3D Cell Culturing by Magnetic Levitation, The Next Generation of Cell Culturing?

Presented in the Embryo Physics Course
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By

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3D Cell Culturing by Magnetic Levitation, The Next Generation of Cell Culturing?

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CANCER CENTER
Making Cancer History®

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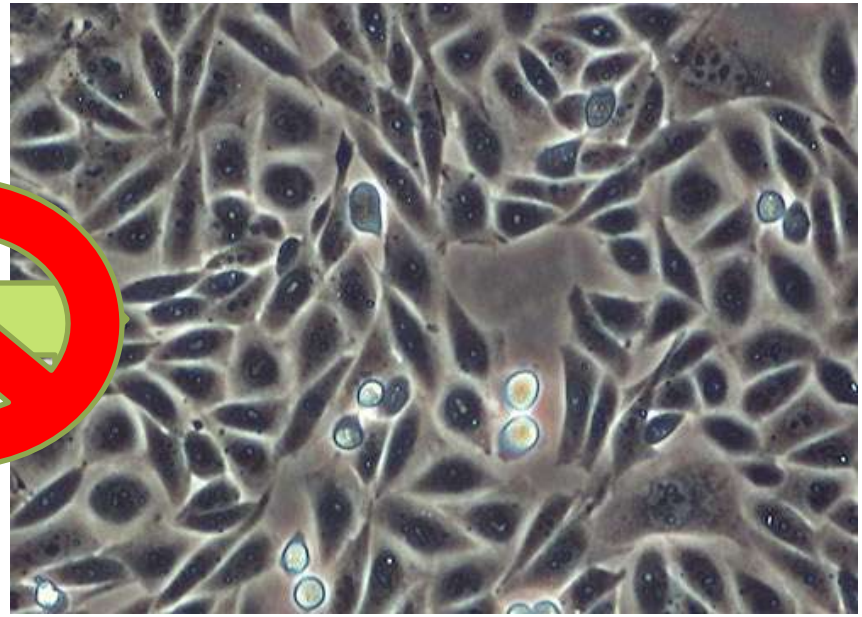
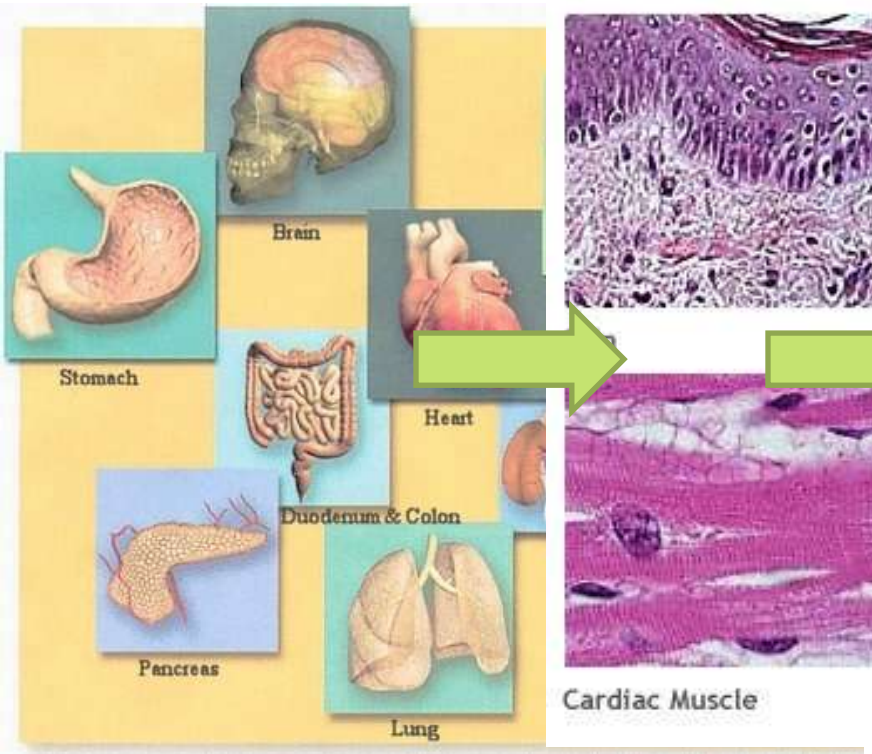
RICE

What is Cell Culture?



http://www.microscopyu.com/staticgallery/phasecontrast/images/chocellspositive.jpg
http://www.sociology.ccsu.edu/images/benton_mural.jpg

What is Cell Culture?



in vivo:

Inside the body

in vitro:

Outside the body

http://www.ptei.org/assets/HistoryofTissues.jpg

http://www.freewebs.com/bnip1/segmentation.htm

http://universe-review.ca/I10-35-organs.jpg

Why 3D Cell Culturing?

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http://www.neosurrealismart.com/3d-artist-gallery/3d-artworks/3d-fantasy-art/353d-Final-Frontier-B.jpg

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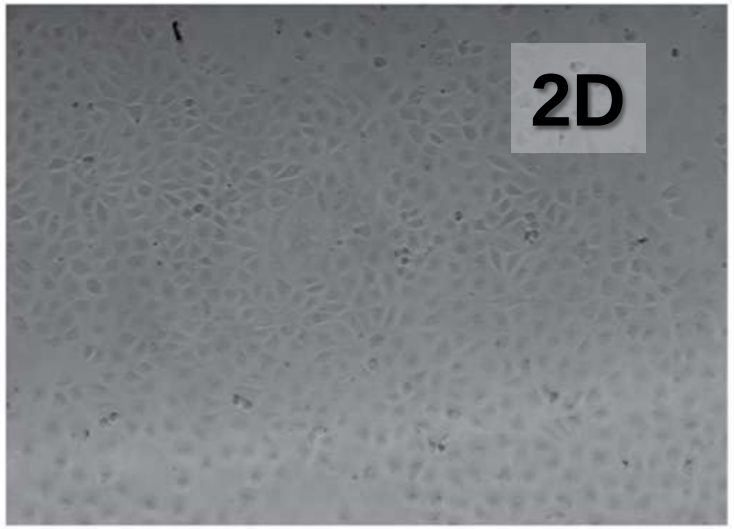
Paradigm Shift



- Culturing cells remains essential to all work in life sciences.

- Now widely recognized that cells grown in 2D inaccurately represents real tissue.

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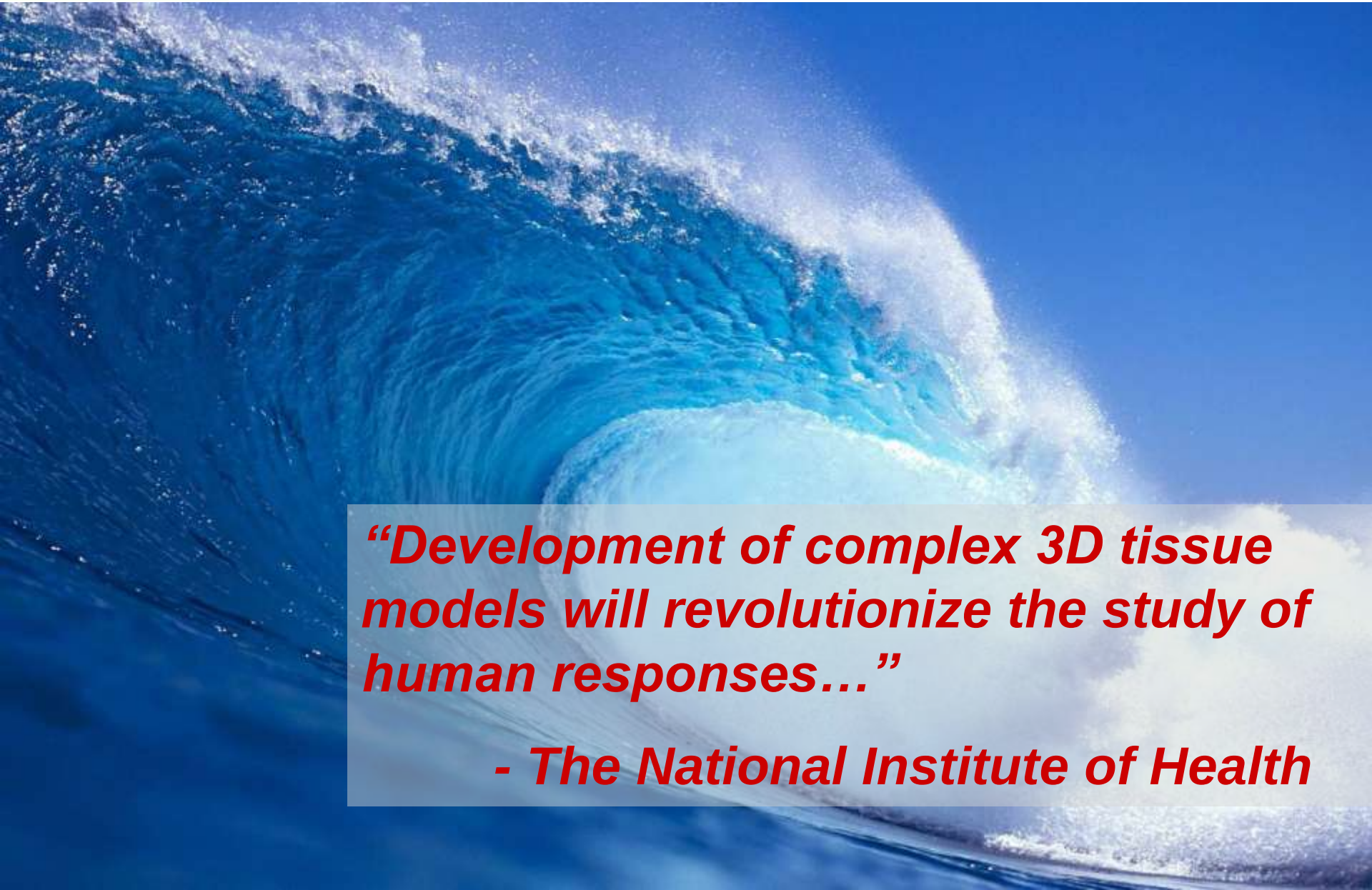
Start of the 3D Wave



"Development of complex 3D tissue models will revolutionize the study of human responses…"

- The National Institute of Health

Today's 3D Cell Culturing?

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Today's 3D Cell Culturing

- Gel, such as Matrigel
- Rotary Bioreactor
- Polymer Scaffolds

BD

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Gel and Matrigel

- ✦Today's gold standard
- ✦Extensive body of literature
- – Often harvested from animals - rats
- – Exogenous extracellular matrix proteins – no translational application
- – Batch-to-batch variability
- – Laborious
- – Poor co-culturing capability
- – Difficult to handle cells post-culture

BD

Rotary Bioreactors

- ✦ Cell expansion
- ✦ Mimics microgravity
- ✦ In vivo traits
- – Difficult to visualize live cultures
- – Excess components
- – Not compatible with high-throughput
- – Poor co-culturing capability
- – No spatial control of cells
- – Poor co-culturing capability
- – Difficult to handle cells post-culture

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Polymeric Scaffolds

- ✦ Compatible with high-throughput
- ✦ Porous polymers
- ✦ Diffusion of nutrients
- – Poor cell-cell interaction - artificial cell migration
- – Poor co-culturing capability
- – Difficult visualization
- – Poor translational applications

Next Generation?

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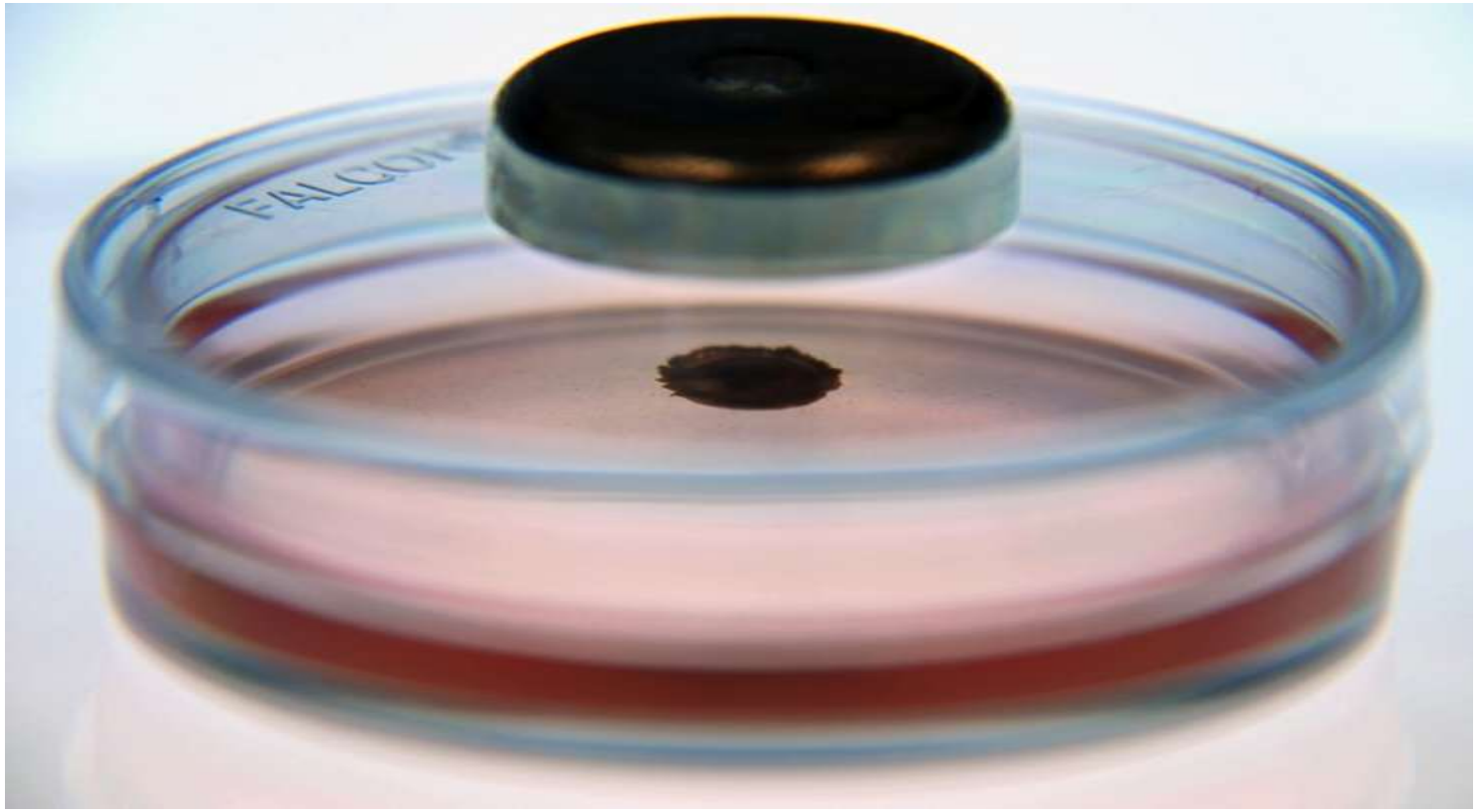
Next Generation: Magnetic Levitation?



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The Genesis Multidisciplinary Collaboration

Spin off from Rice and MD Anderson

"Develop the Bio-Assembler™ into the industry leading standard for 3D in vitro cell culturing and to apply this breakthrough technology in the fields of toxicology screening, drug discovery, and regenerative medicine."

n3D

- Glauco R. Souza, Ph.D. (**Physical Chemist**, CSO)
- David Lee (**Business**, President)

Rice University

- Thomas Killian, Ph.D. – Professor of **Physics** and Astronomy (co-inventor)
- Robert Raphael, Ph.D. – Professor of **Bioengineering** (co-inventor)

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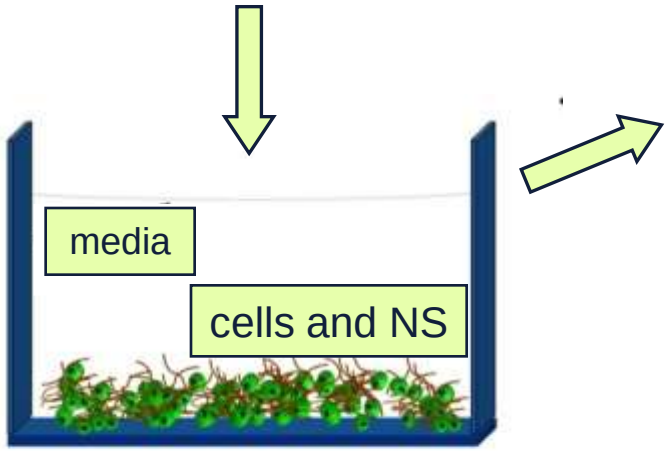
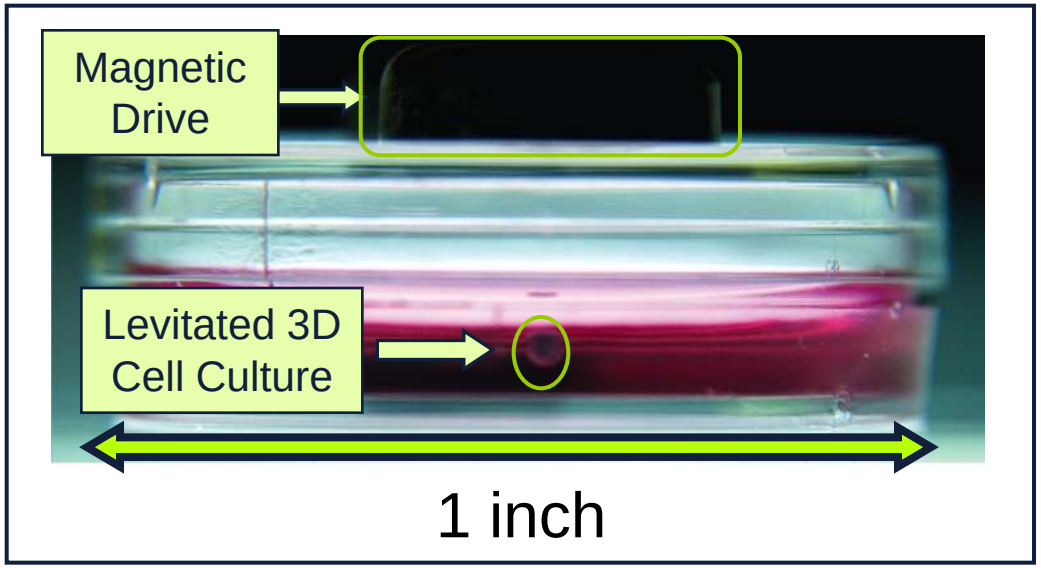
How?

We “decorate” cells with magnetic nanoparticles

Nanoparticle Assembly: Nanoshuttles (NS)



The Bio-Assembler™



Souza *et al.* Nature Nanotechnol. April 2010

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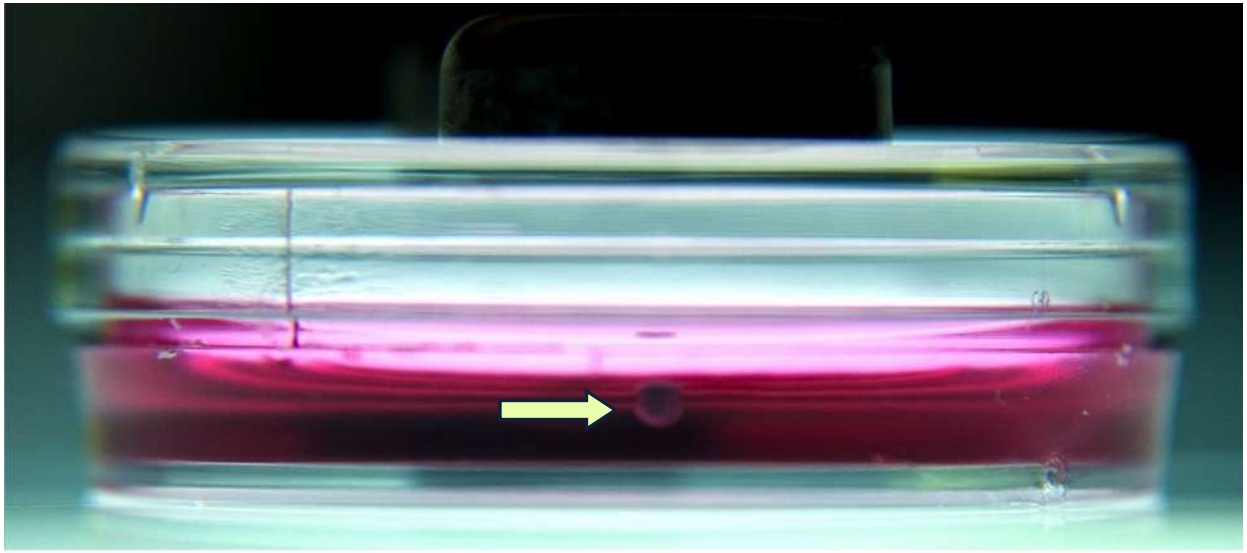
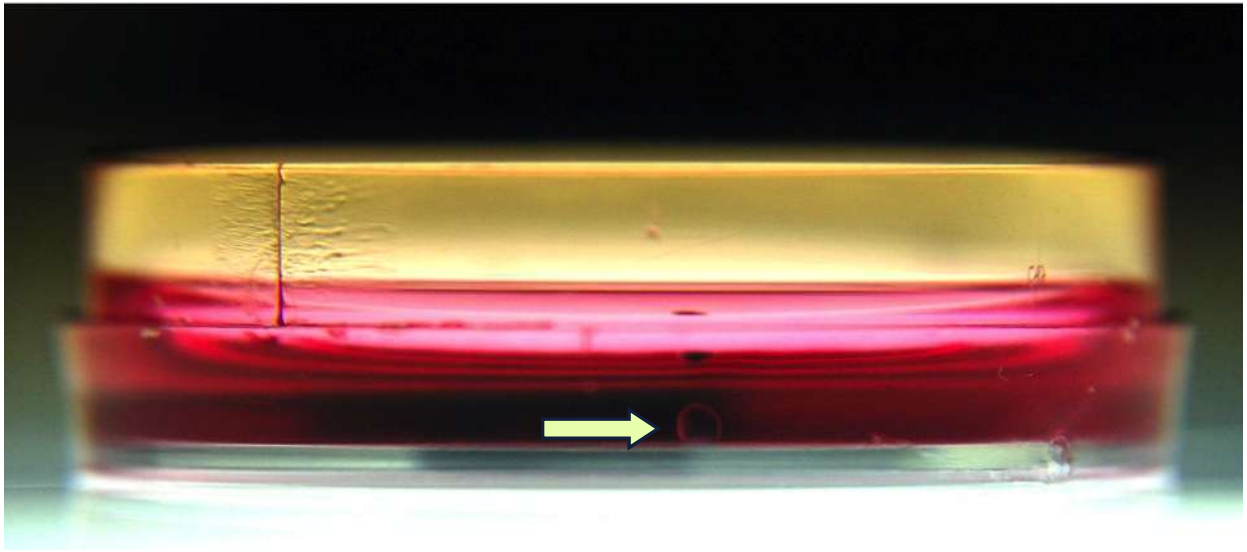
Magnetic Levitation

