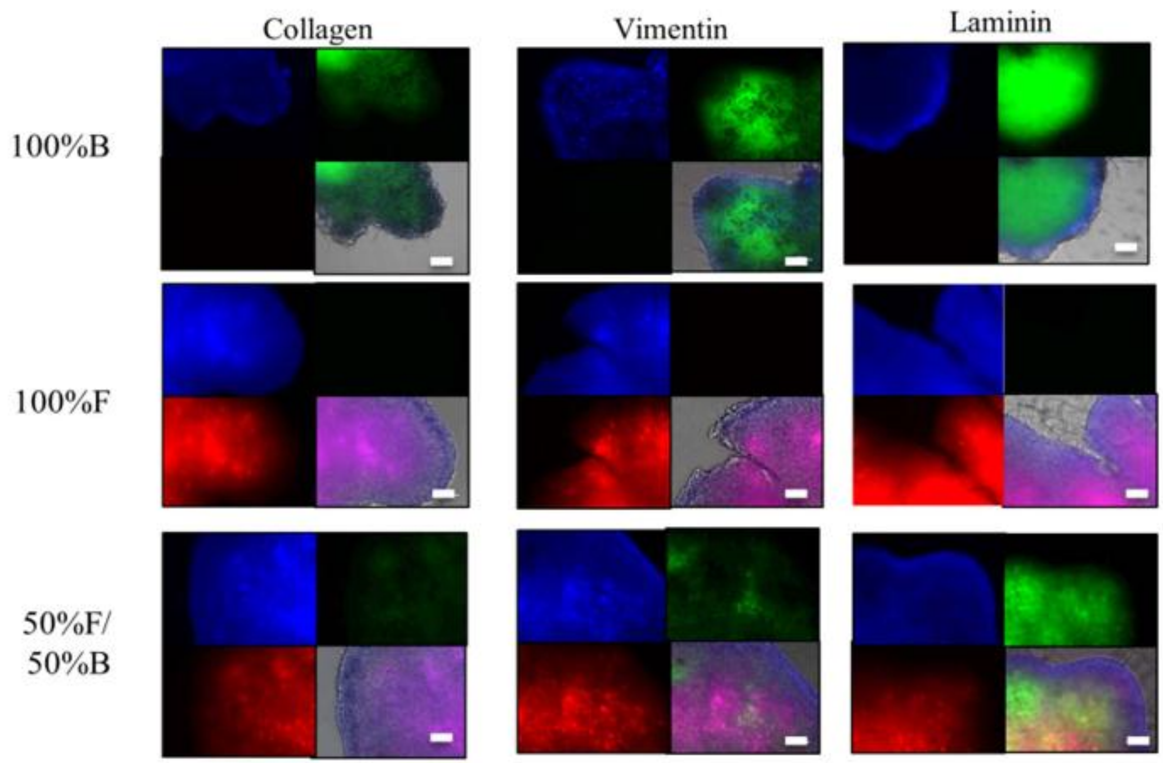
MDA-MB-231/
CAF



➔ **Hs578bst and CAF grow in sync with the cancer cells**

Characterization of in vitro 3D co-cultures



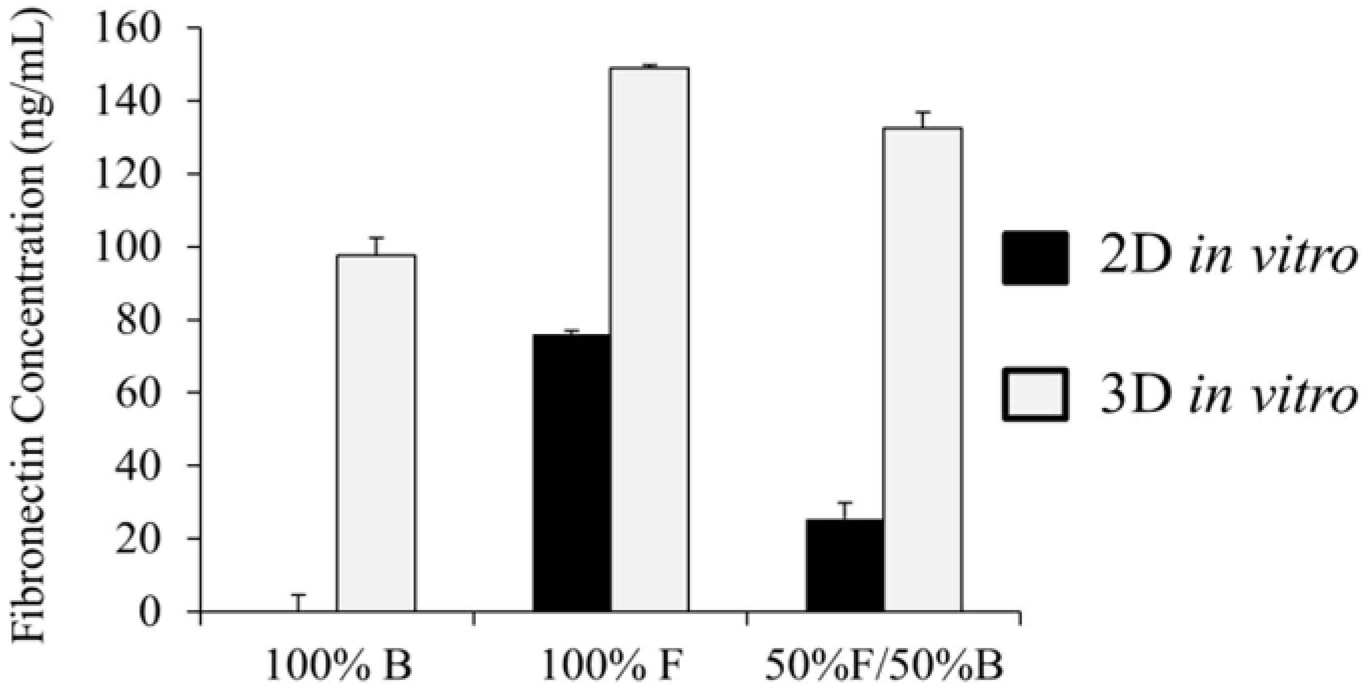


Blue – Protein, Green – Breast Cancer Cells, Red - Fibroblasts