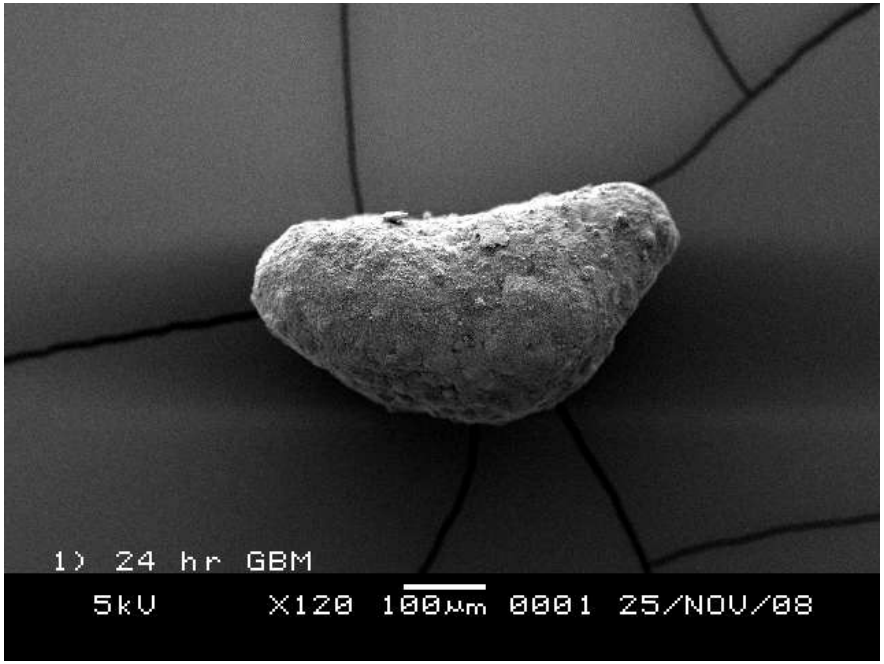




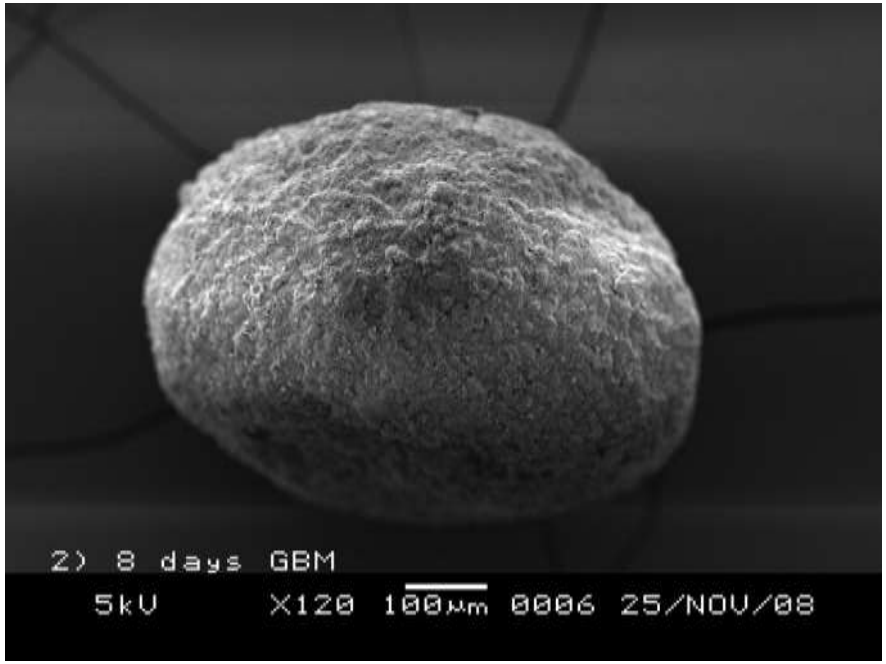
3D Structure

SEM – 24 hours vs. 8 days GBM Cell Cultures

24 hours



8 days



Souza *et al.* Nature Nanotechnol. April 2010

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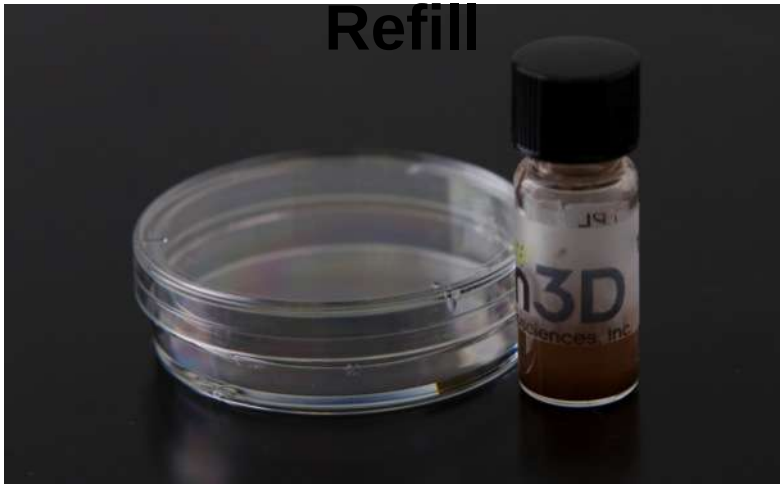
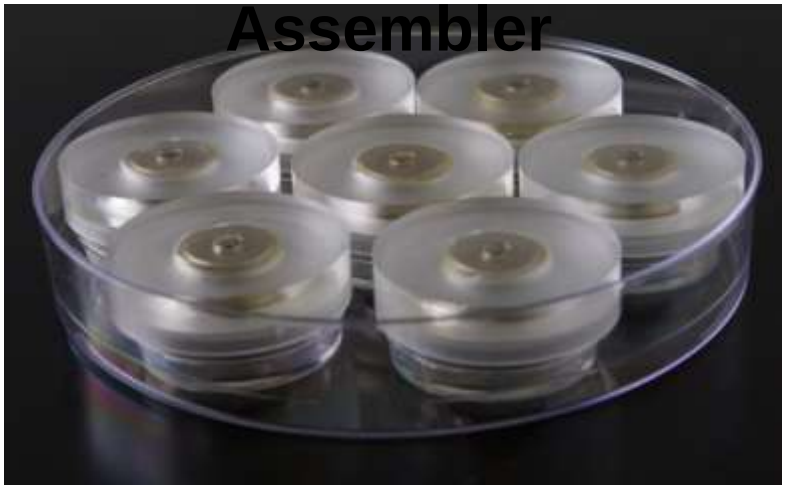
Bio-Assembler™ Kit

NS Refill



Single-Well Bio-Assembler



6-Well Bio-Assembler

24-Well Bio-Assembler

What about *in vivo* like?

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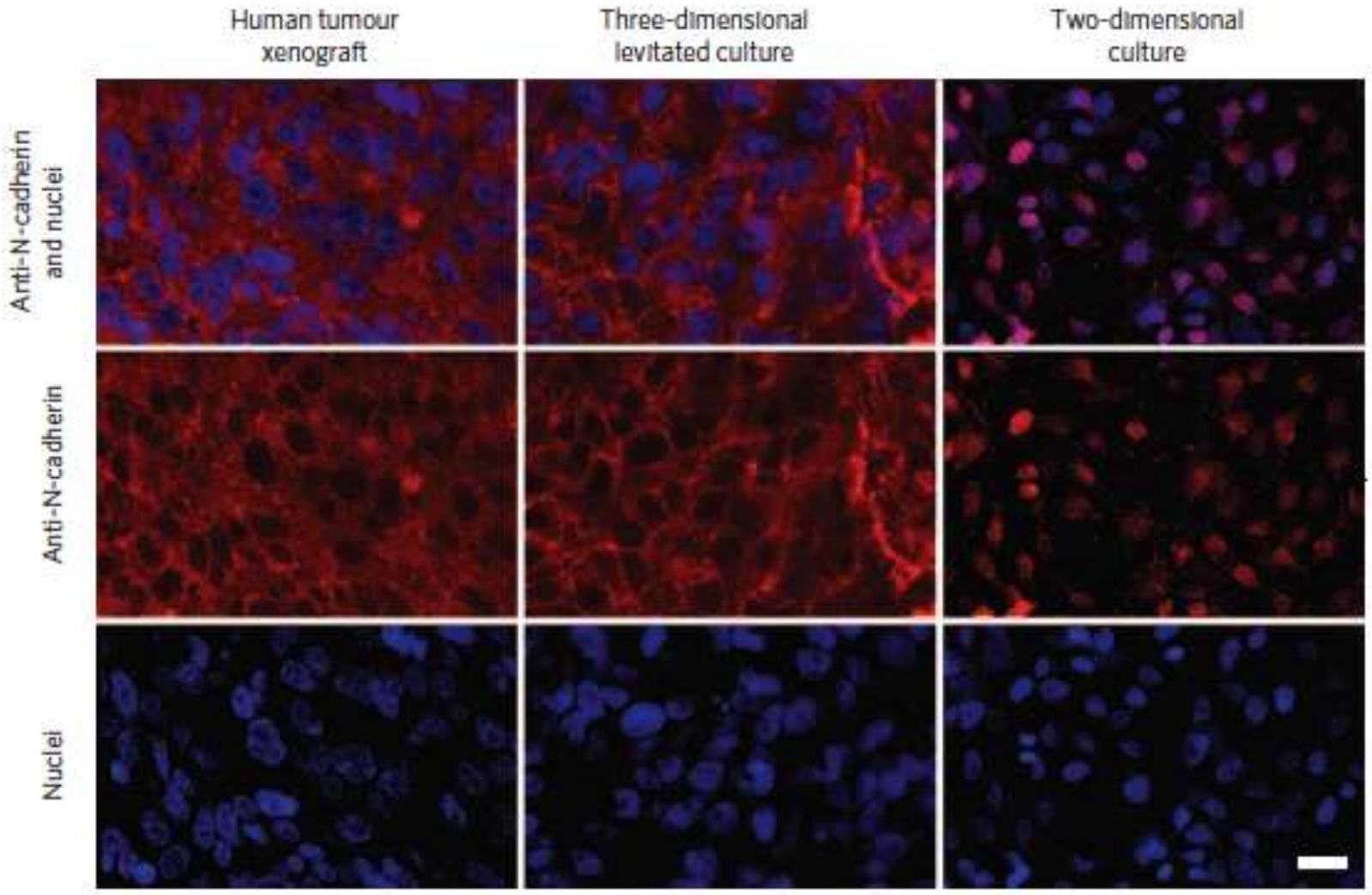
In Vivo like *In Vitro*

Mouse Brain Xenograft Comparison - Glioblastoma



Souza *et al.* Nature Nanotechnol. April 2010

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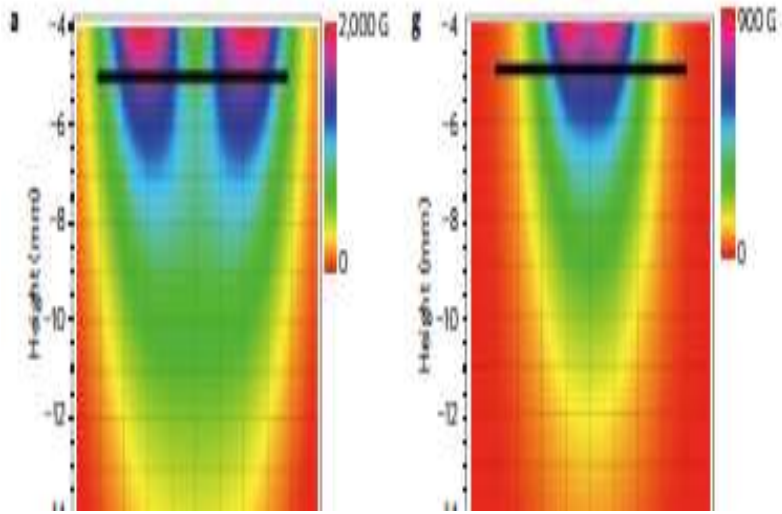
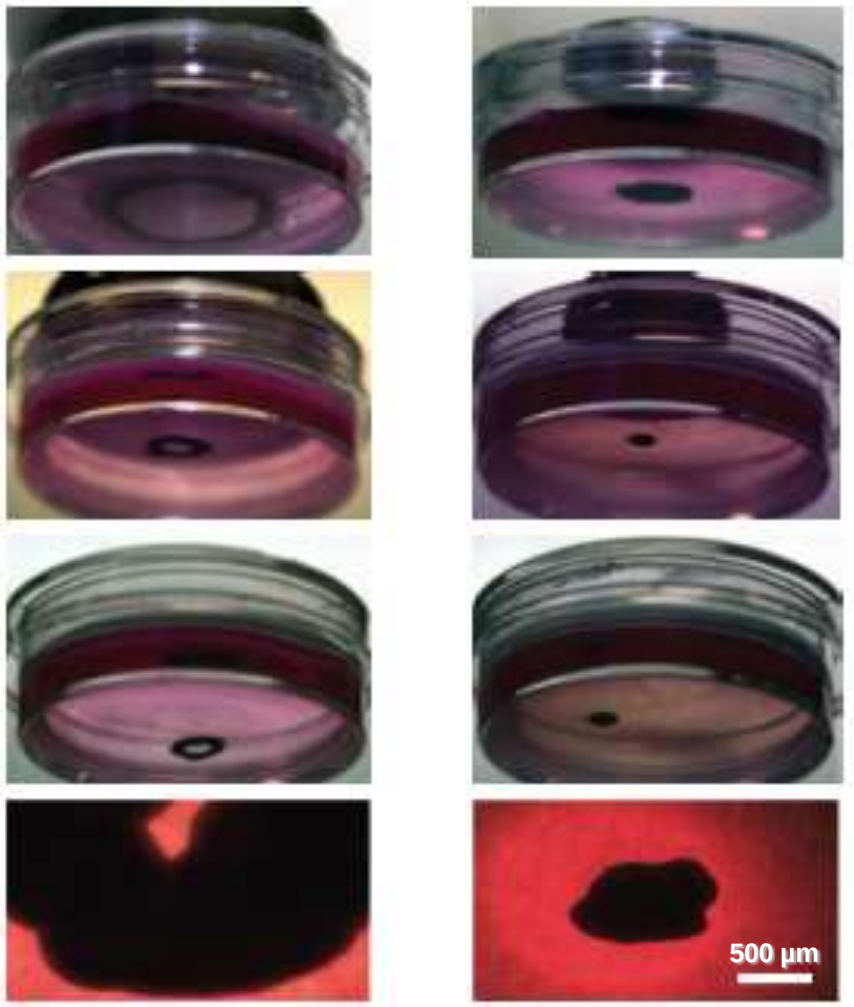
Shape but Scaffoldless: The New Paradigm

Levitated Magnetic Pattern





Souza *et al.* Nature Nanotechnol. April 2010

New paradigm

Scaffoldless tissue engineering with shape

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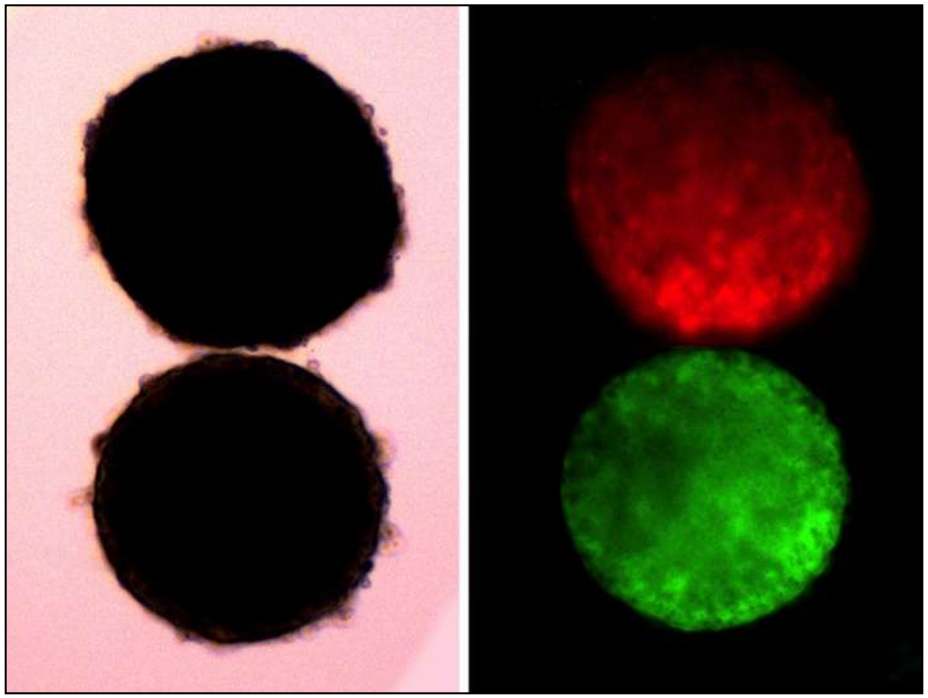
Co-Culture, Spatial Control, Invasion Assay

Invasion Assay & Co-Culture

Magnetic Guidance = Control

t = 0



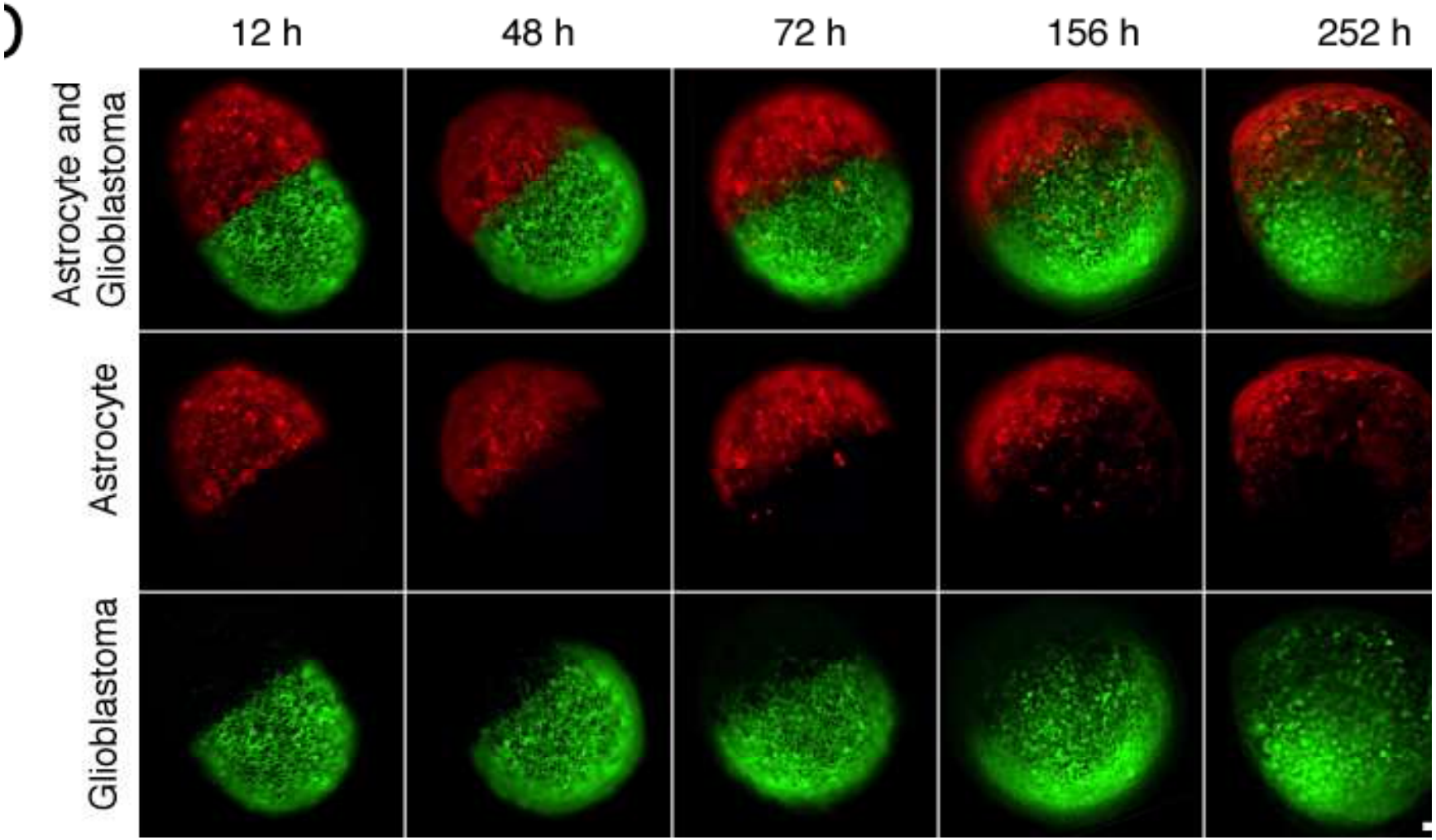
Normal Human Astrocyte
mCherry fluorescence

Human Glioblastoma
GFP fluorescence

Souza *et al.* Nature Nanotechnol. April 2010

Invasion Assay & Co-Culture

Magnetic Guidance = Control



(Scale bar, 200 µm.)

Souza *et al.* Nature Nanotechnol. April 2010
Molina *et al*. Neoplasia, May 2010

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What About the Nanoparticles?