- **fibroblasts at the tumor edge is higher than in the core, which corresponds to the fibrotic capsule phenomenon observed in vivo**
- **3D in vitro tumors expressed different levels of common ECM proteins**

Day 7

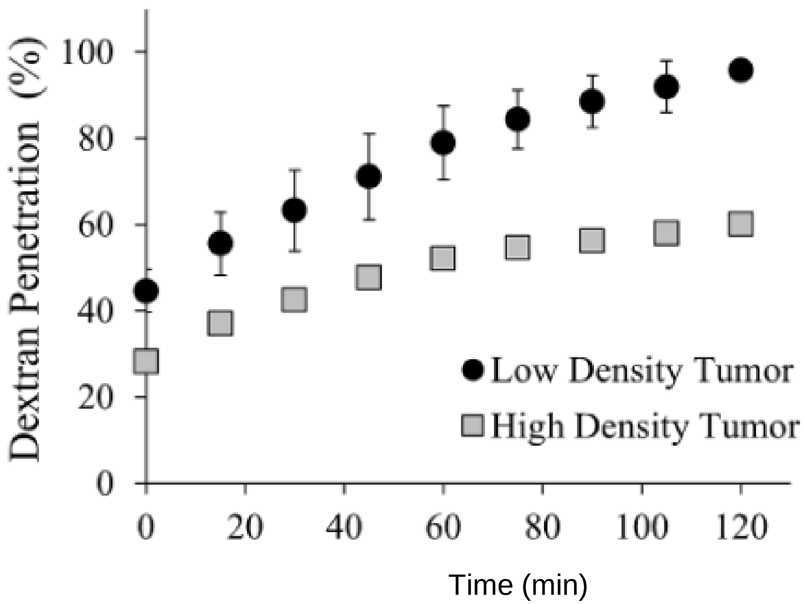
higher concentration of fibronectin in 3D than 2D

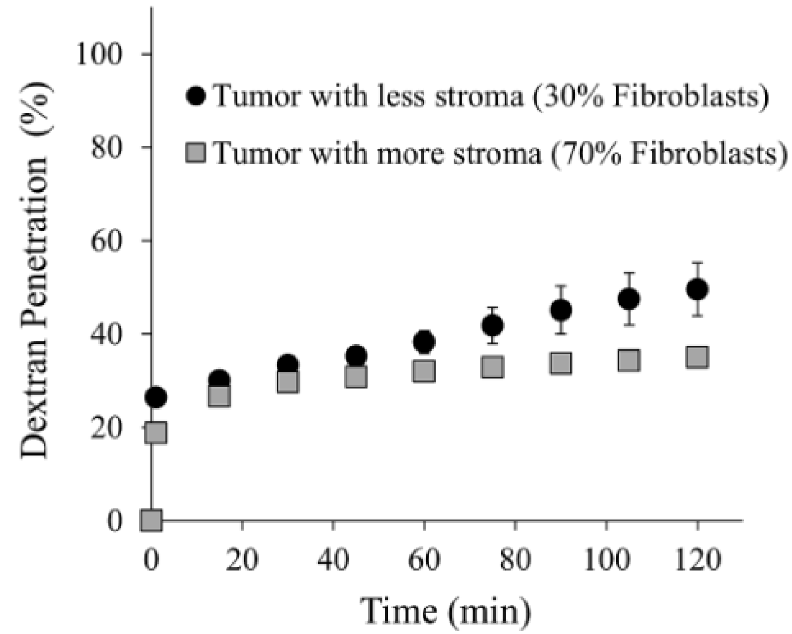


F = fibroblasts (293T)
B = breast cancer cells (SUM159)

➡ **evidence that co-culture of breast cancer and fibroblasts cells can produce an ECM matrix without a scaffold**