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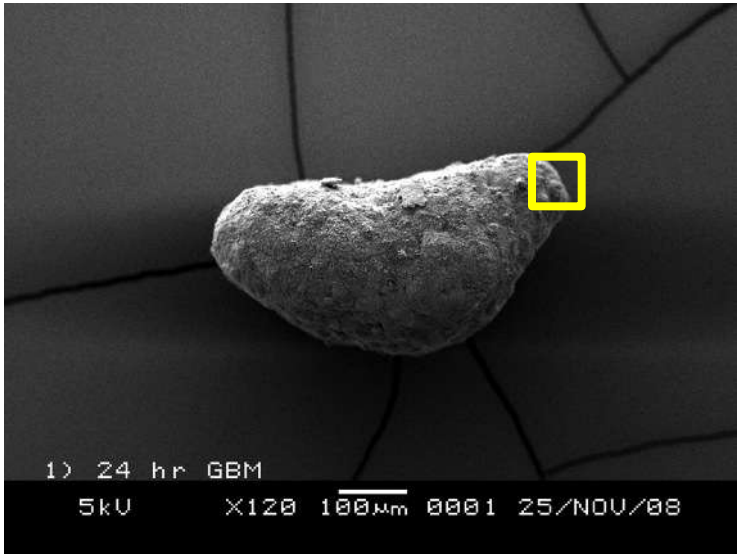
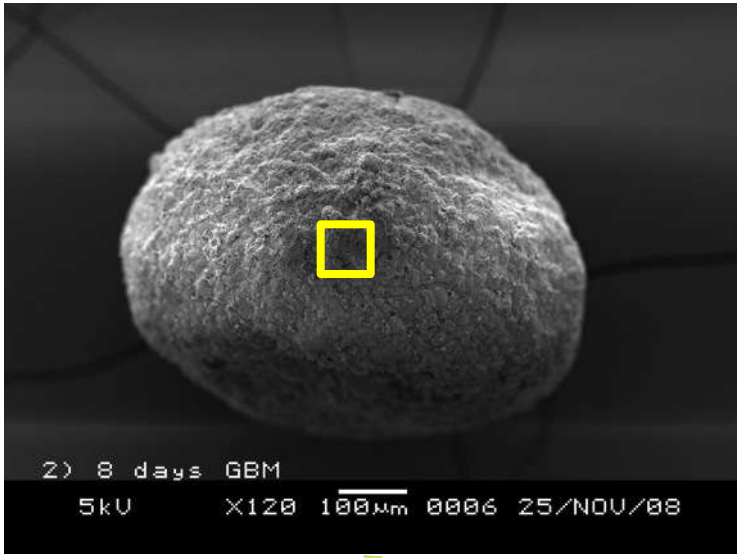
SEM & TEM - 1 day = Intracellular NP
8 days = Extracellular NP

24 hours

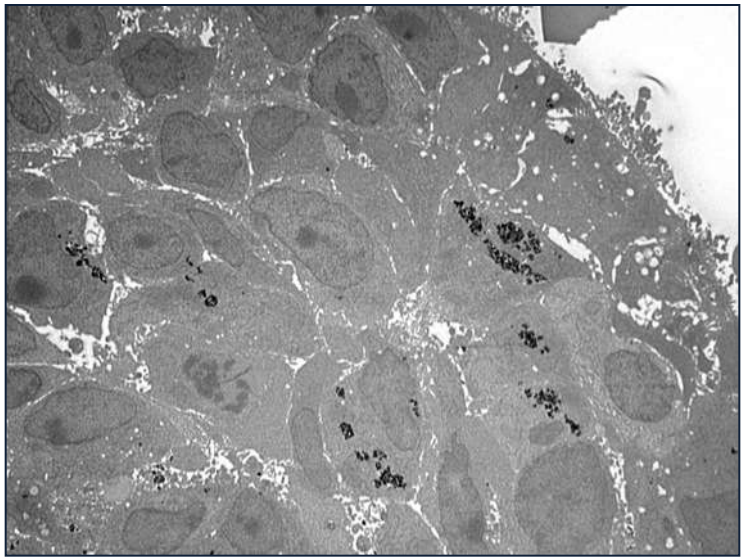
8 days

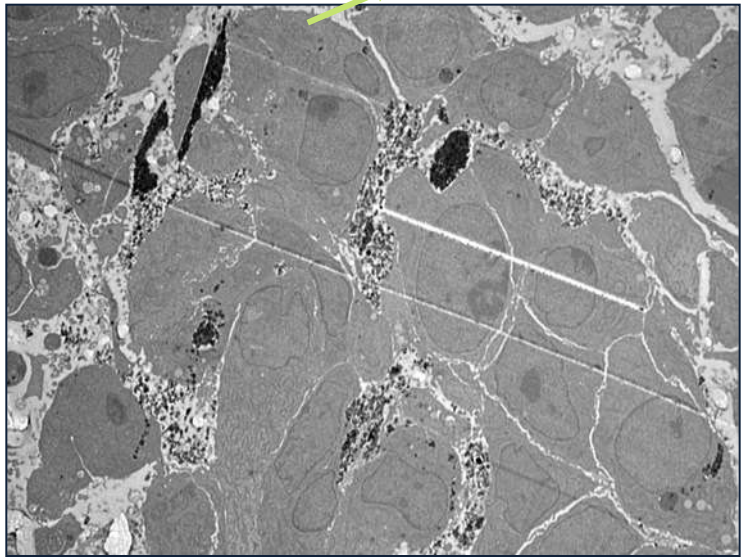
SEM





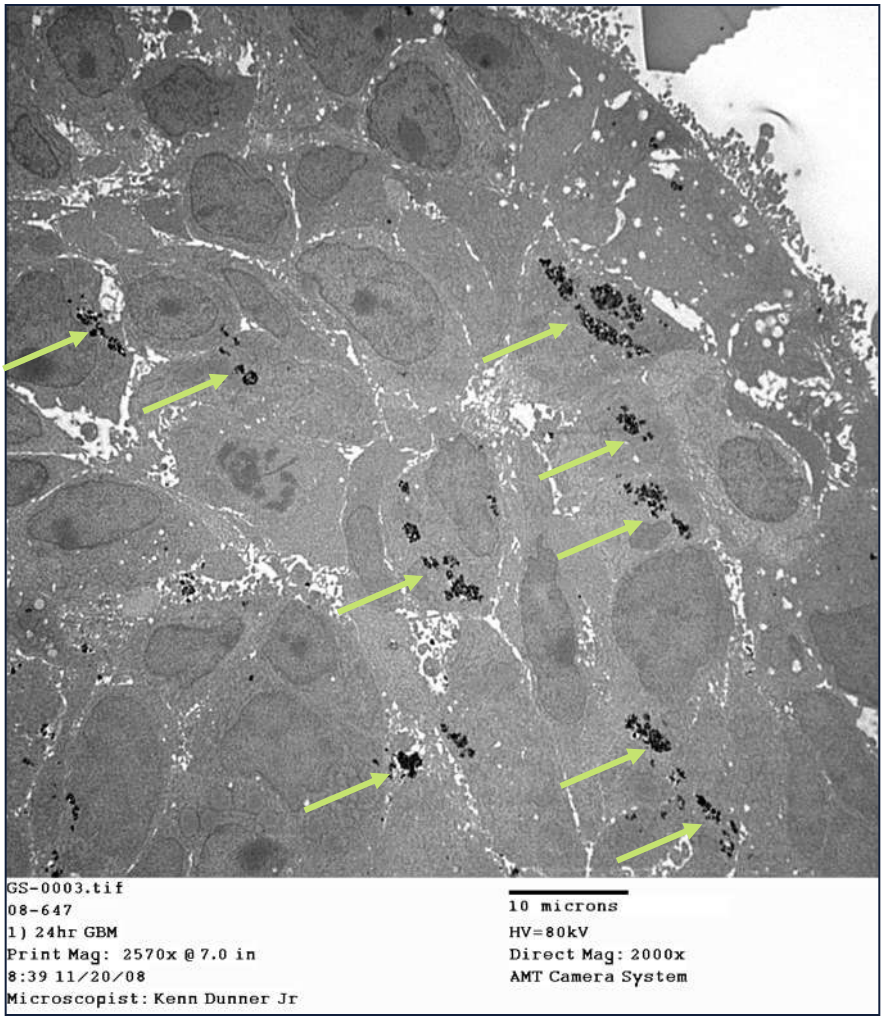
TEM



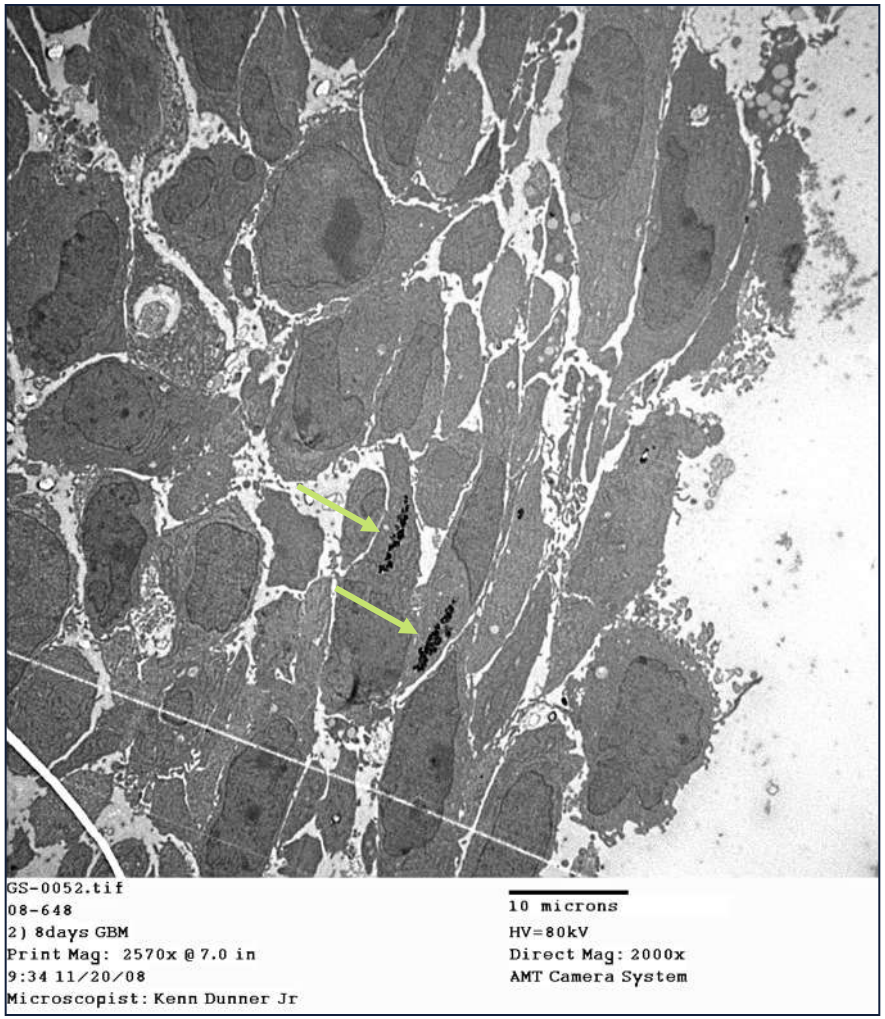


TEM of Cells Closer to the Edge

24 hours



8 days



Nanoshuttles

- All components of the reagent mix are individually **FDA**.
- Nanoshuttle was tested in mice and **no acute toxicity was found**.
- **Over 20 different cells types have been cultured** with the Bio-Assembler, including primary cells
- We have **not found a cell type that did not culture** in the Bio-Assembler.
- Healthy cell cultures have been **maintained for as long as 2 months**. They were terminated at the end of experiment.
- Comparative Genome hybridization (CGH) profile was comparable between Nanoshuttle treated and non-treated human primary cells, indicating that the nanoparticles do not cause any genomic instability.
- No difference in viability and proliferation between cells in 2D treated and not-treated with Nanoshutlle
- Western blotting showed no difference in gene expression between primary cells treated and not-treated with Nanoshutlle cultured in 2D: fibronectin, laminin, N-Cadherin, E-Cadherin, smooth muscle α-actin

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Step-by-Step

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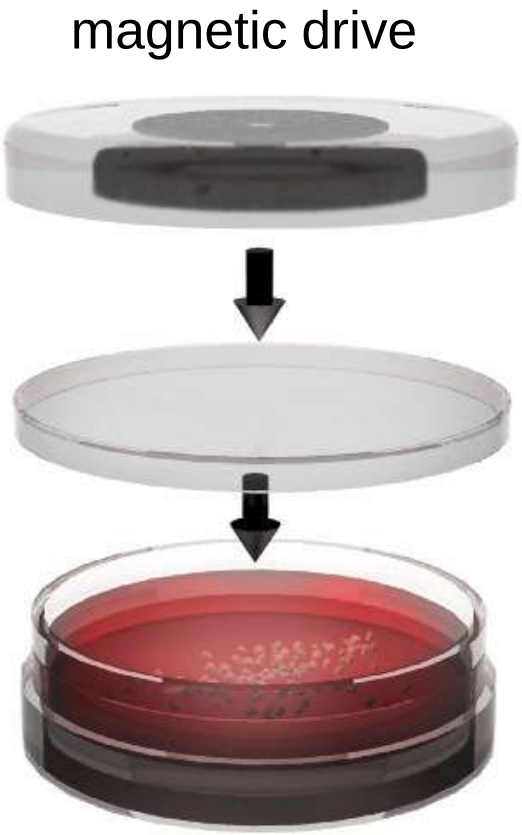
As Simple As 2D

Add Nanoshuttle to media & cells

Trypsinize cells



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Preparing Neural Stem Cells for Magnetic Levitation

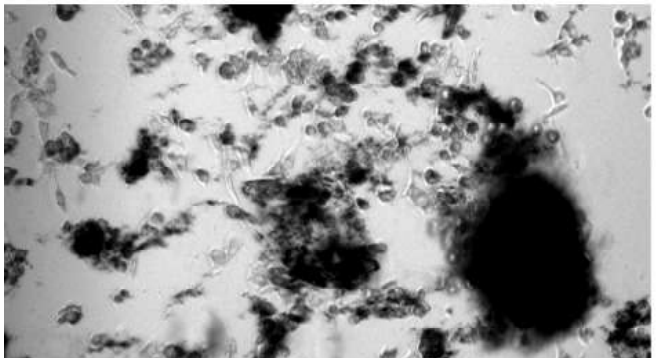
Add Nanoshuttle to media & cells

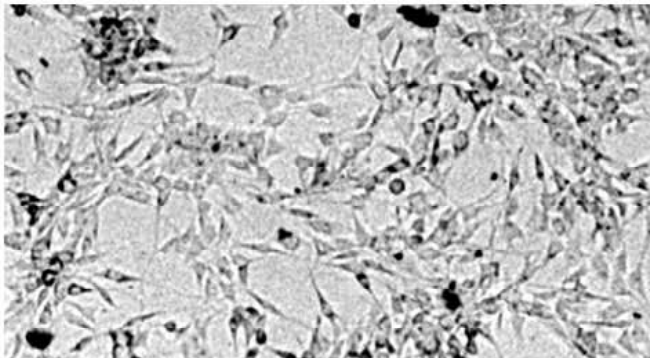
First Step

Trypsinize cells

Add & Incubate
Neural Stem cells with Nanoshuttle

PBS Wash
Remove Excess Nanoshuttle





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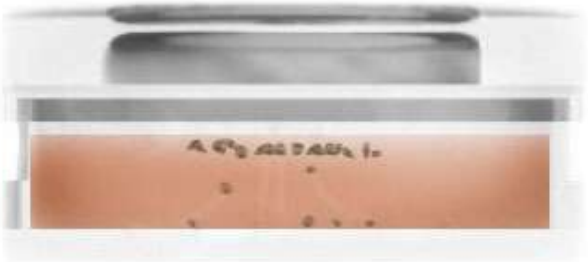
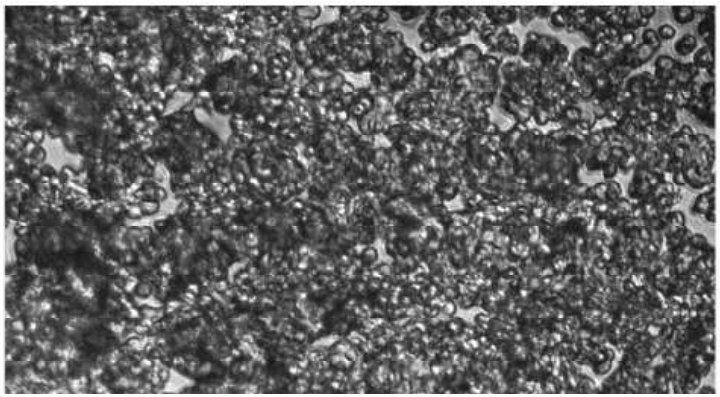
Souza *et al.* Nature Nanotechnol. April 2010

Levitating Cells

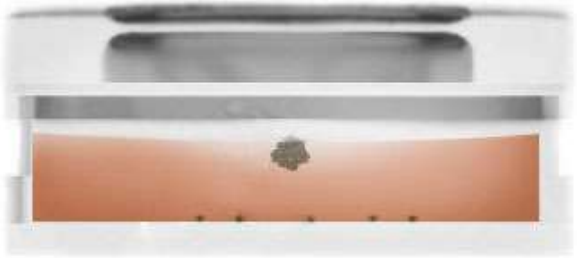
15 minutes



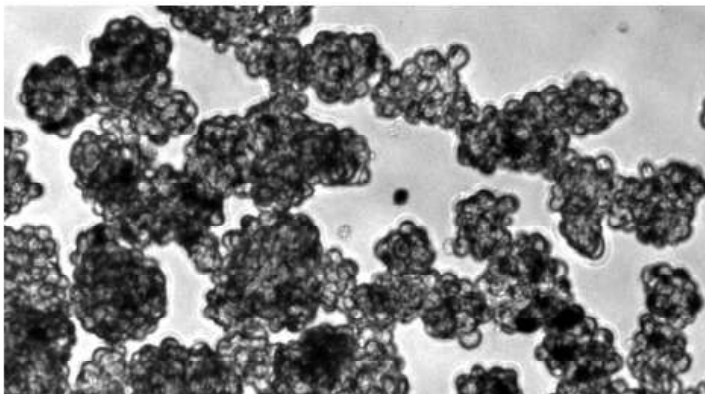
Levitated NSC



12 hours



3D NSC Culture



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**NSC = Neural Stem cells*

Tuning the Culture

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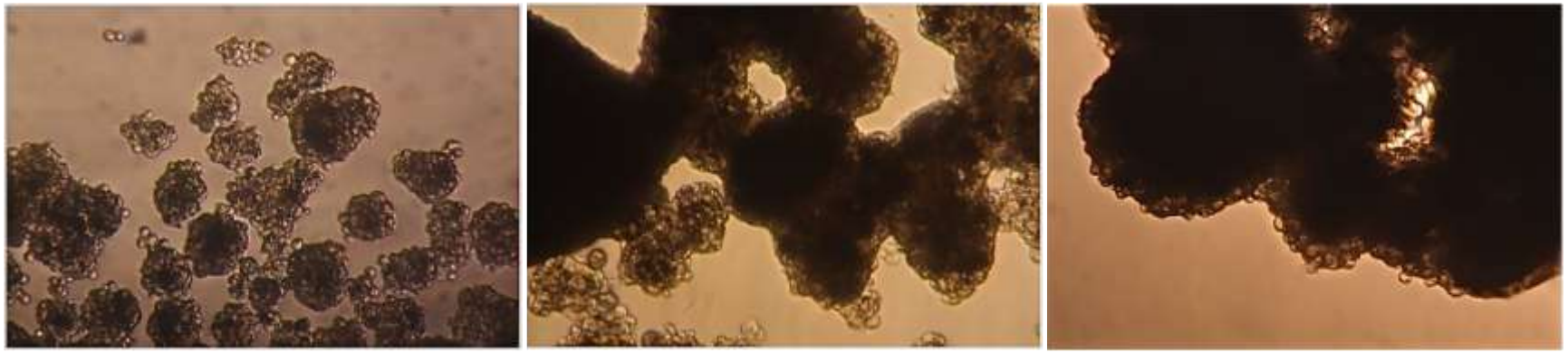
Levitation Time

Chondrocytes

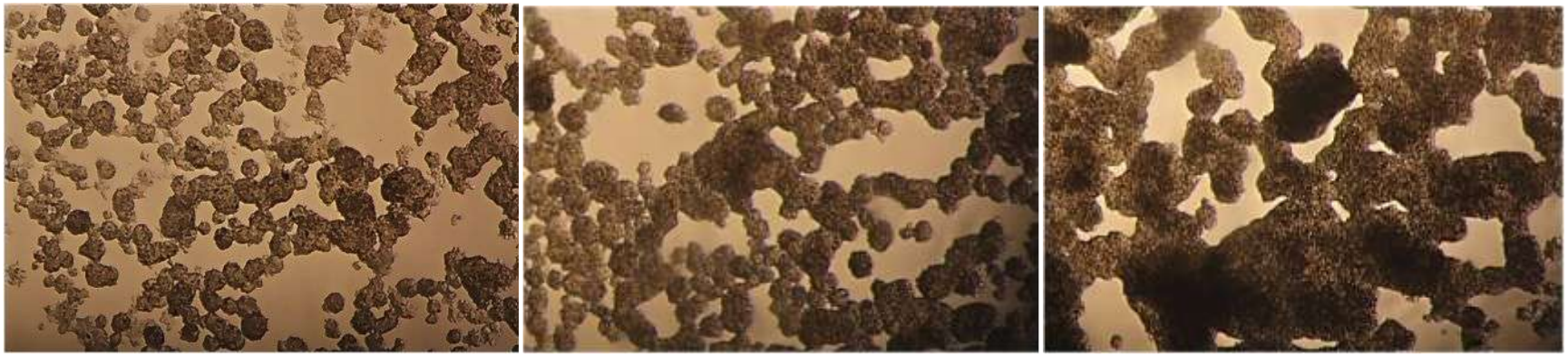
24 hours | 48 hours | 10 Days



Number of Cells – 24 hours

Hepatoma

200k | 400k | 880k



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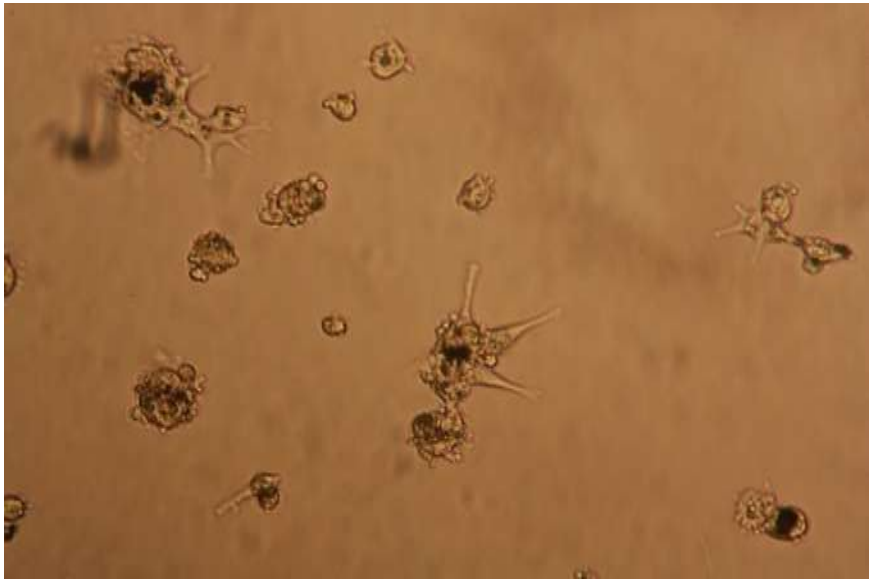
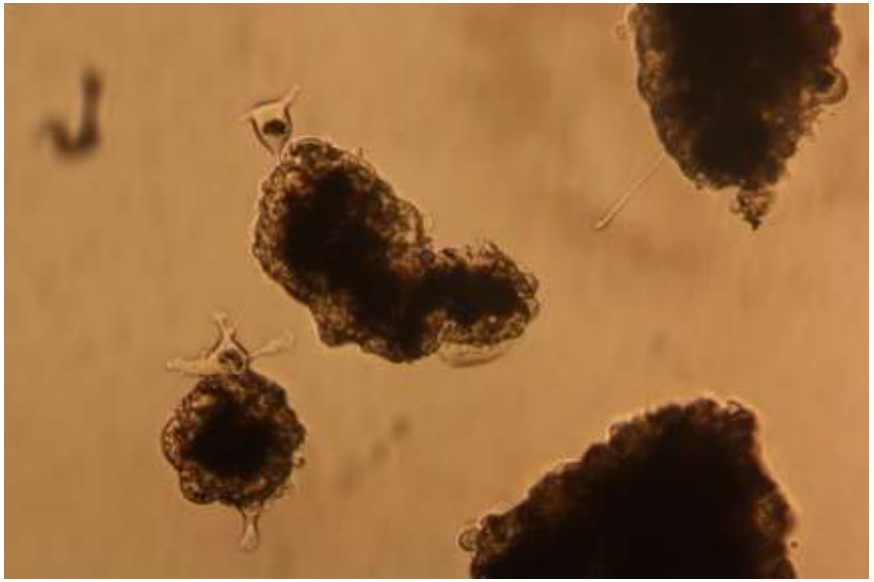
Human Mesenchymal Stem Cells