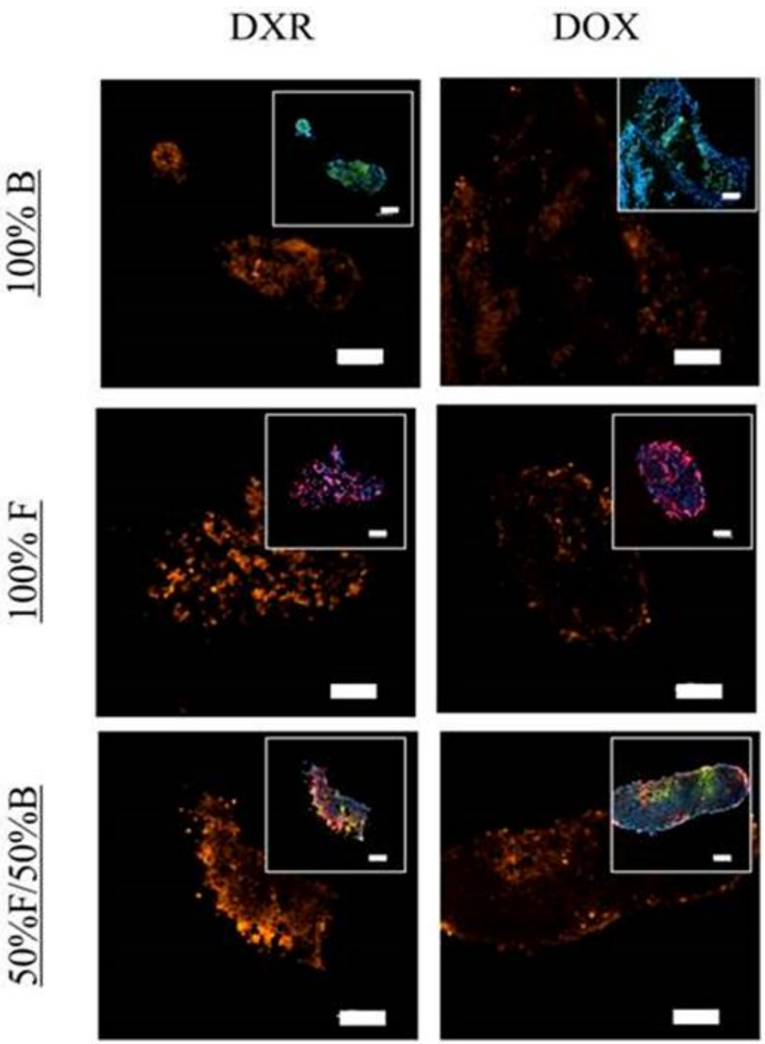
Penetration of TRITC-tagged dextran

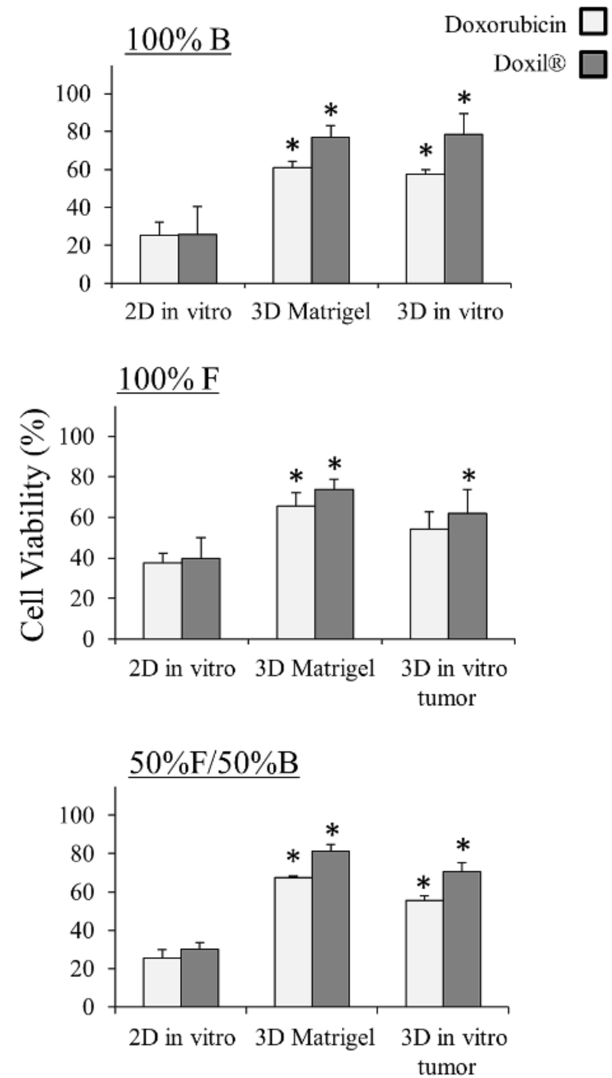




- **Better penetration of dextran through low density tumors/ tumors with less fibroblast cells than high density tumors**

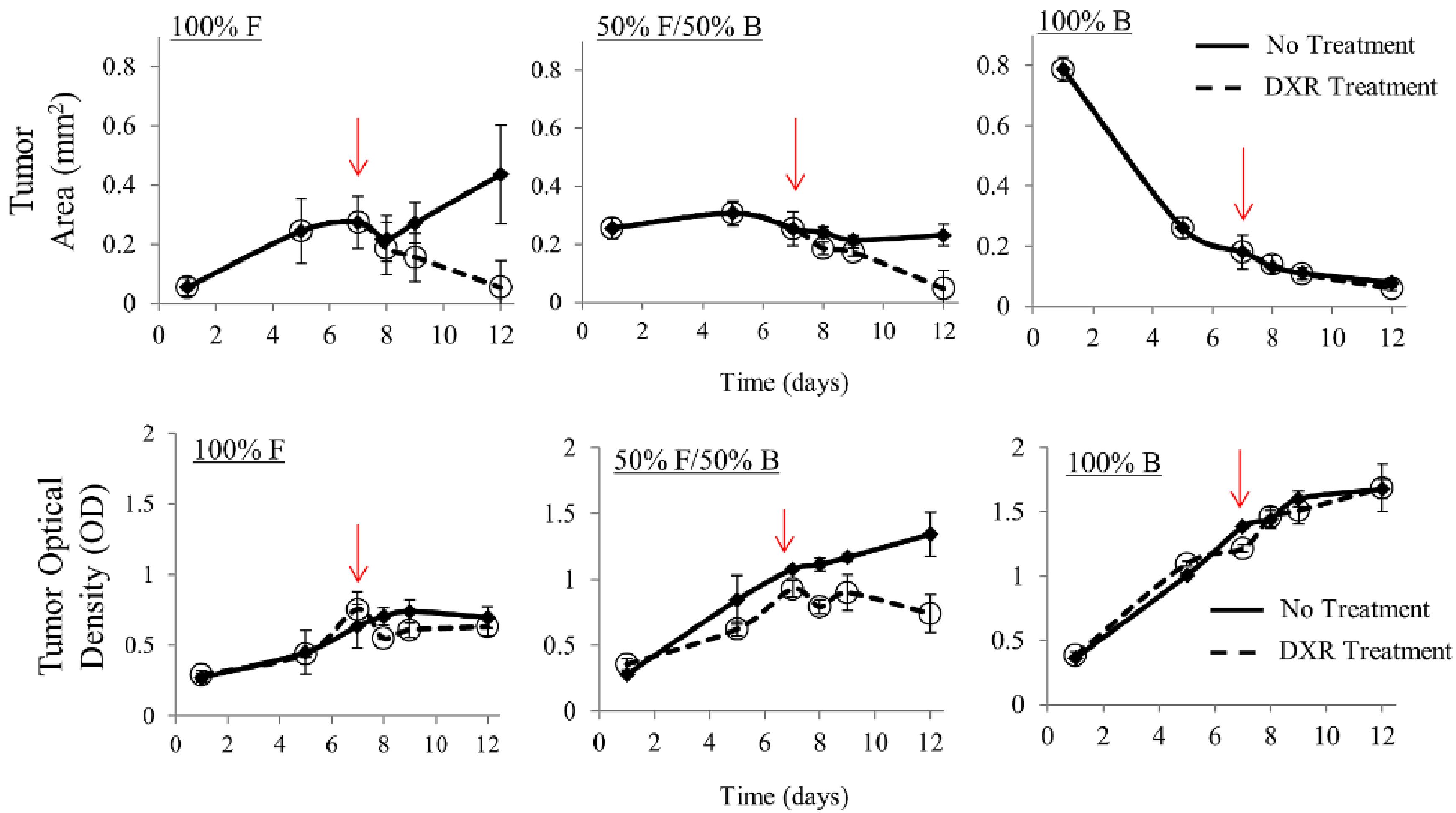
Distribution and therapeutic efficacy of doxorubicin and Doxil on 3D in vitro tumors



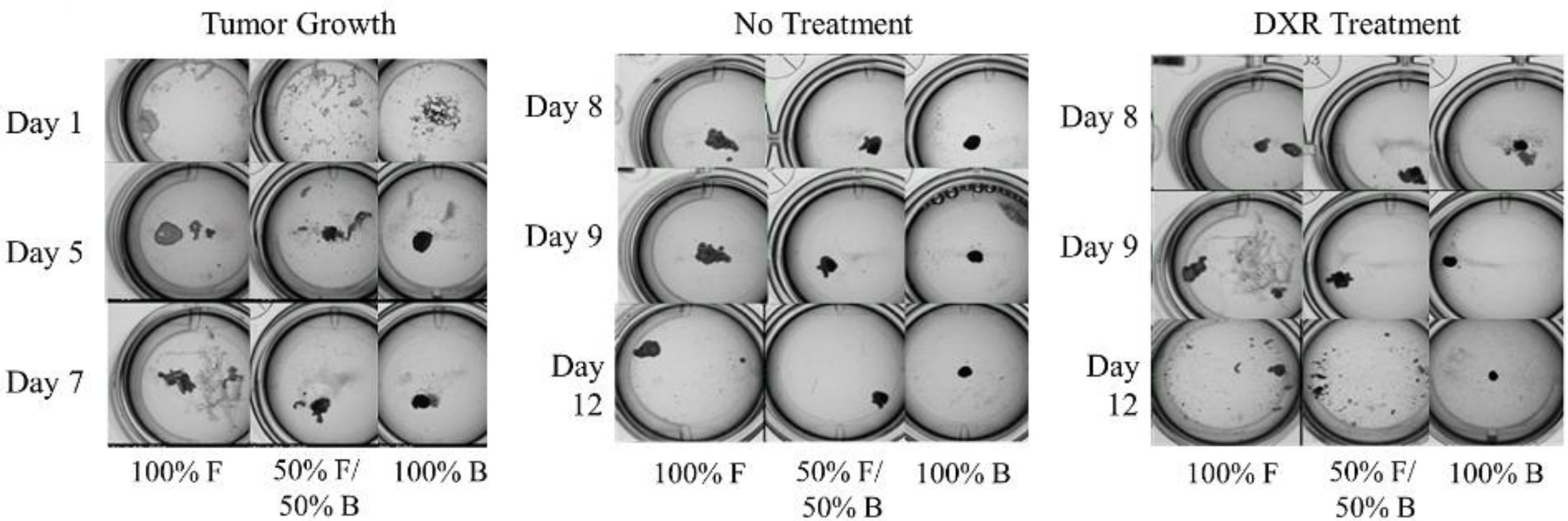


➡ **Doxorubicin and Doxil significantly affects viability in 3D/2D systems**

Effect of doxorubicin treatment on 3D in vitro tumors



Effect of doxorubicin treatment on 3D in vitro tumors



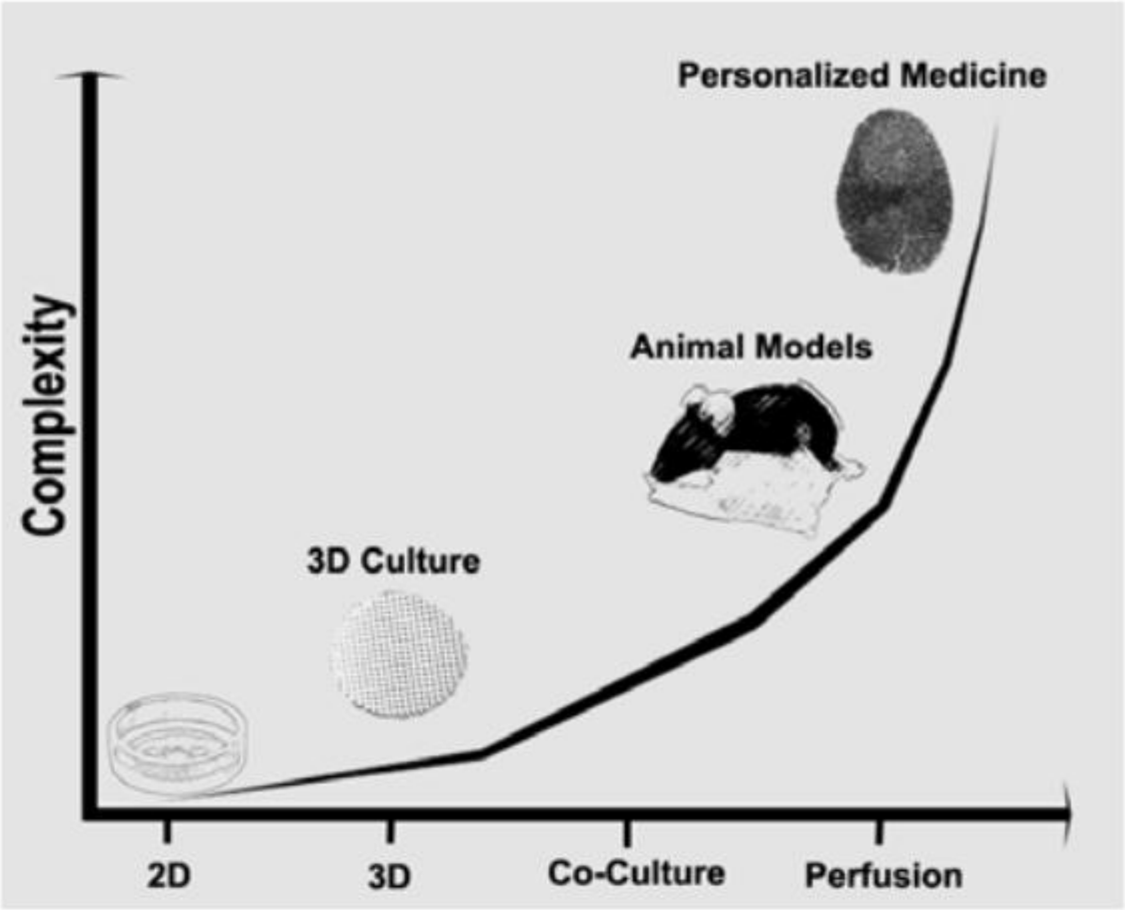
➜ **decrease of tumor area and density after doxorubicin treatment**

summary

- form large-sized breast tumor within 24h
- formation of heterogeneous 3D in vitro breast tumors at various sizes, densities and compositions by controlling the number and type of cells
- mimic the in vivo tumor microenvironment
- decrease of tumor area and density after drug treatment

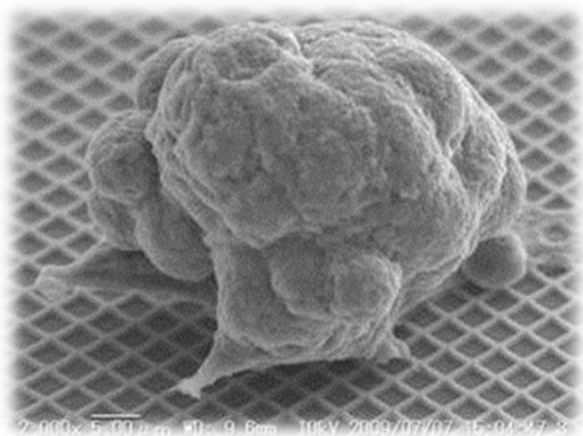
➡ **Method provides a 3D in vitro brest tumor model to test drug efficiency**

Progression of in vitro Cell-Based Models



Caicedo-Carvajal et.al., Translational Medicine 2012

Thanks for your attention



http://www.nationalgeographic.de/wissen/biologie-nadeln-gegen-stress