100k cells



200k cells



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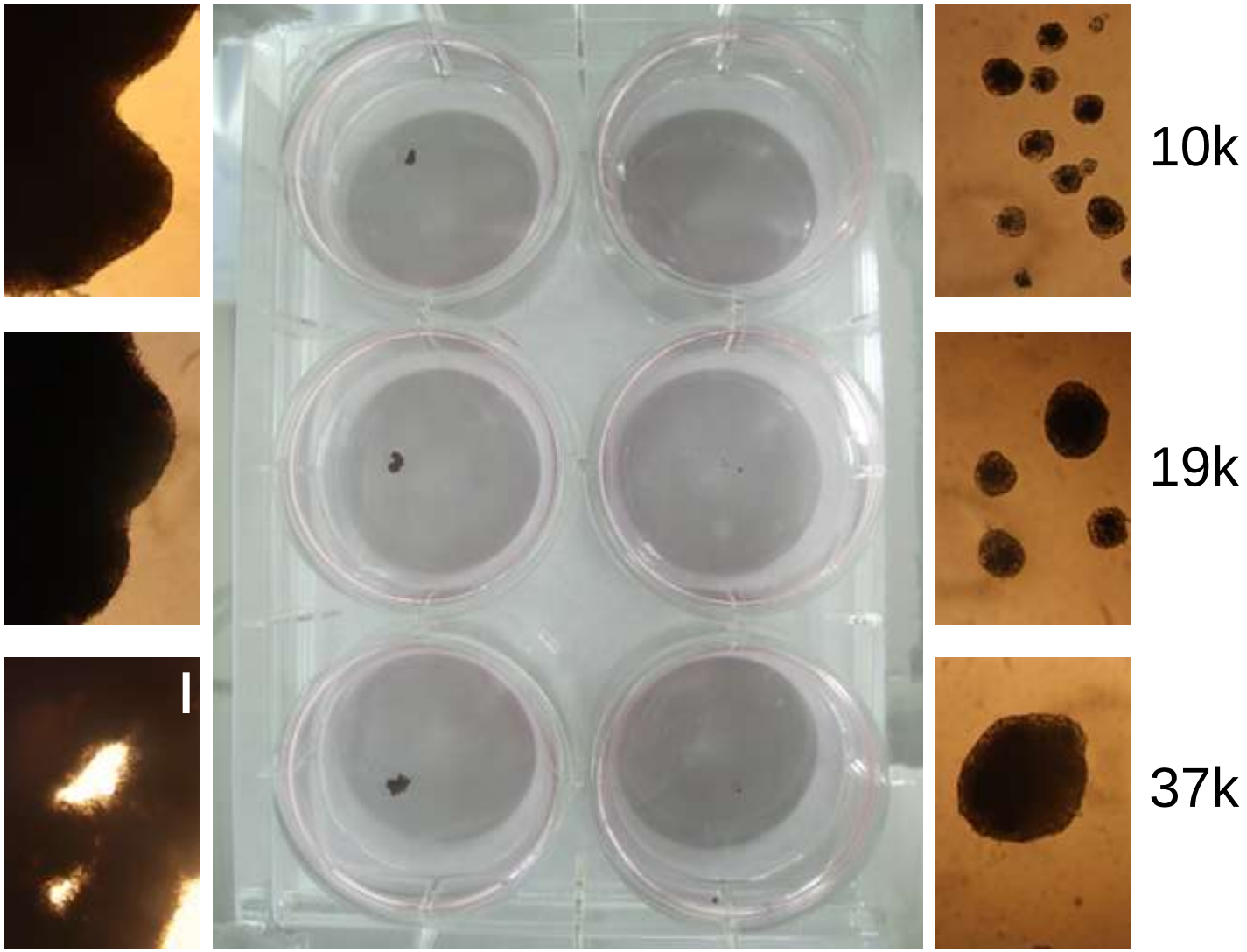
Number of Cells – Human Primary Pulmonary Fibroblast



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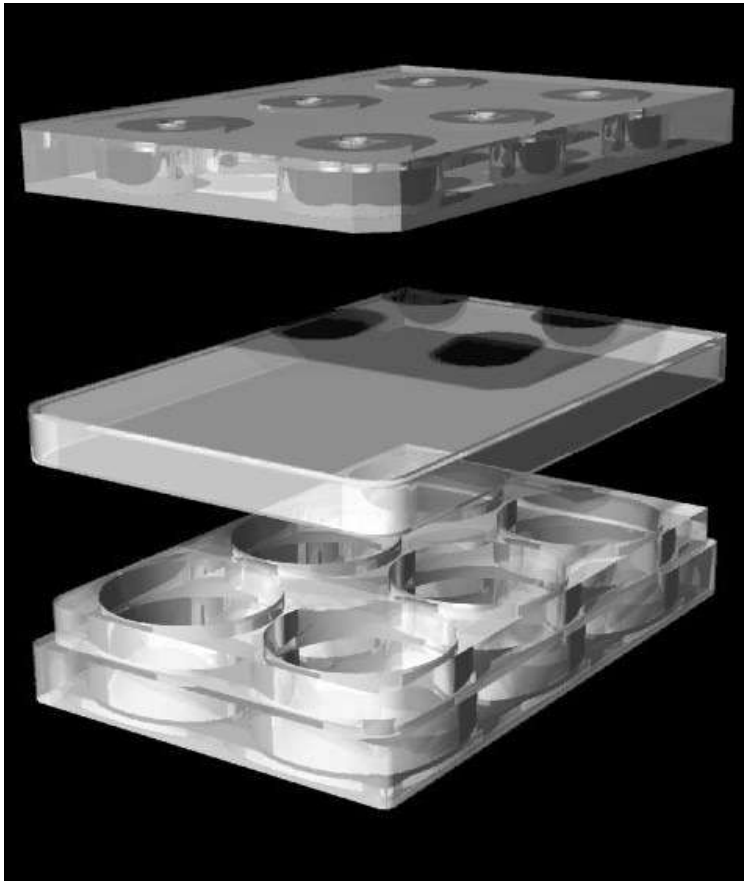
6-Well Bio-Assembler™



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**Submitted for Publication*

Lung Primary Cells

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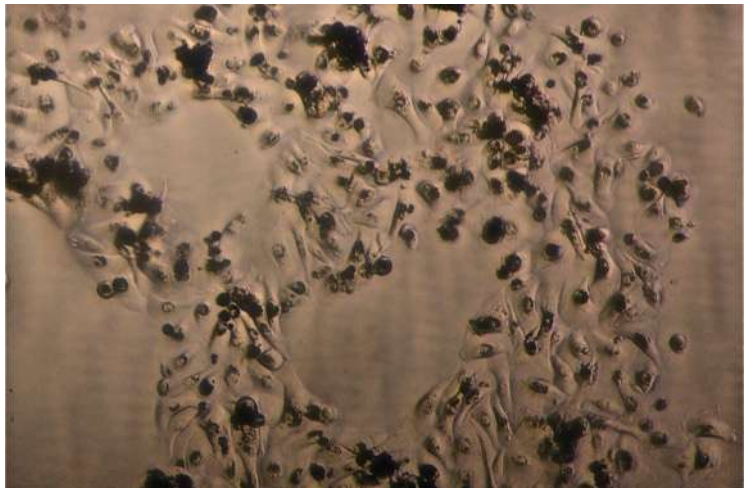
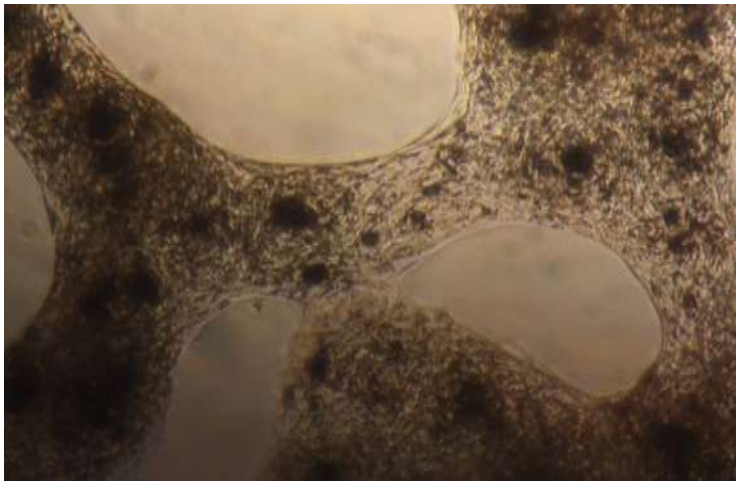
Human Lung Primary Cells

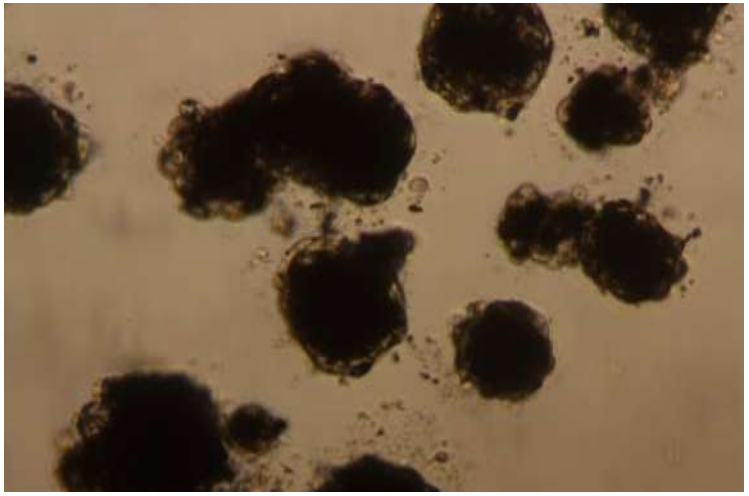
Epithelial



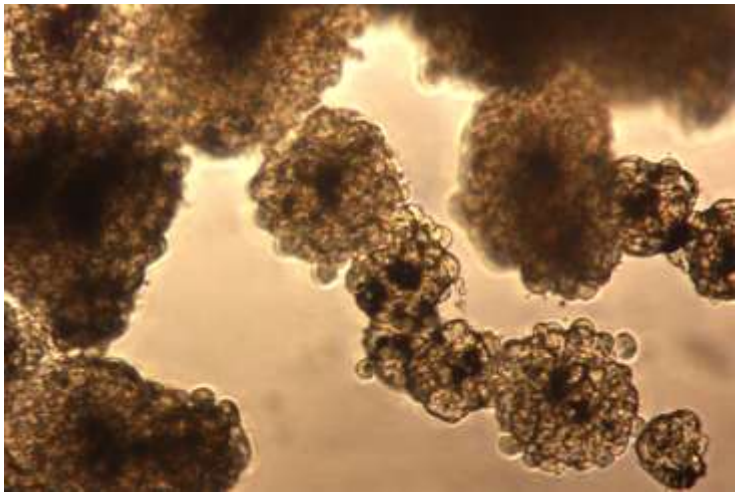
Smooth Muscle



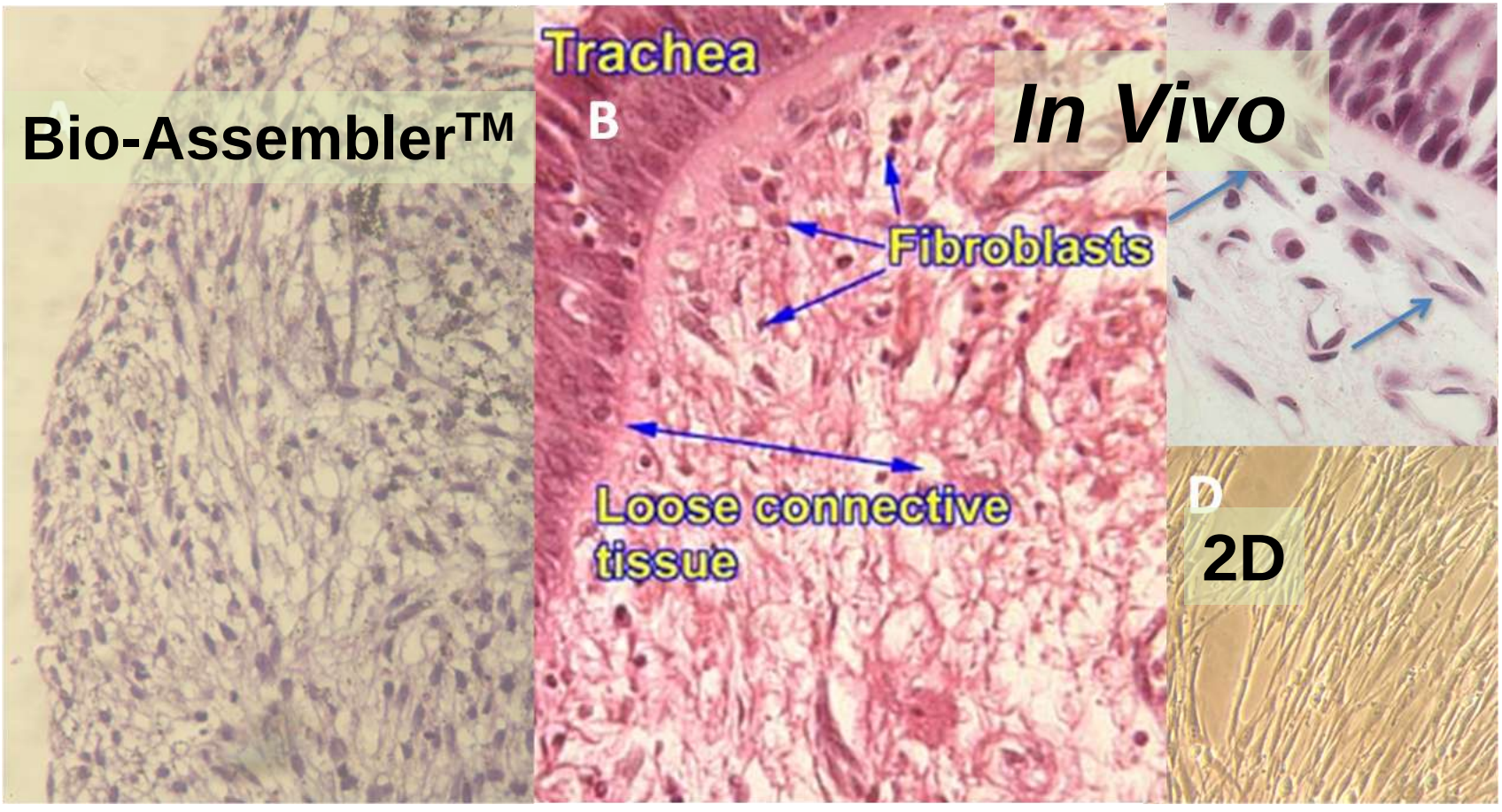
Endothelial



Fibroblast



Primary Pulmonary Fibroblasts



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Human Umbilical Vein Cells
HUVEC

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HUVEC – Macrostructure

*48 hour culture

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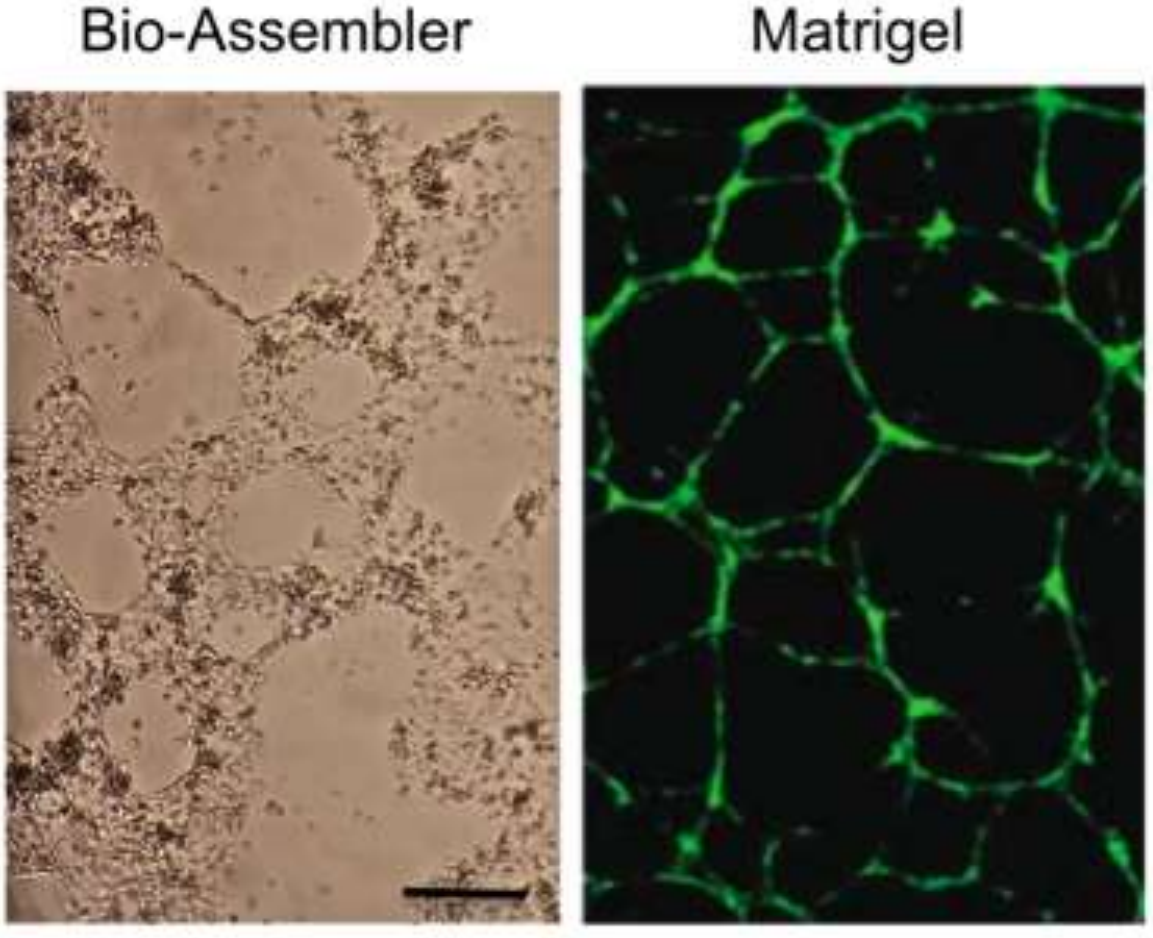
HUVEC - Microstructure



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Rapid 3D Formation by Promoting Cell-Cell Interaction

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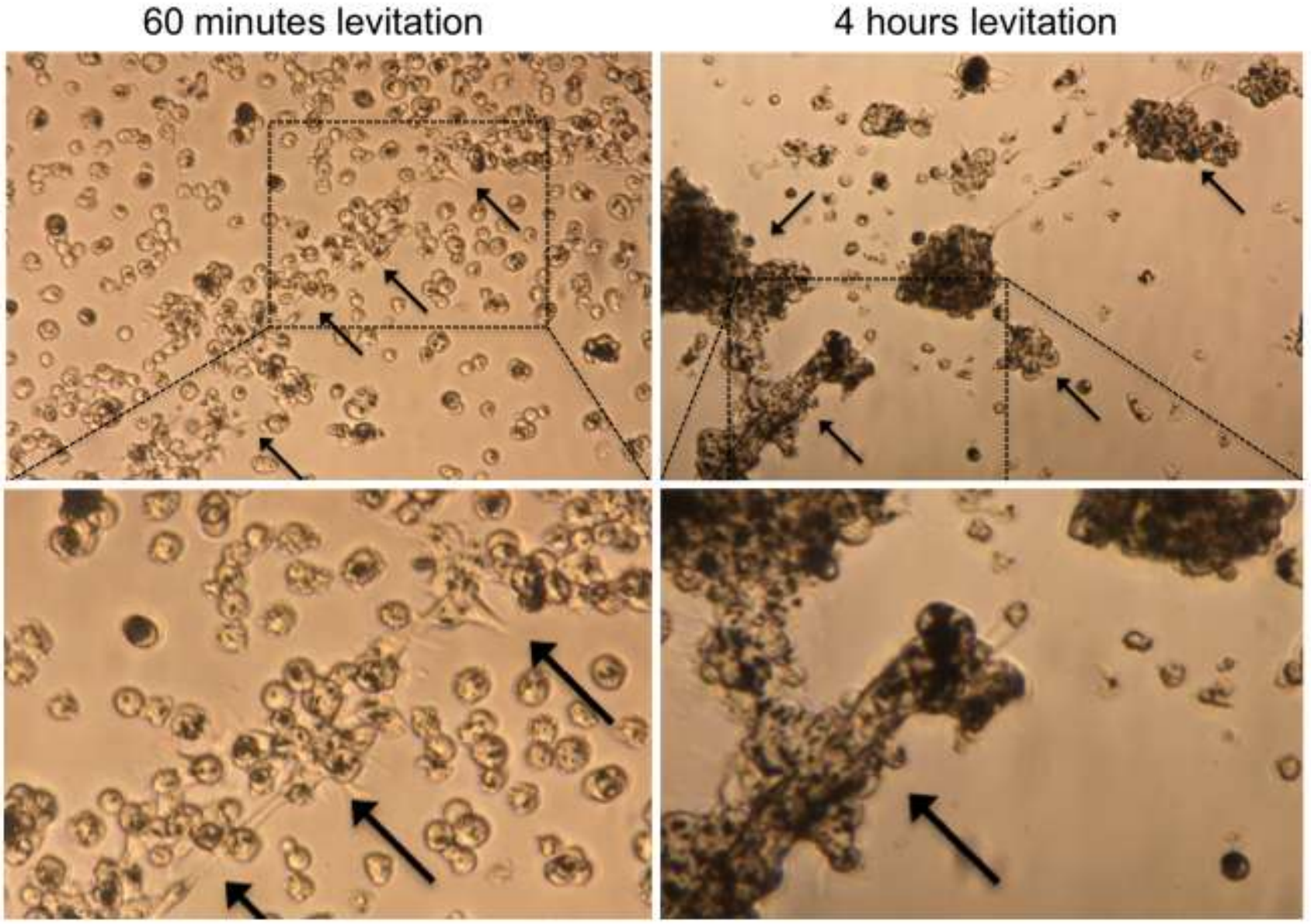
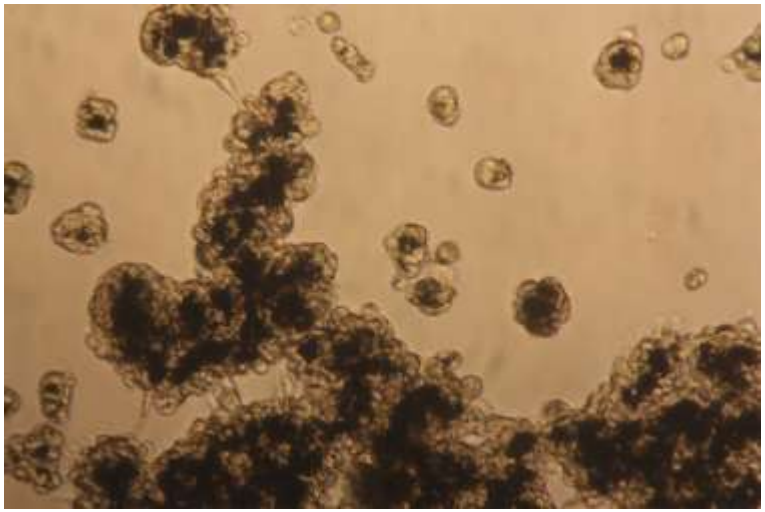
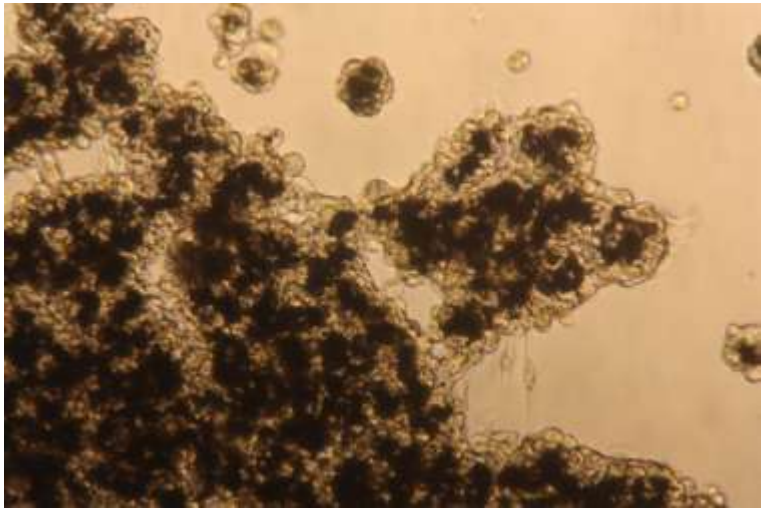
HUVEC 1 & 4 Hours Culture



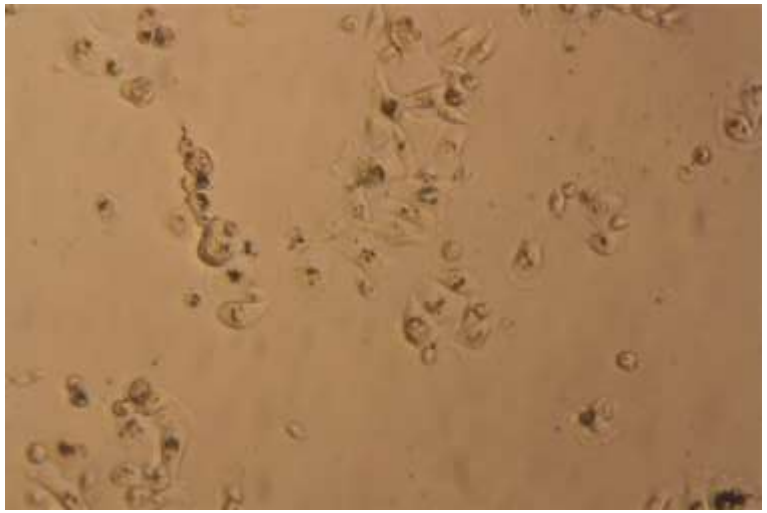
4 Hours of Levitation – Primary Cells

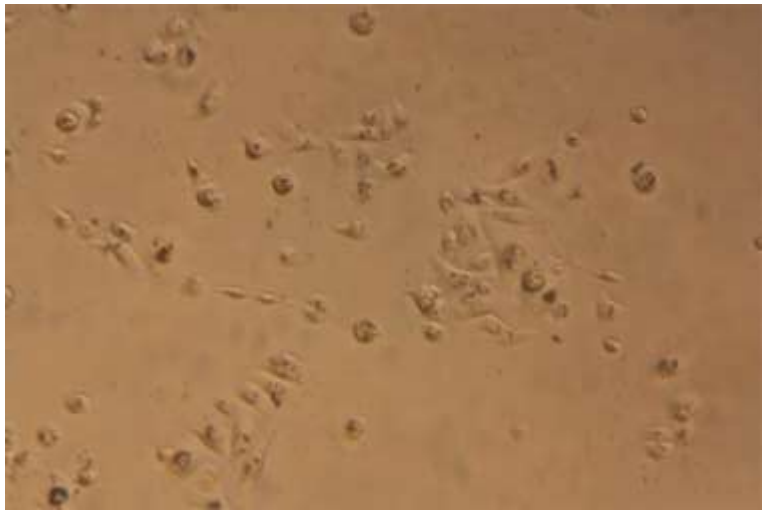
Fibroblast





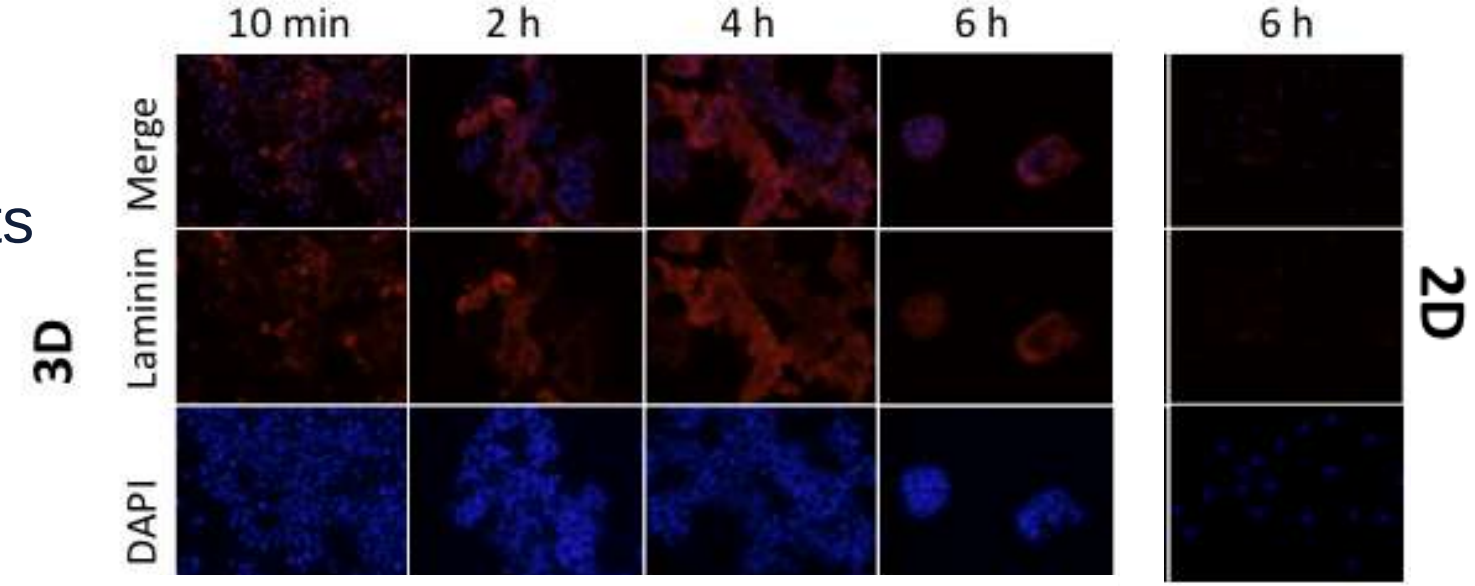
Epithelial



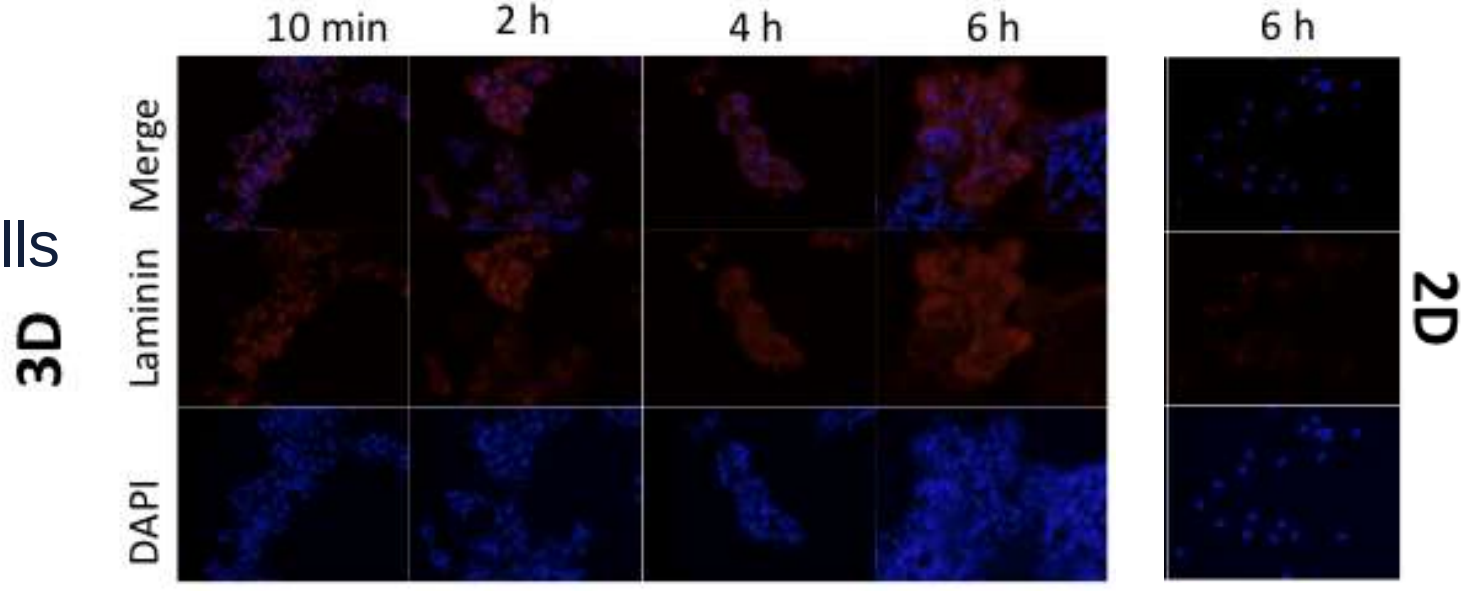


Extracellular Matrix:Laminin Immunohistochemistry

Fibroblasts



Smooth Muscle Cells



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Stem Cells - Dental Pulp

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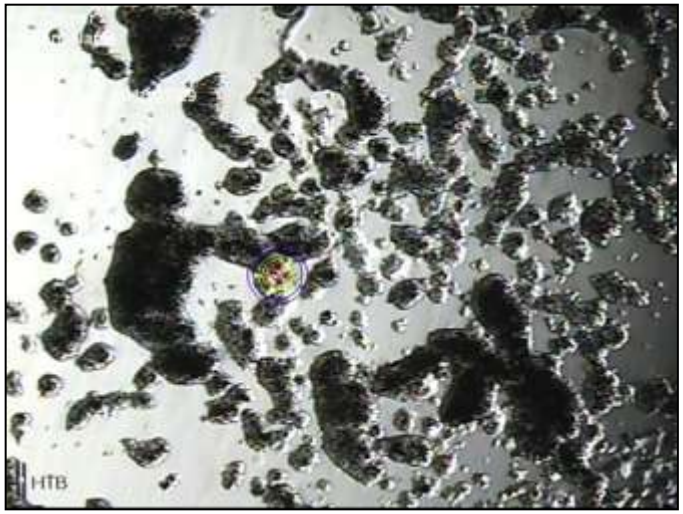
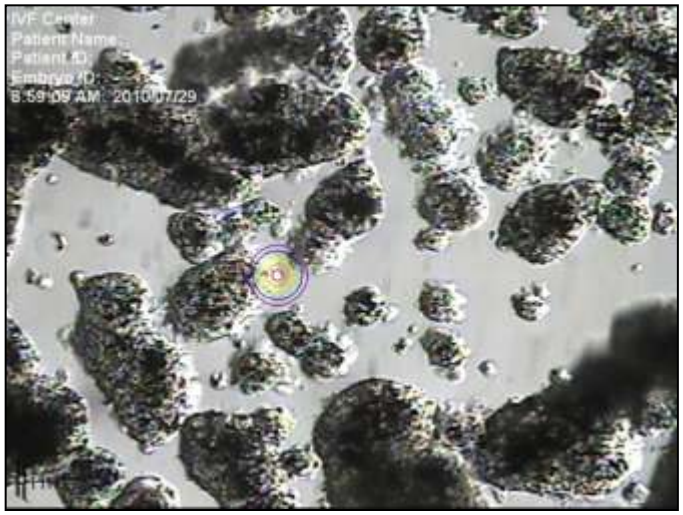
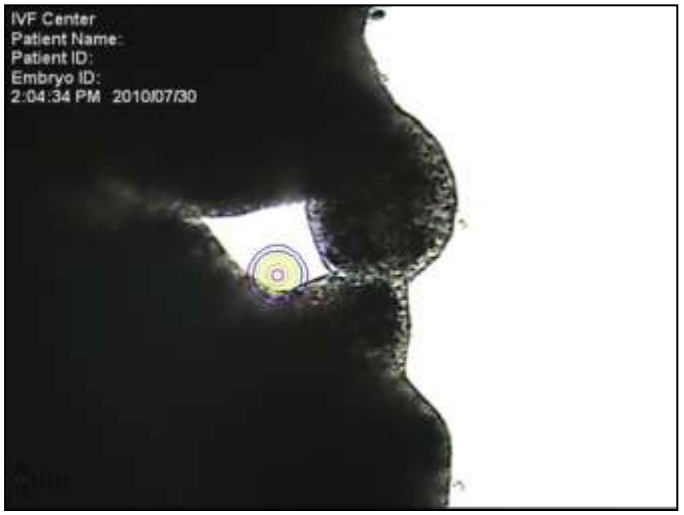
Cells from Dental Pulp

Day 1



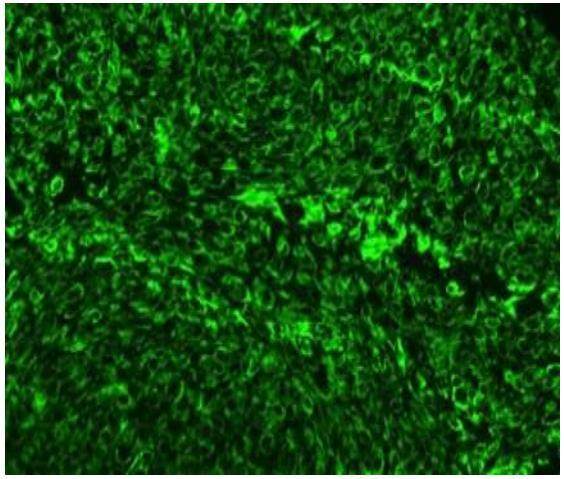
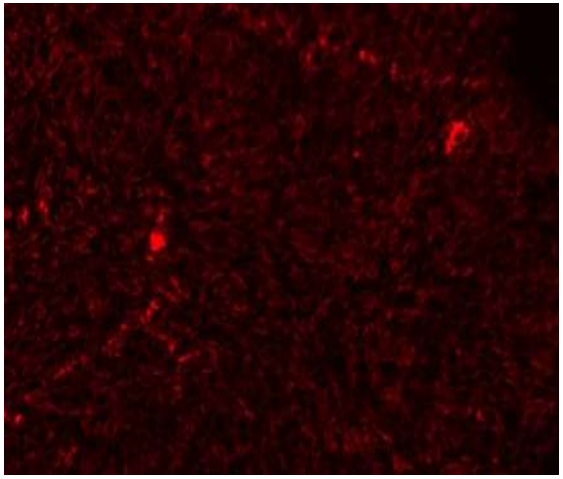
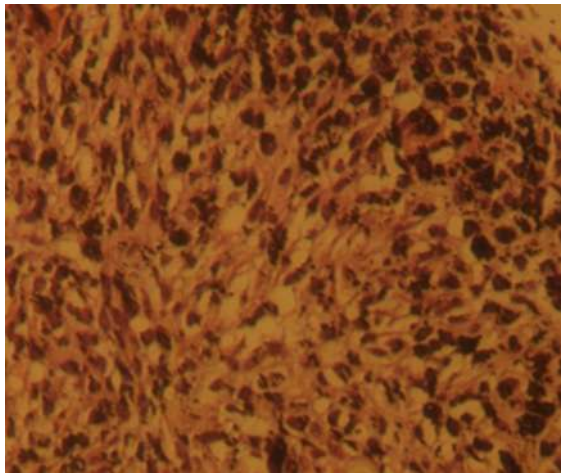


Day 2



In collaboration with Dr. Dozortsev, Director of Advanced Fertility Center of Texas

Stem Cells from Dental Pulp Immunohistochemistry

	Vimentin	Stro-1	H&E
Stem Cells			
Negative Controls*			

*Negative Controls – secondary only

Stem Cells – Adipose Derived

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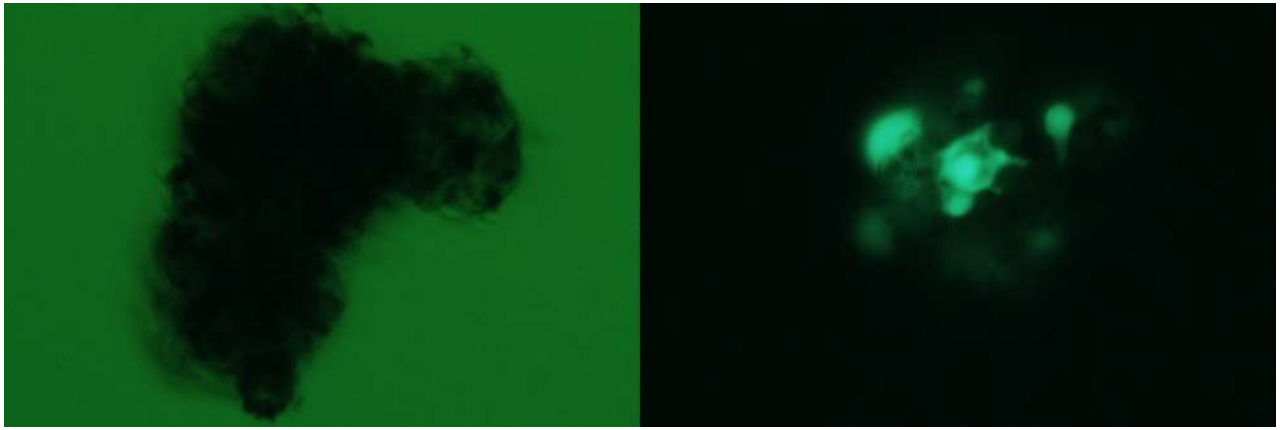
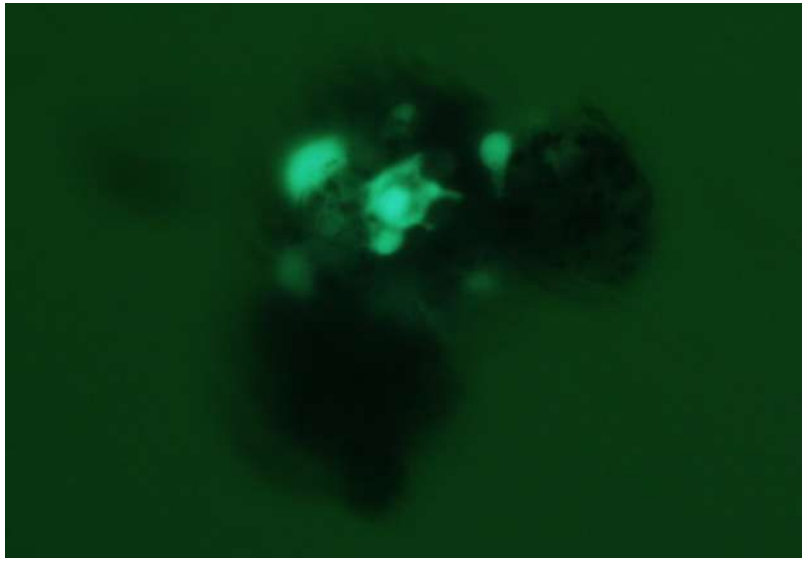
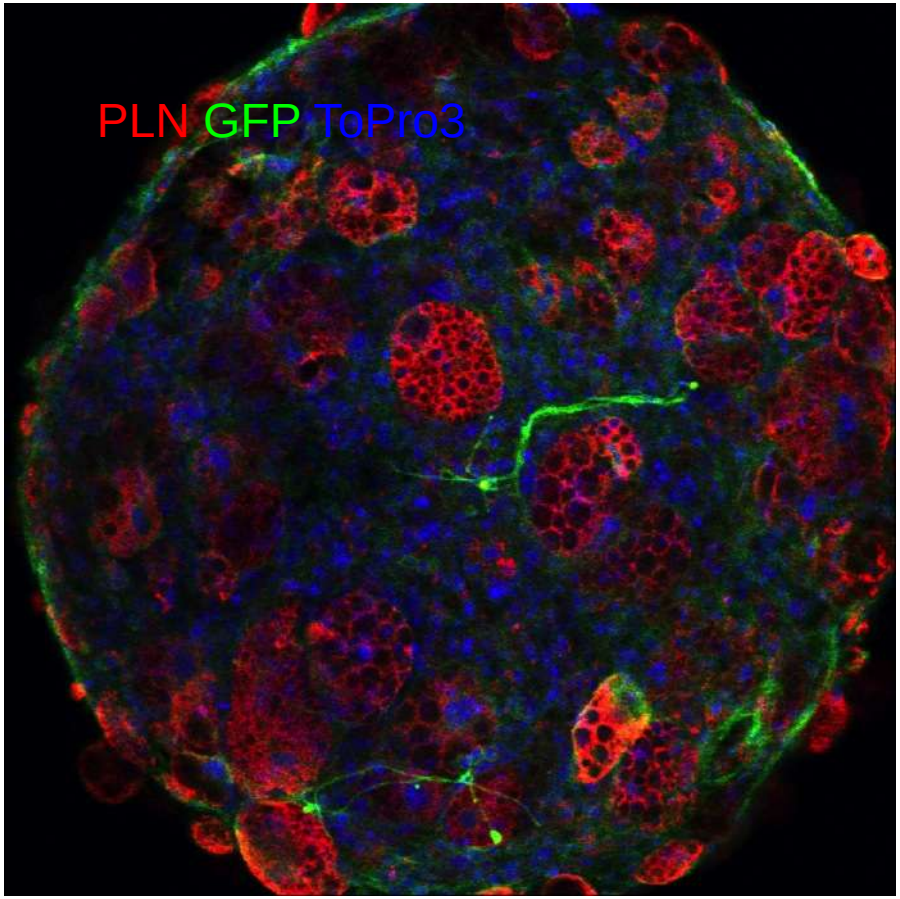
Co-Culturing Endothelial (GFP) and Fibroblast





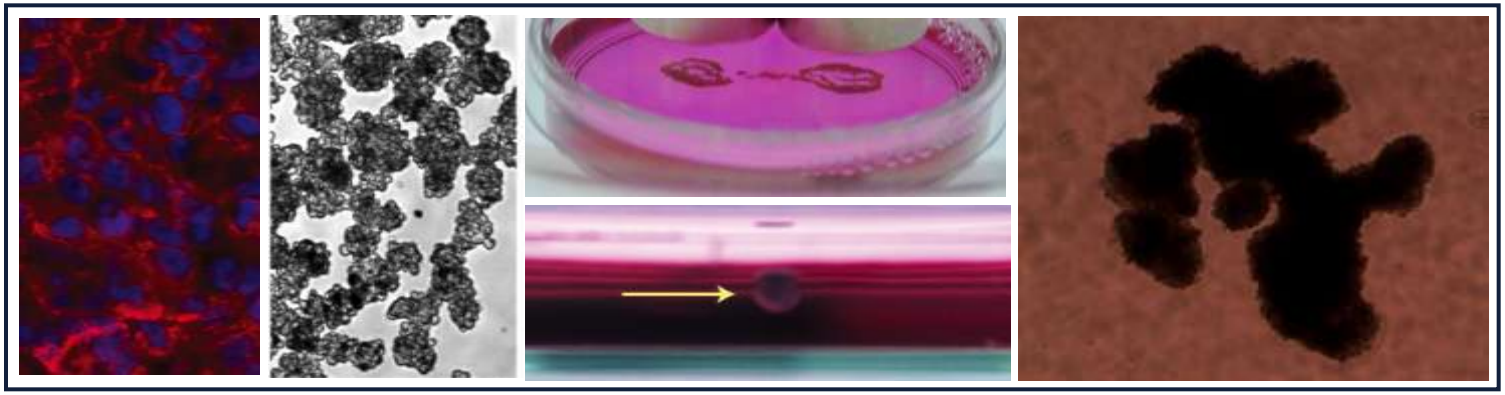
Adiposphere organoid composed of differentiated 3T3-L1 in co-culture with bEND.3-GFP



Day 14 after induction of adipogenesis

bEND.3-GFP endothelial cells formed microvessels within the adiposphere. Larger lipid droplet formations are also observed.

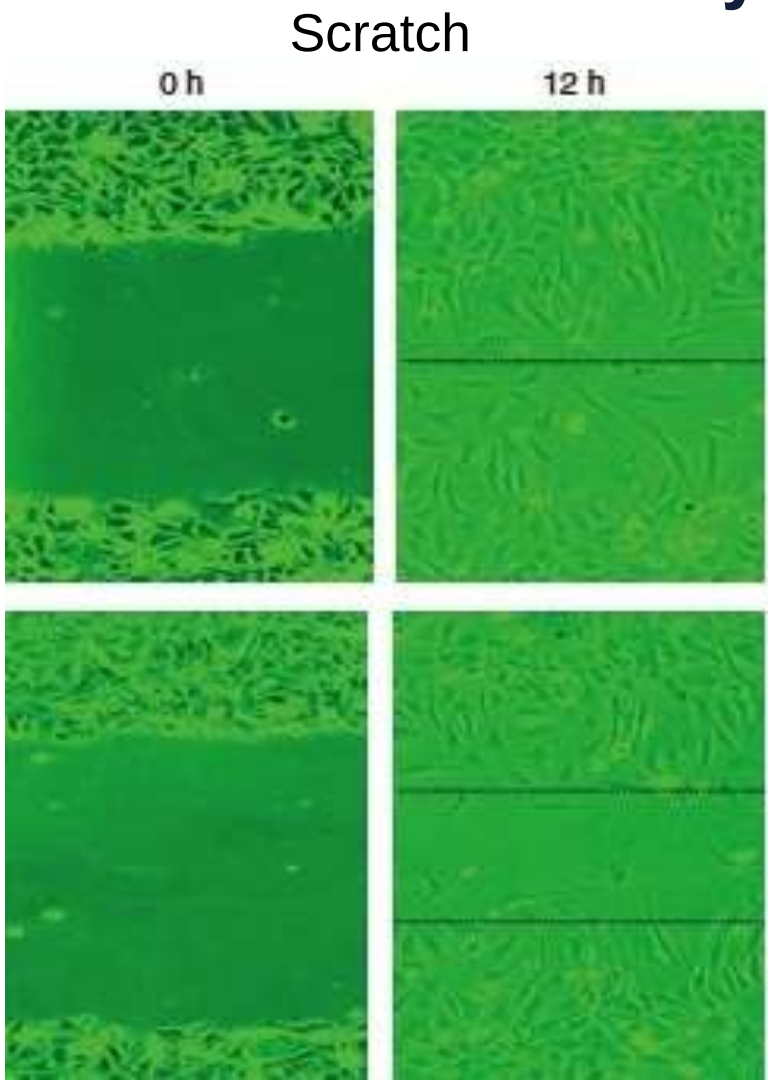
Levitated Cell Types



- **Human Primary Cells**
  - Pulmonary Fibroblast
  - Pulmonary Endothelial & HUVEC
  - Small Air Way Epithelial
  - Tracheal Smooth muscle
  - Mesenchymal Stem Cells
  - Dental Pulp Stem Cells
  - Murine Adipose Tissue
- **Bone Marrow Endothelial**
- **Heart Valve endothelial**
- **Human Mammary Epithelial - MCF10A**
- **Pre-adipocytes Fibroblasts**
- **Adipocytes**
- **Neural Stem Cells**
- **HEK 293**
- **Melanoma**
- **Astrocytes**
- **Glioblastomas**
- **T-Cells and Antigen Presenting Cells**
- **Chondrocytes**

New Tool
3D Wound Healing Assay

2D Scratch Assay
Today's *In Vitro* Wound healing Model

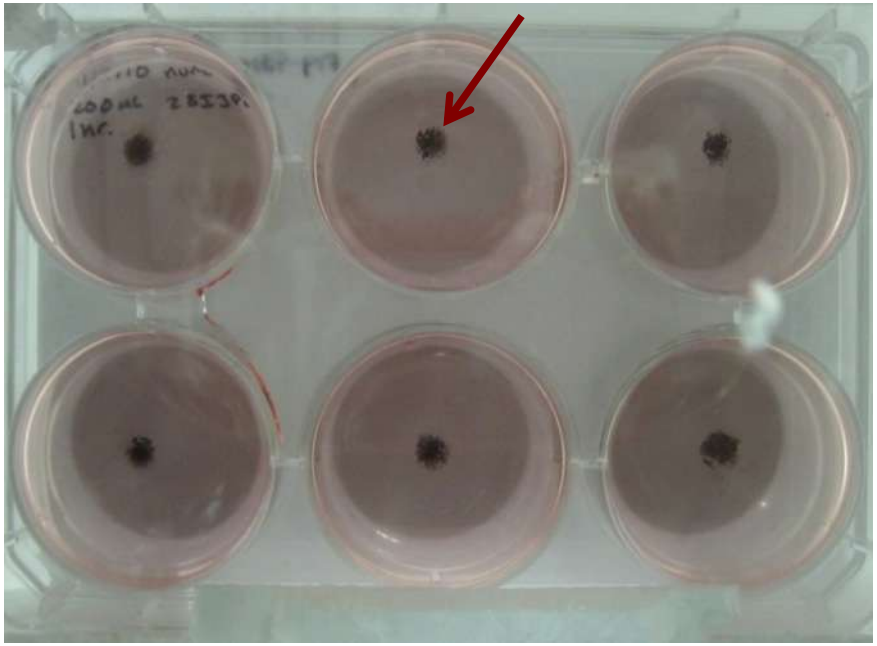


- 2D culture
- Poor cell-cell interaction representation
- Interaction of cells with plastic or coated surface = **NOT *in vivo* like**
- Difficult to co-culture different cell types
- No "wound" contraction

Liang et al., Nature Protocols, 1 March 2007

New Tool
3D Wound Healing Assay

6-Well Bio-Assembler™



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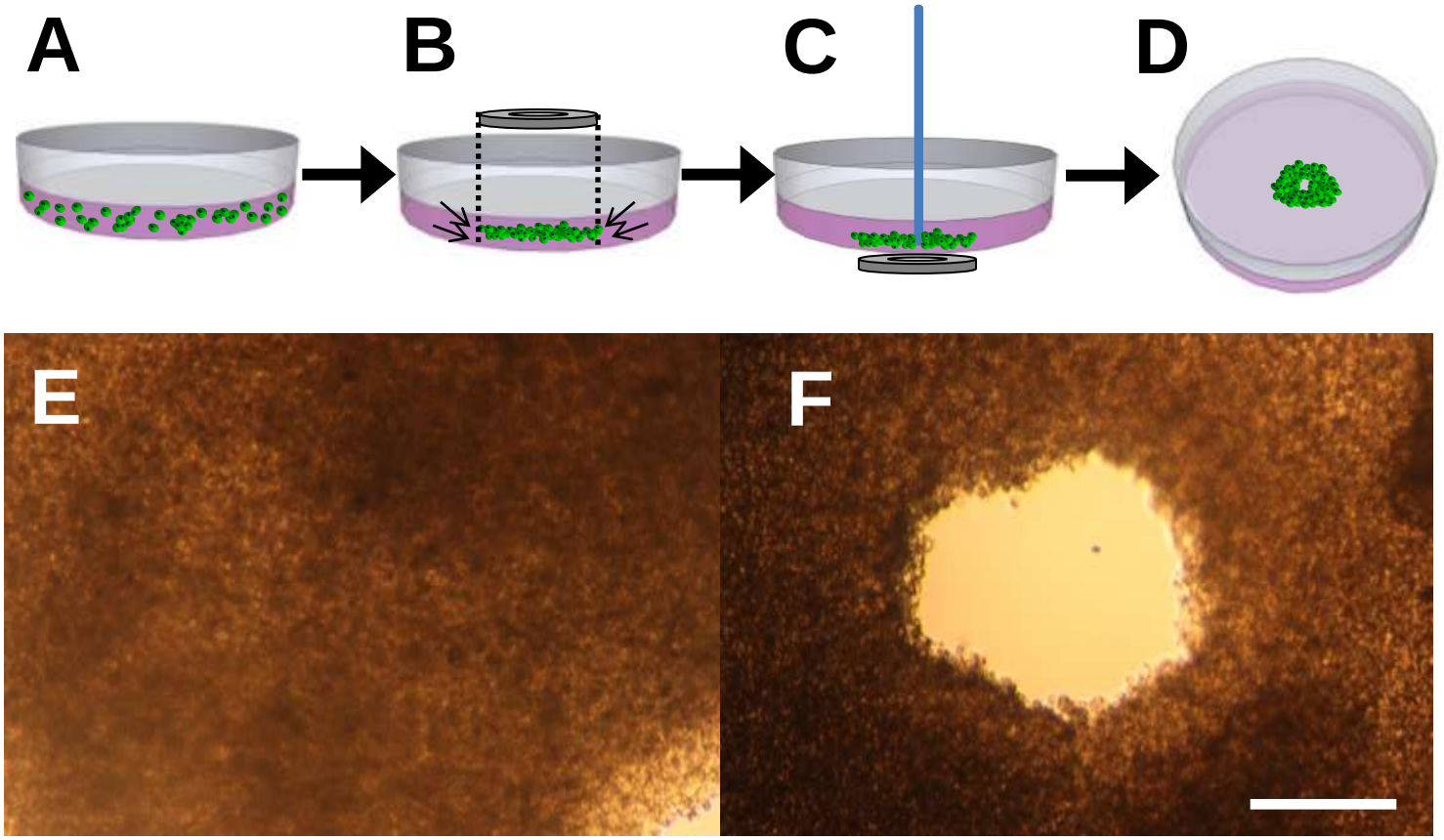
*Submitted for Publication

Puncturing Wound



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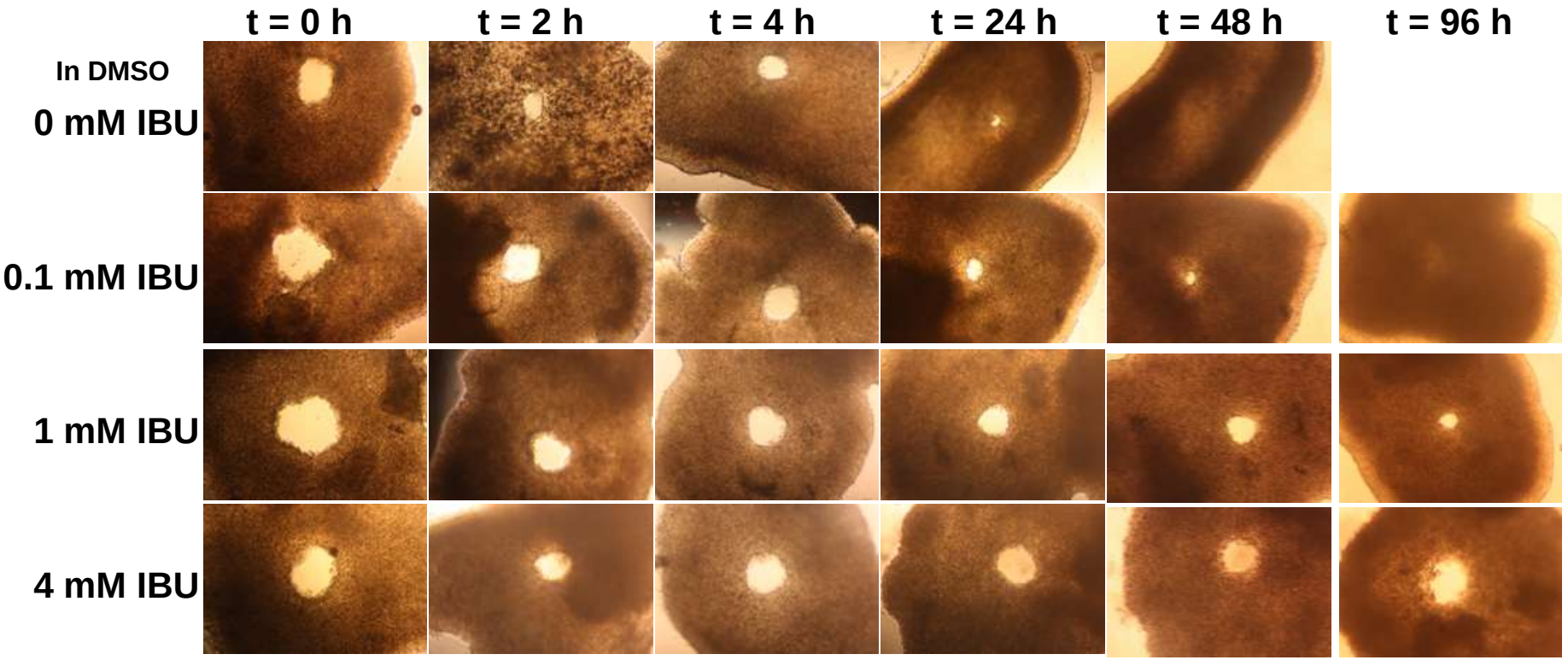
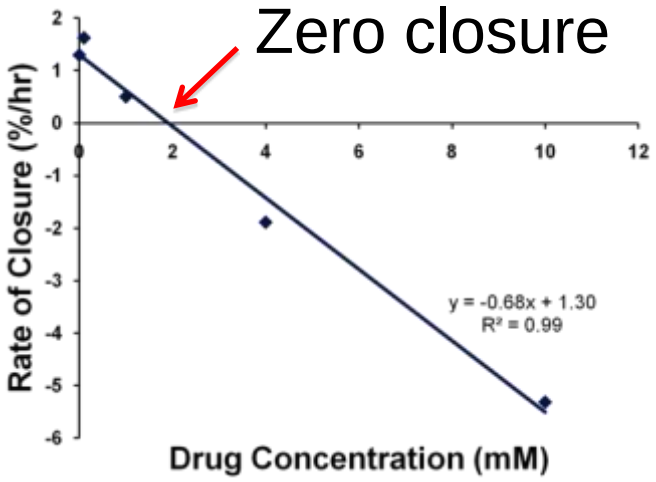
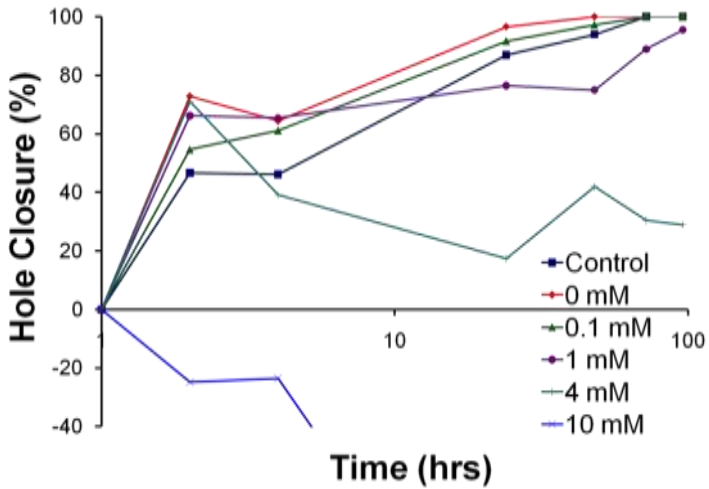
**Submitted for Publication*

HEK293 Wound Healing vs. Ibuprofen Concentration



*Submitted for Publication





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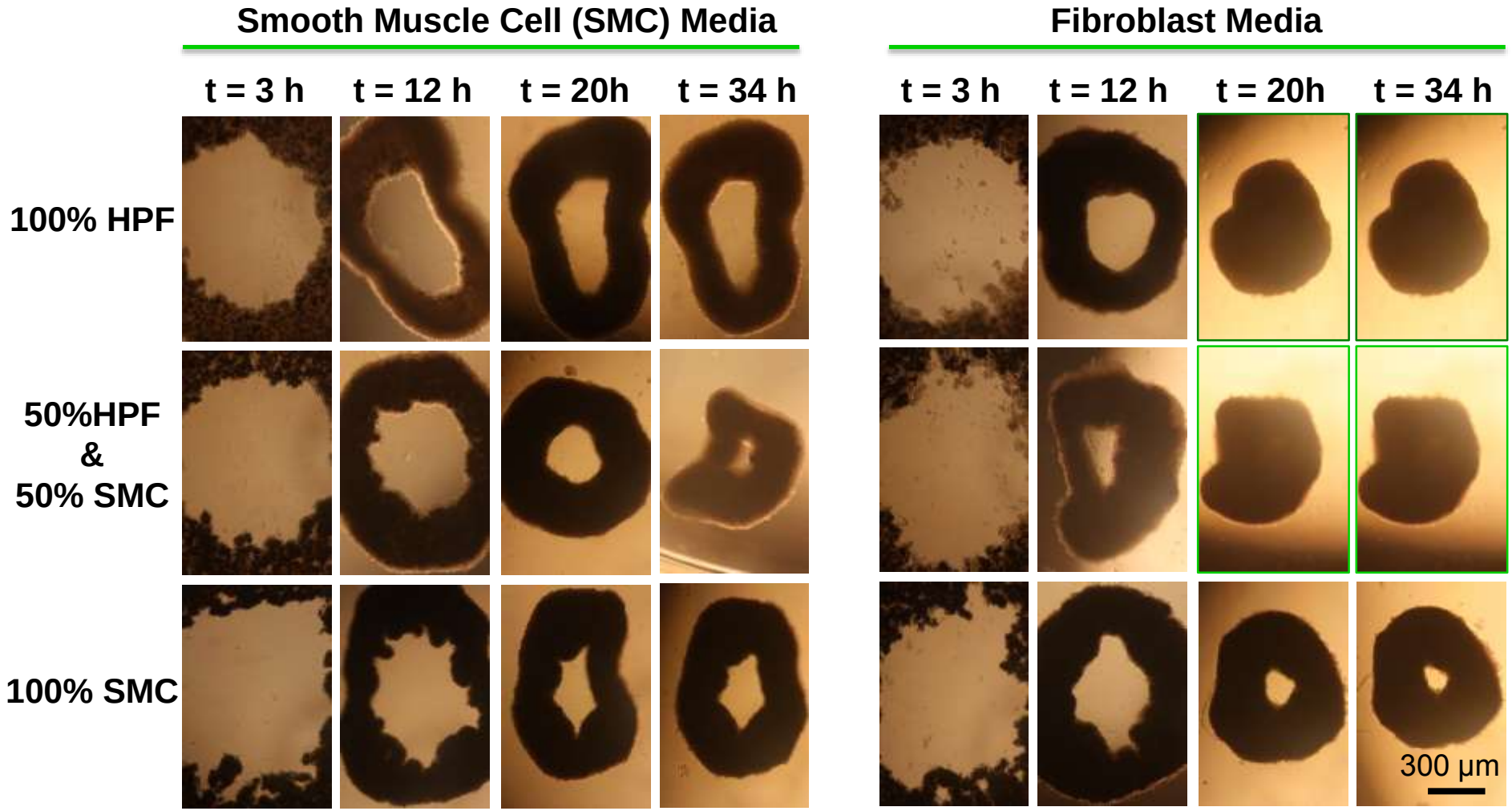
3D Wound Healing Assay by Co-Culturing Primary Cells



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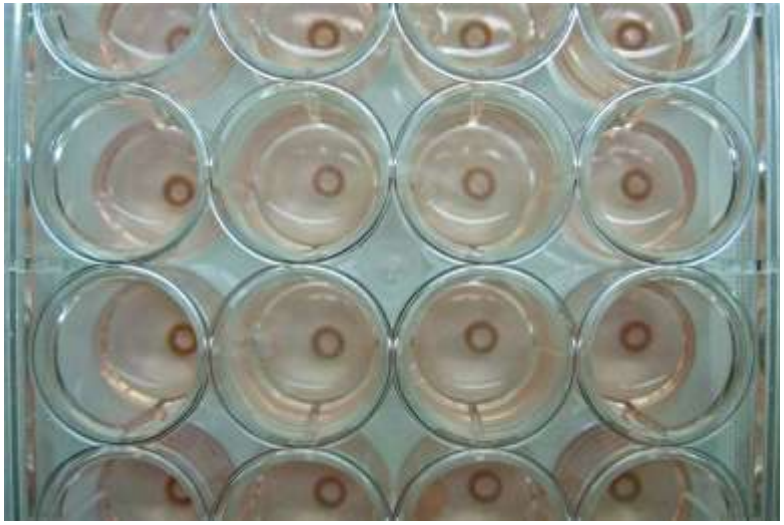
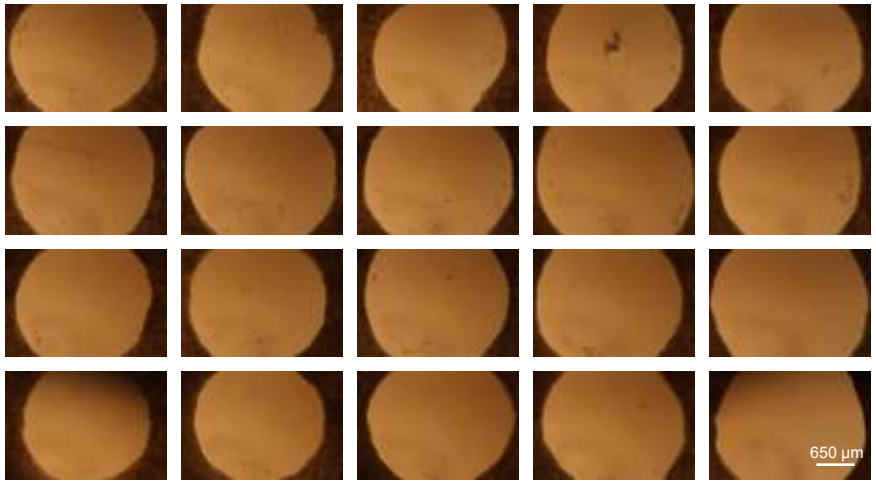
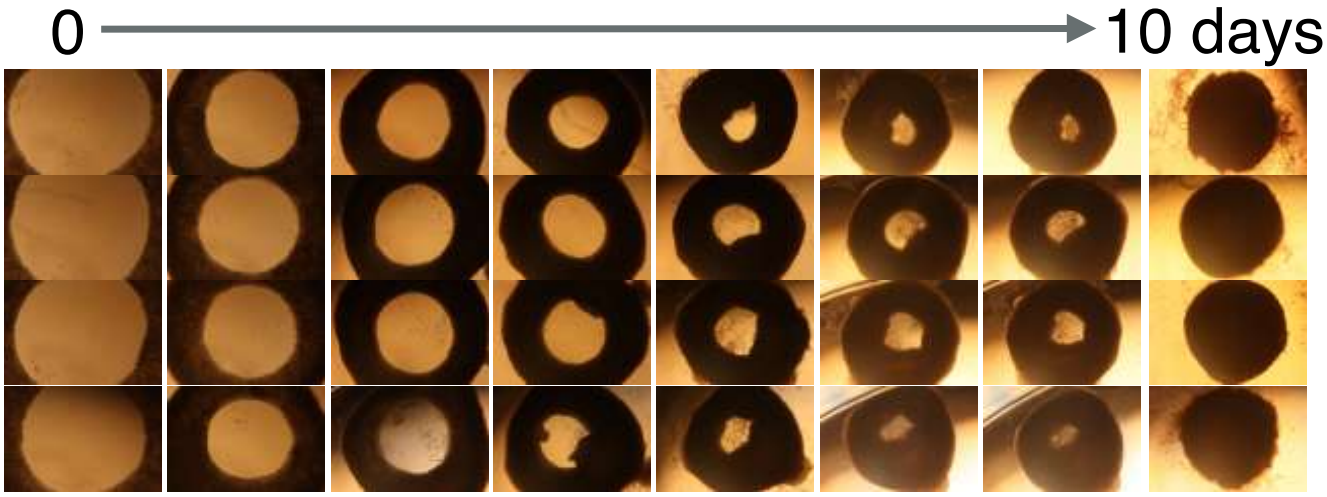
Magnetic Printing Method



Average Area of opening = 1.18 ± 0.18 mm^2





Future Tools

Tissue Layering with The Magnetic Pen

24- & 96-Well Plates

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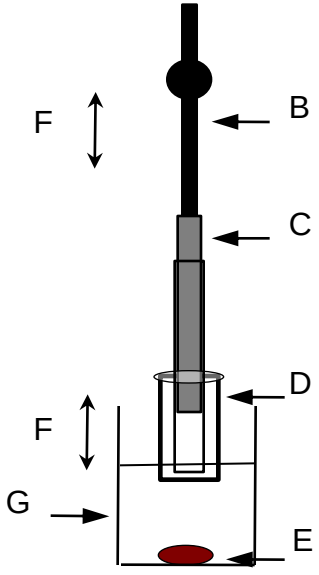
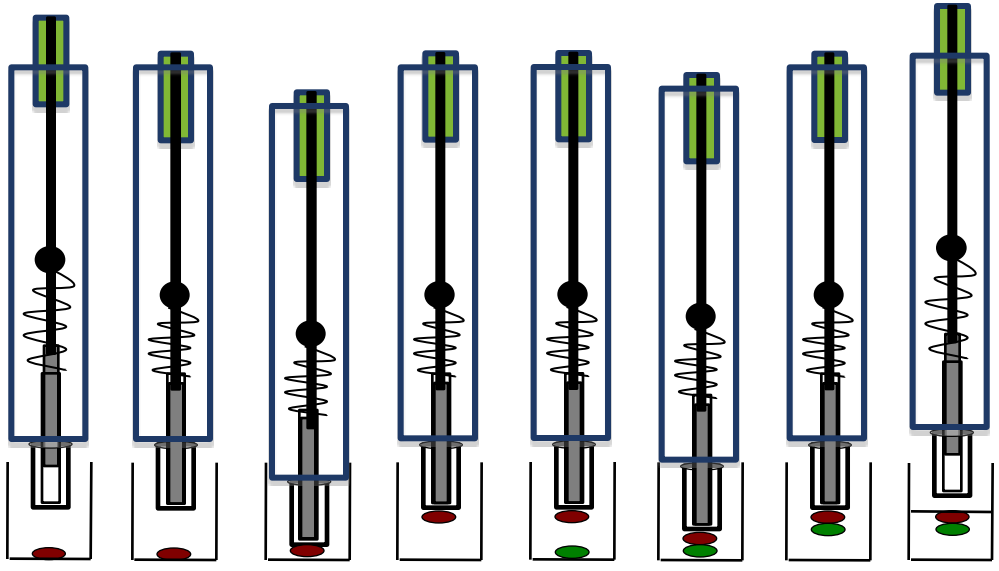
Magnetic Pen





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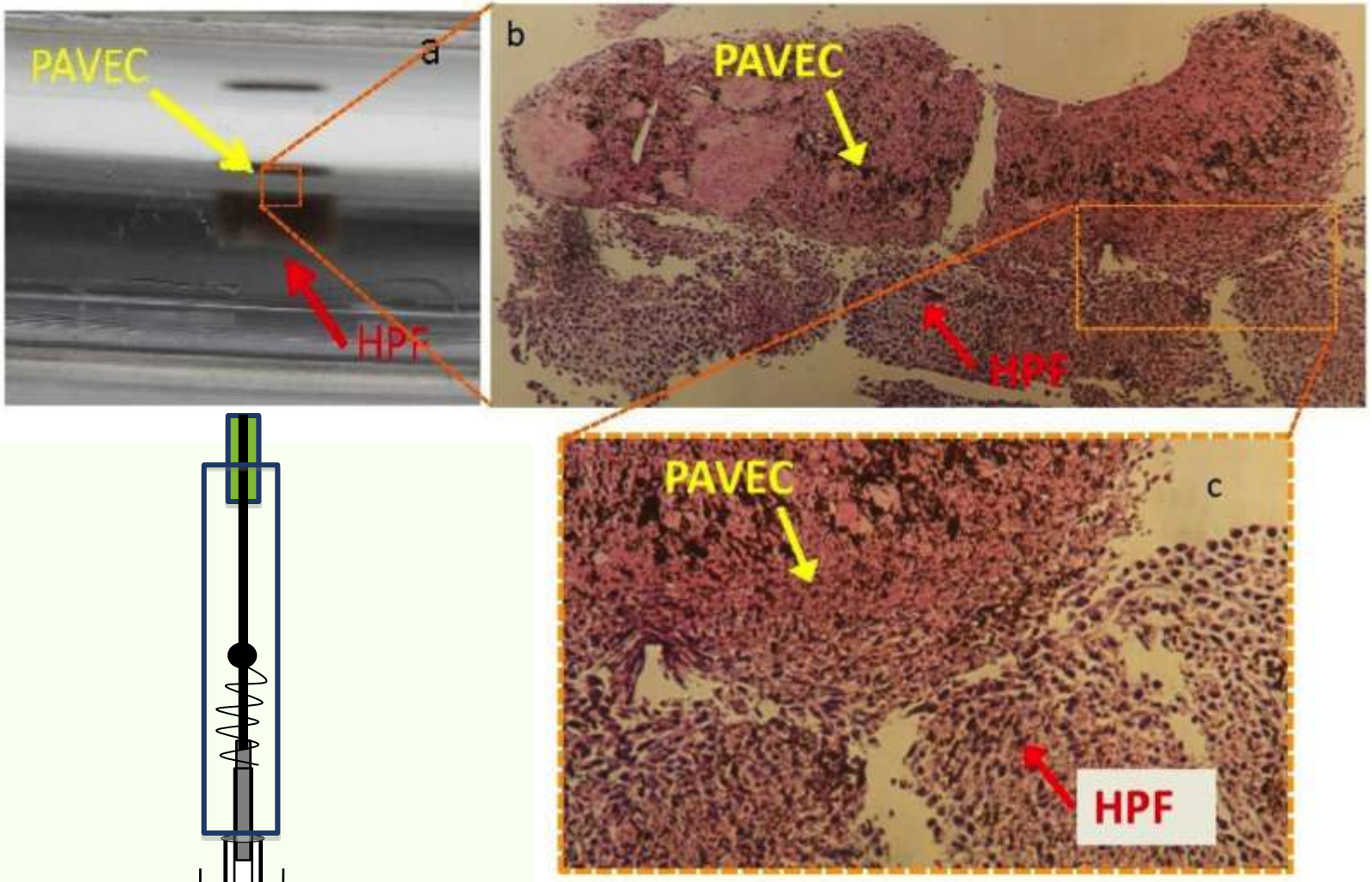
Layered Co-Culture



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In collaboration with **Dr. Jane Grande-Allen – Rice Bioengineering*

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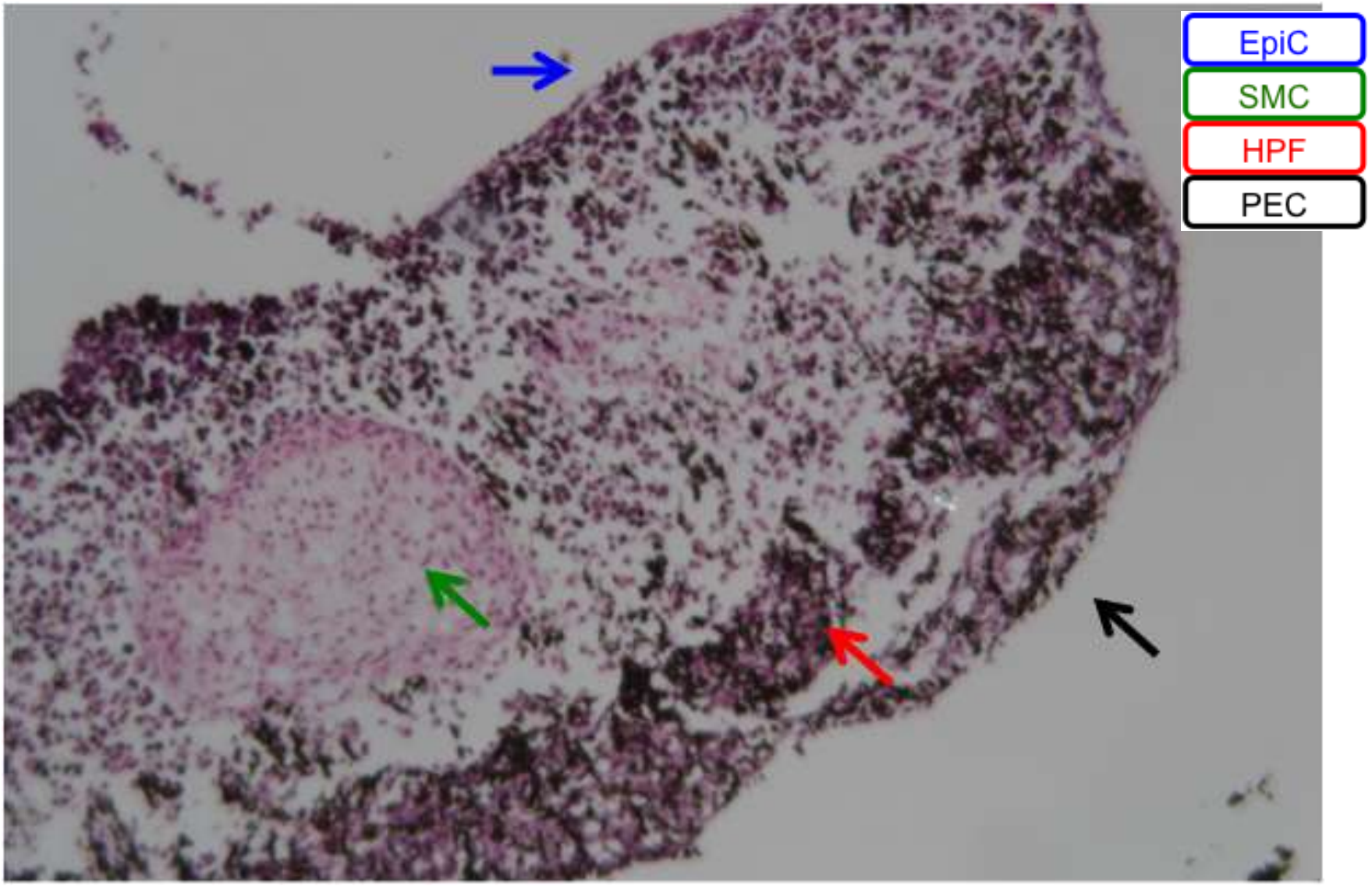
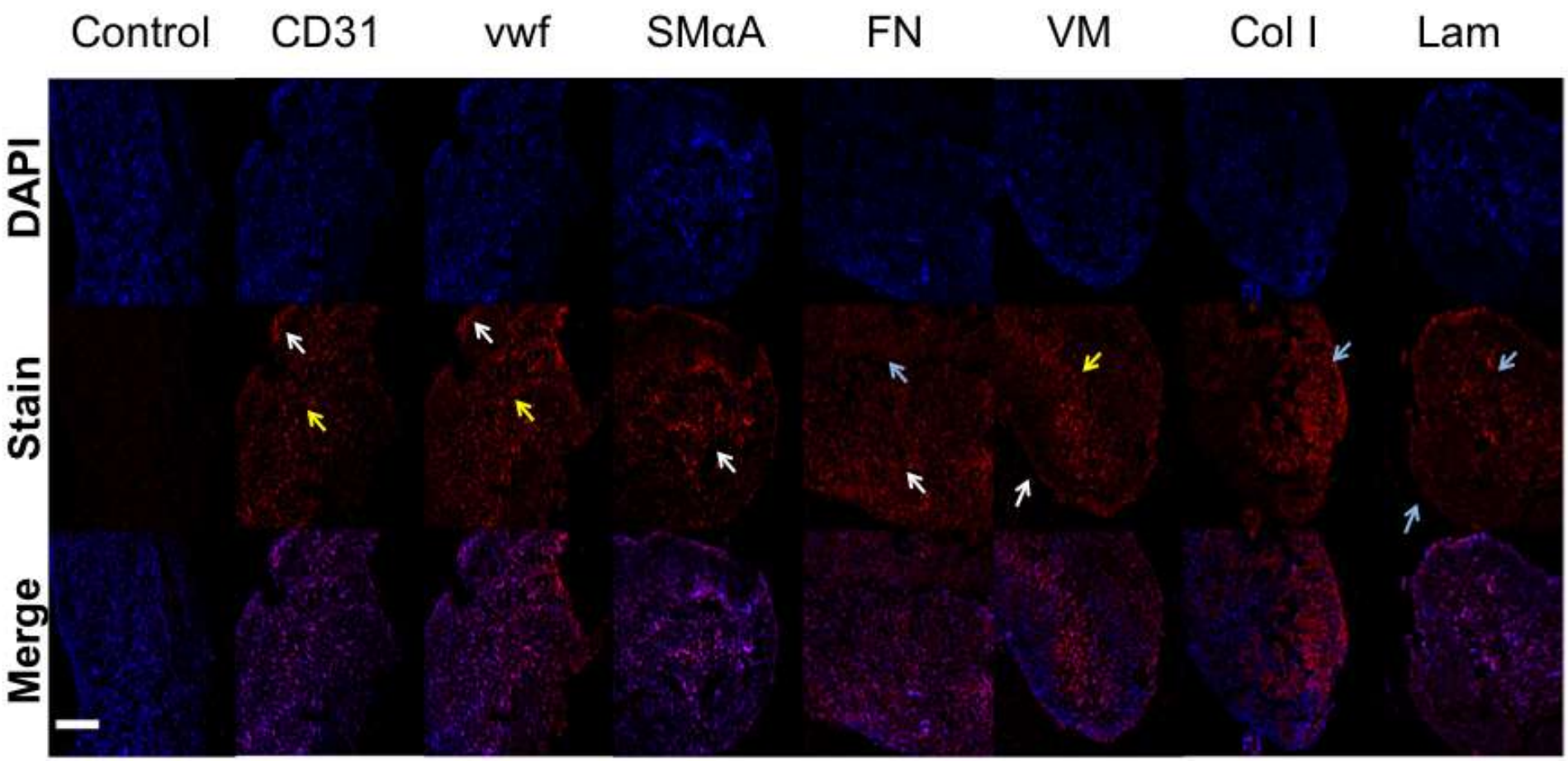
Layered Co-Culture
Four Primary Cell Types



In collaboration with **Dr. Jane Grande-Allen – Rice Bioengineering*

Immunohistochemistry of Four Cell Types Layered Co-Culture



Antigen	Purpose
CD31	PEC phenotypic markers
Von Willebrand's factor (vwf)	
Smooth muscle α-actin (SMαA)	SMC phenotypic markers

Fibronectin (FN)	HPF phenotypic marker
Vimentin (VM)	EpiC phenotypic marker
Collagen I (Col I)	Extracellular matrix components
Laminin (Lam)	

In collaboration with* ***Dr. Jane Grande-Allen* *– Rice Bioengineering*

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“Viability” Assay

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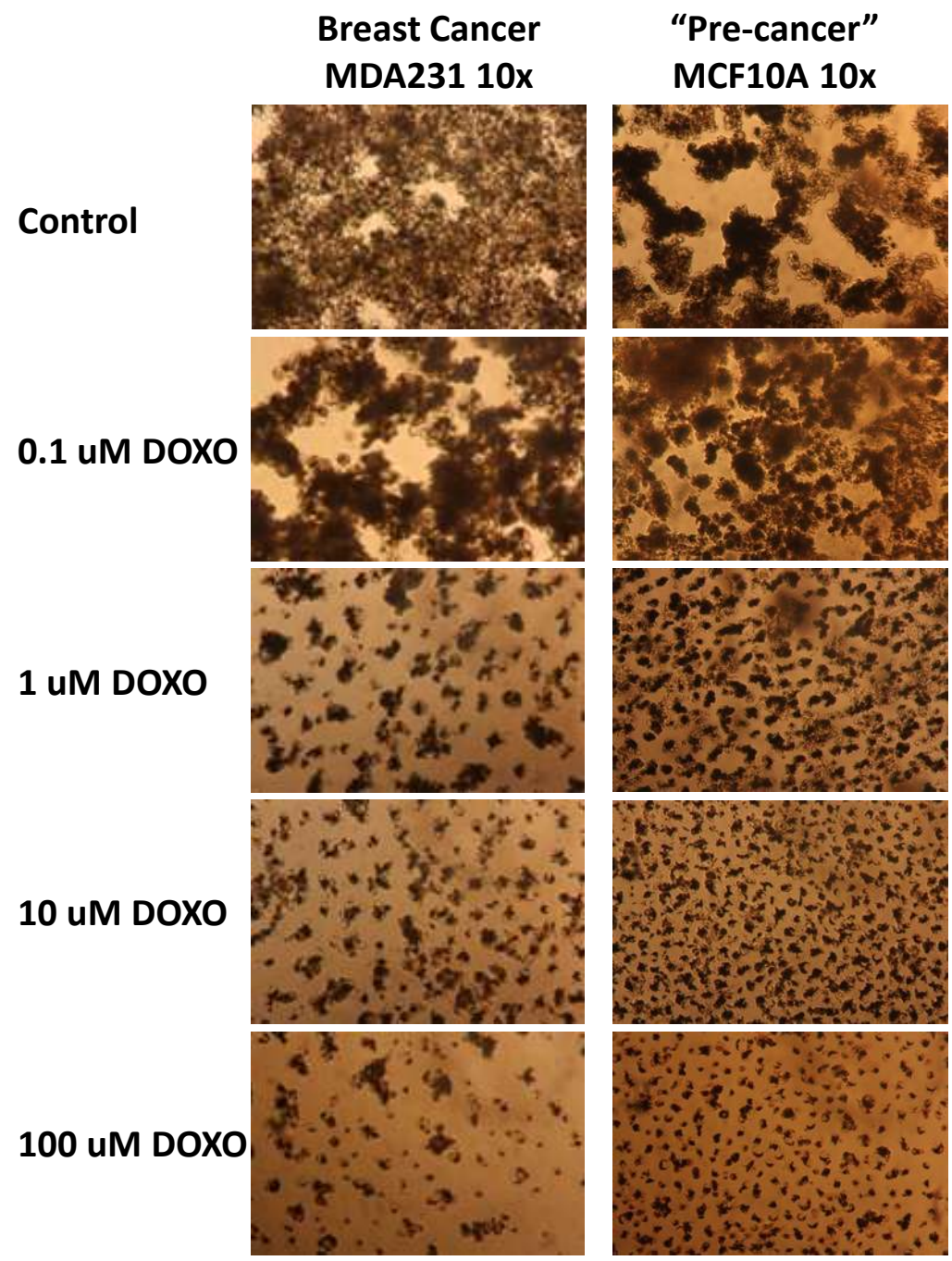
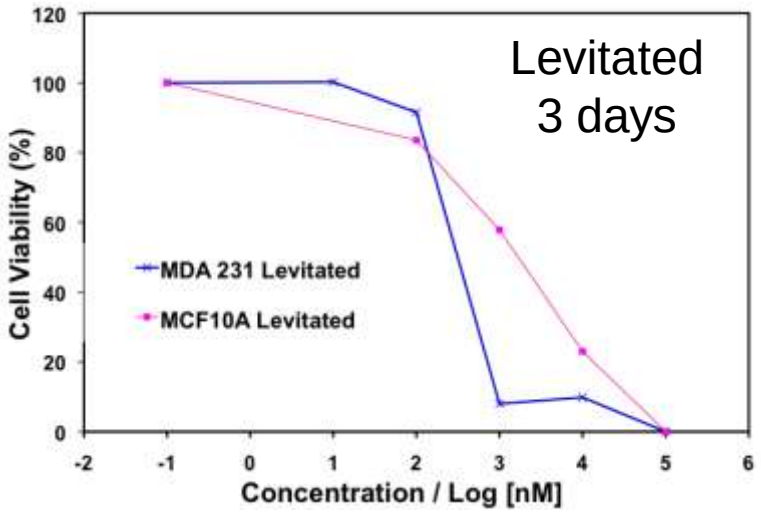
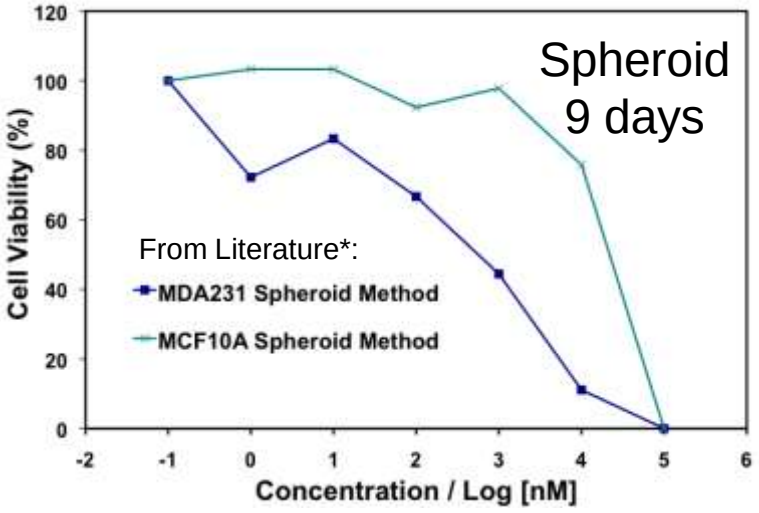
Traditional *In Vitro* Cell Viability Testing A Challenge in 3D Cell Culturing

- Metabolic assays - MTT
  - Poor permeability through 3D culture
  - underestimate cell viability
- DNA content – picoGreen
  - Challenging to extract DNA – requires extensive cell manipulation
  - underestimate cell viability
- Cell counting
  - Requires dispersing cells by enzimatic digestion which compromises cell viability
  - underestimate cell viability
- Poor correlation with side-by-side 2D results

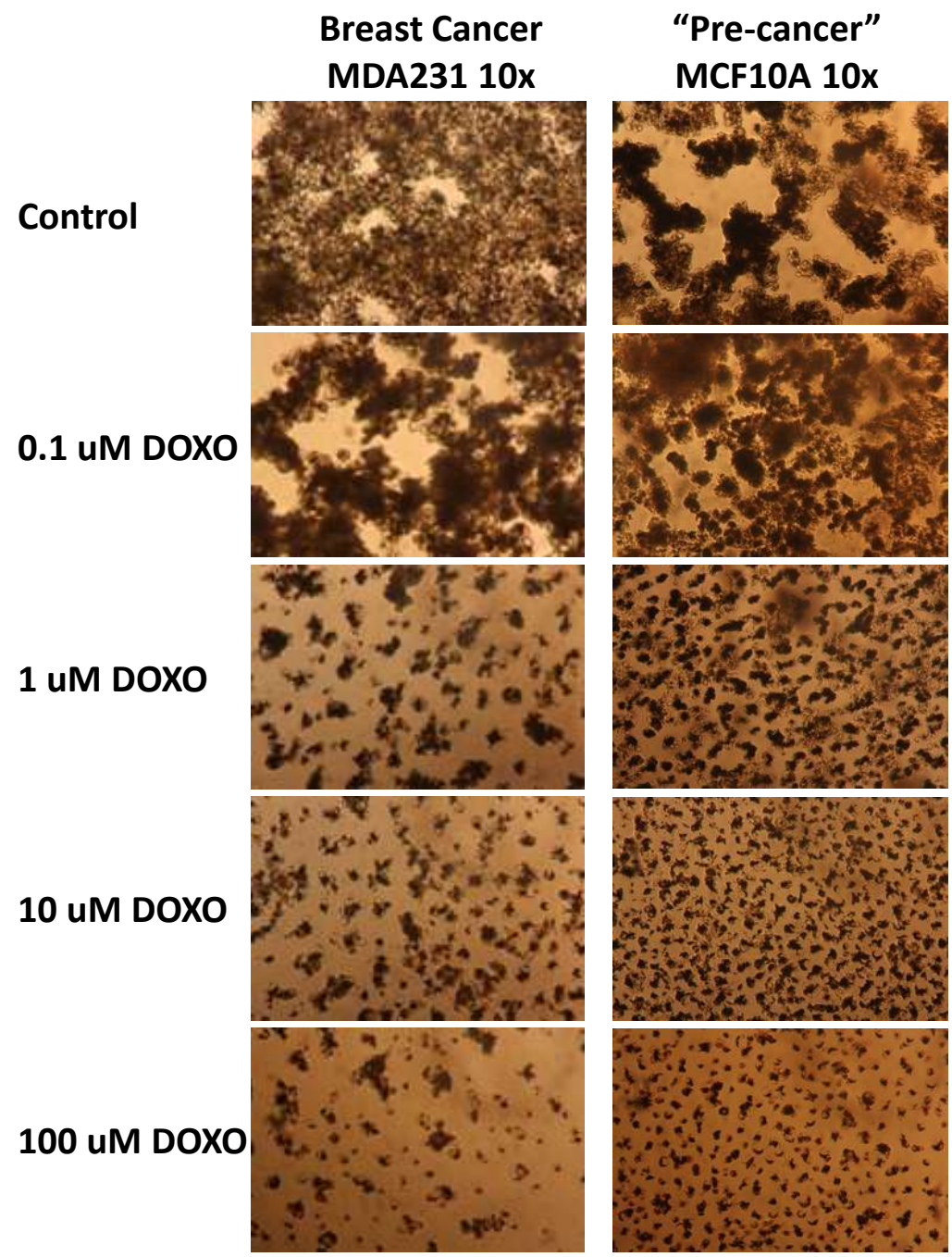


Pre-Cancer more resistant to Doxorubicin Chemotherapy



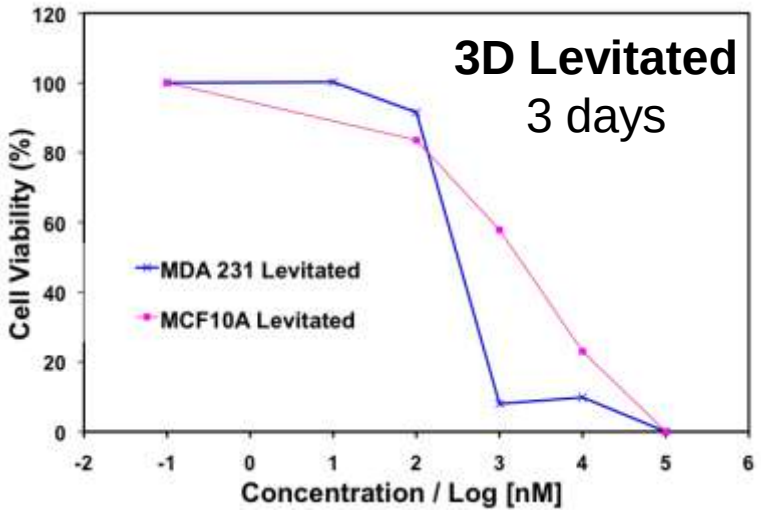


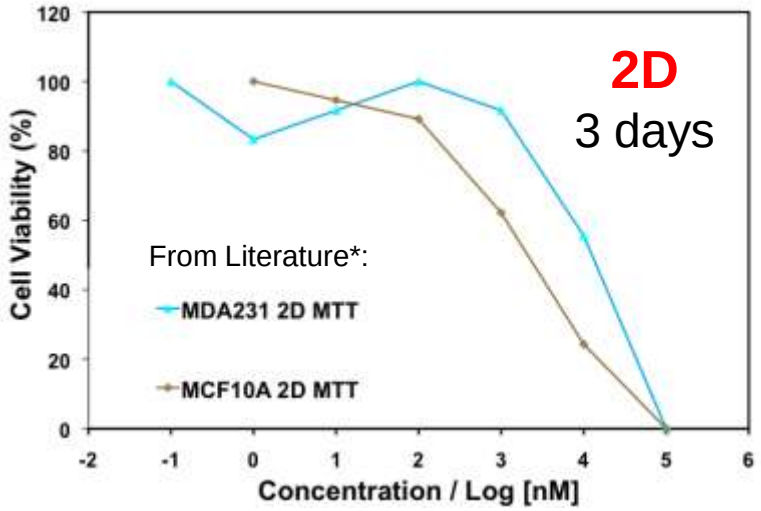
*Li et al. *J. Pharmacol. Exp. Ther.* ***2010***, *332, 821-8.*



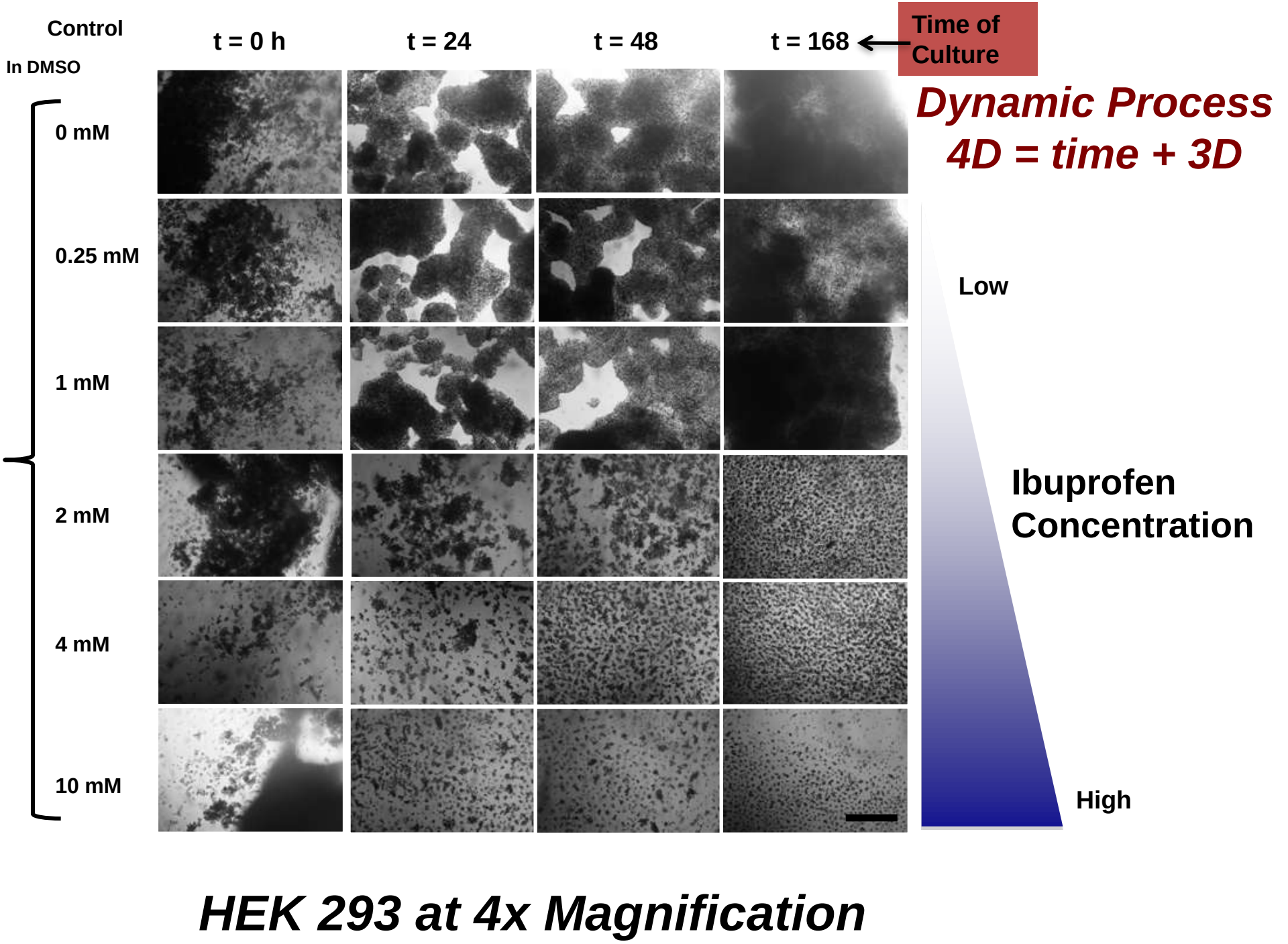
2D showed opposite response to Doxorubicin Chemotherapy

NOT PREDICTIVE





*Li et al. *J. Pharmacol. Exp. Ther.* ***2010***, *332, 821-8.*


Control
In DMSO
t = 0 h
t = 24
t = 48
t = 168
Time of Culture
0 mM
0.25 mM
1 mM
2 mM
4 mM
10 mM
Dynamic Process
4D = time + 3D
Low
Ibuprofen Concentration
High
HEK 293 at 4x Magnification

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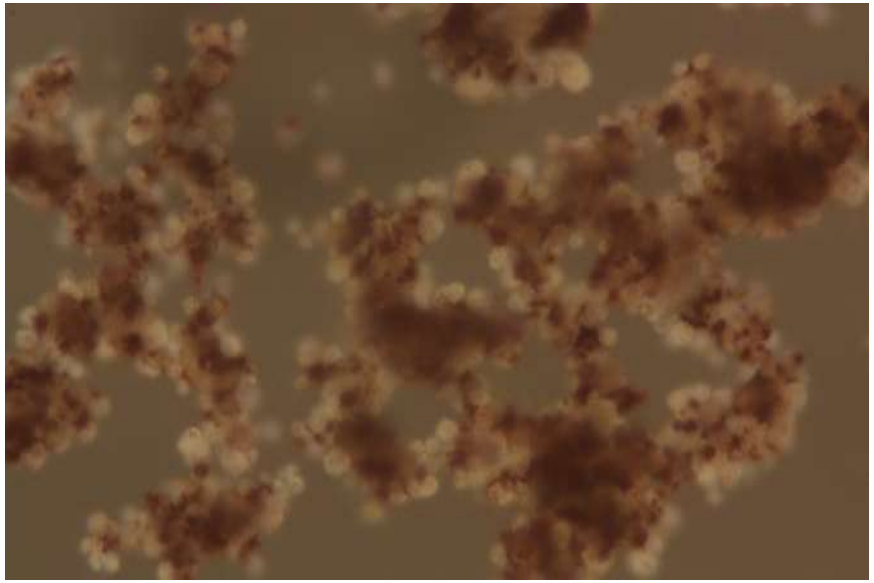
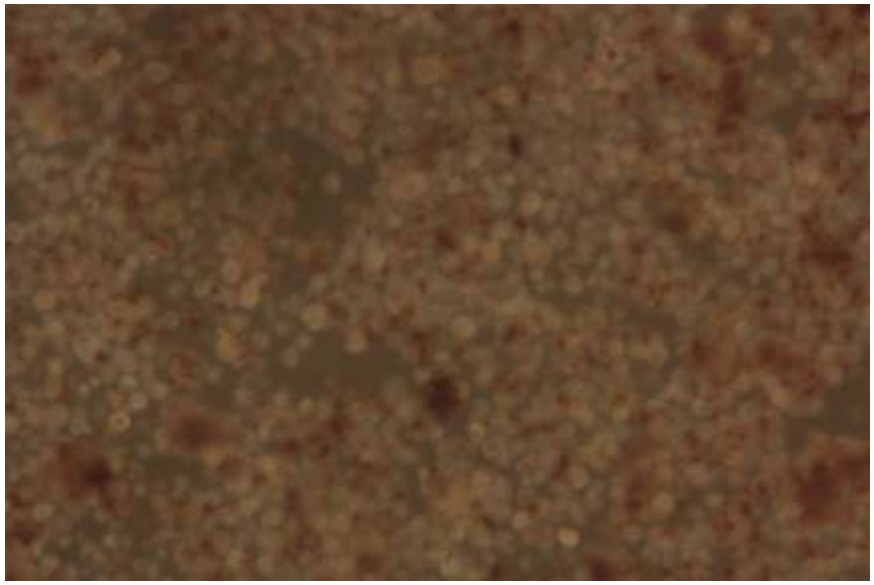
LNCaP – siRNA Treated

Control



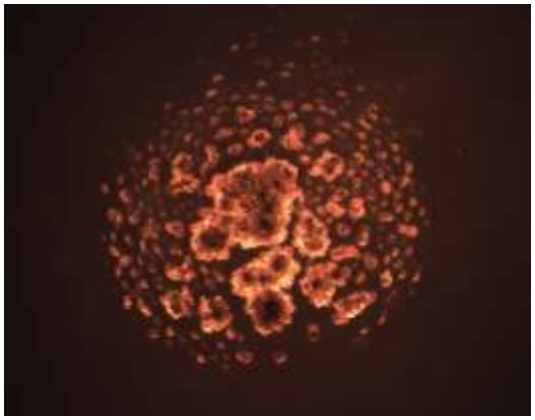
Df = 1.7

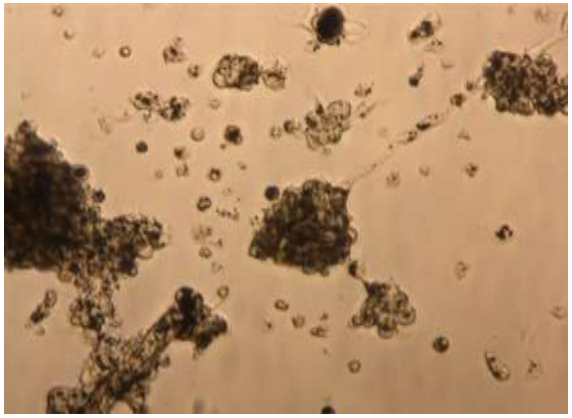
siRNA Treated

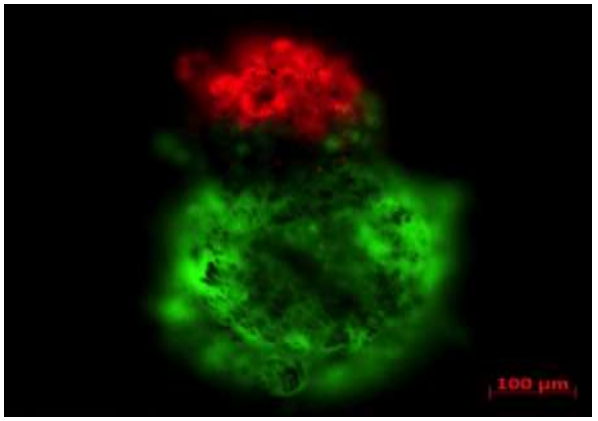


Df = 1.9

The Next Generation!
Needed Research & Operational Impact







- Rapid formation of 3D *in vivo*-like multicellular structures
- Promote cell-cell interaction
- Co-Culturing capability
- Easy to handle, pre- and post- culture
- Compatible with standard diagnostics
- Fast set-up time, as simple as 2D
- Minimum deviation from general protocols

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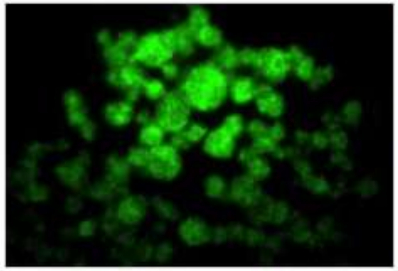
Metal Nano-Particles Suspend Hu Cells In Magnetic Scaffolding For Easy Organ Manufacturing

By Stuart Fox Posted 03.16.2010 at 12:44 pm 9 Comments

While scientists have become rather adept at transforming generic skin cells into spe organ cells, crafting the organs themselves has proven far more difficult. Since the 3- architecture of most organs is as important to their function as their cellular makeup, cultures are not very useful for building a replacement heart from scratch. To solve tha most organ makers **create a scaffolding** for the cells to grow on.

For a team of researchers at Rice University, even a biodegradable scaffolding wasn't good enough. By injecting cells with a metallic gel, the researchers have succeeded in **suspending cultured cells in a three-dimensional magnetic field**. With this magnetic scaffolding, organs can be grown in the right shape, and with no foreign material.



The researchers used bacteriophages, special viruses that inject themselves into cells, to insert a poly the c scie And 2-D mor field

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Levitate cancer cells for rapid 3D tissu

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ls when "levitated" by magnet lab – and produce replaceme balls into shapes that resemb

nature nanotechnology

Acknowledgements

Rice University
Tom Killian
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Jane Grande-Allen
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Veronica Leautaud
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