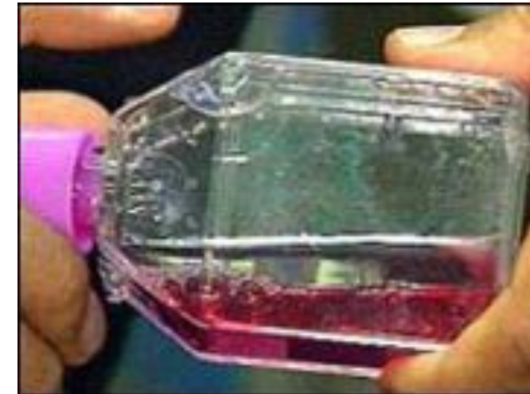
Experimentação em cultura de células de mamíferos

Cultura de células de mamíferos

- Células, previamente crescendo em um humano ou animal (*in vivo*), que são modificadas para crescimento em plástico ou vidro (*in vitro*).
- Manutenção em meio de cultura especial e incubadora (temperatura corporal).



- Conjunto de técnicas que permitem cultivar ou manter células isoladas fora do organismo onde existem, mantendo as características próprias.

- Podem fazer-se culturas a partir tecidos humanos, outros animais.

- A cultura de tecidos implica a prévia desagregação (mecânica ou enzimática) do tecido original e em que as células são cultivadas em placas ou garrafas.

- Objetivo: procedimento adequado para manter as características morfológicas e bioquímicas (ex: devem produzir as mesmas proteínas).

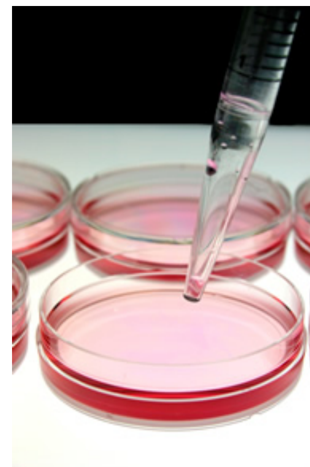
- Motivação: estudo em animais é complexo.
  - Muitos tipos celulares distintos
  - Grande diversidade de proteínas

  interações contínuas

  - Dificuldade de observação de efeitos individuais *in vivo.*
    - Problema: sofrimento é usualmente induzido nos animais

Meio de cultura

- DMEM (Dulbecco’s modified Eagle’s medium)
  - Composição: aminoácidos, sais, glicose, vitaminas, fenol RED). Soro (porção do sangue sem células)
    - Antibióticos, compostos enriquecedores
  - Maioria das células: humano, macaco, roedores, porco, coelho, galinha, peixe...



Composição de um Meio Típico Adequado para o Cultivo de Células de Mamíferos

Aminoácidos	Vitaminas	Sais	Outros	Proteínas (necessárias em meios sem soro, quimicamente definidos)
Arginina Cistina Glutamina Histidina Isoleucina Leucina Lisina Metionina Fenilalanina Treonina Triptofano Tirosina Valina	Biotina Colina Folato Nicotinamida Pantotenato Piridoxal Tiamina Riboflavina	$NaCl$ KCl NaH_2PO_4 $NaHCO_3$ $CaCl_2$ $MgCl_2$	Glicose Penicilina Estreptomicina Vermelho de fenol Soro	Insulina Transferrina Factores específicos de crescimento

Fig.9 – Tabela de componentes de um meio típico

Algumas células não crescem nem se diferenciam se a placa não estiver coberta com componentes específicos da matriz extracelular

Placa de cultura

- Ao contrário das bactérias, a maior parte das células de tecidos não estão adaptadas para viverem em suspensão e necessitam de uma superfície sólida para crescerem e dividirem-se. Usualmente é uma superfície plástica de uma placa de cultura.

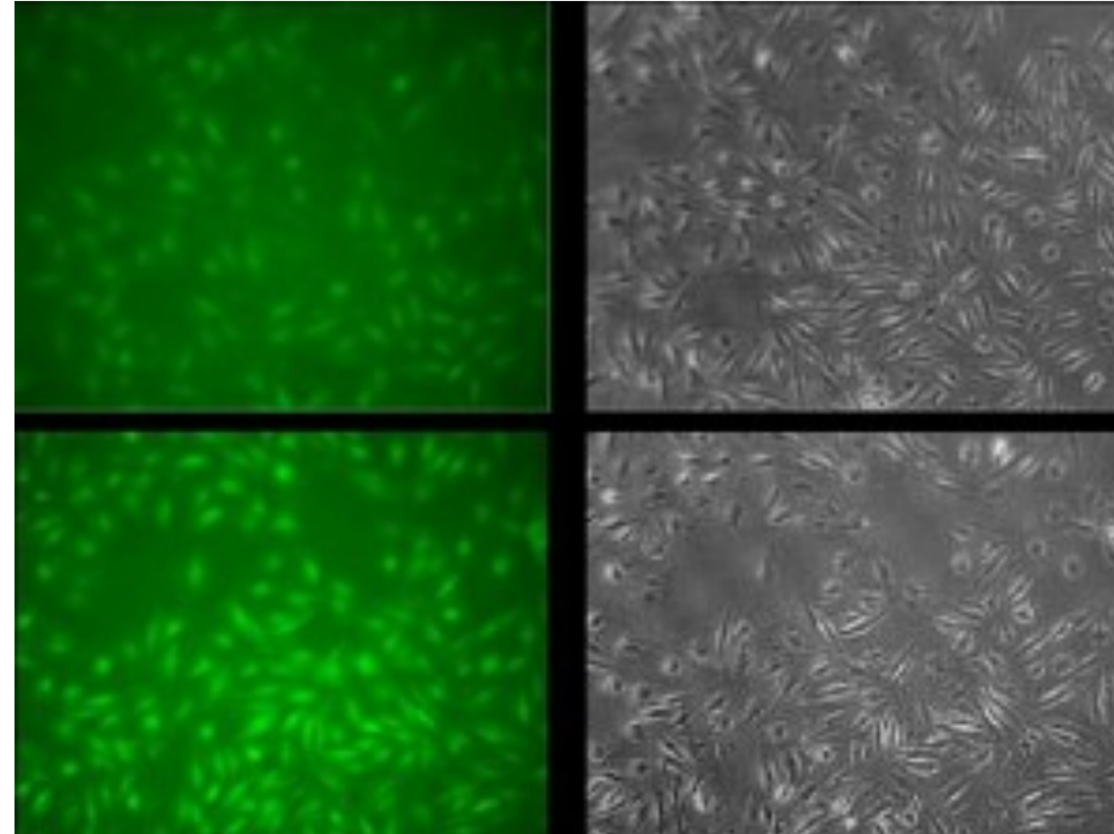
- Alguns meios de cultivo celular incluem:

Fatores de crescimento	+	Transferrina
|		|
|		|
estimulam a proliferação celular		transporta ferro para a célula

Crescimento

- Garrafas (com tampa com “respirador”)
- Incubadora (temperatura e mistura de gases)

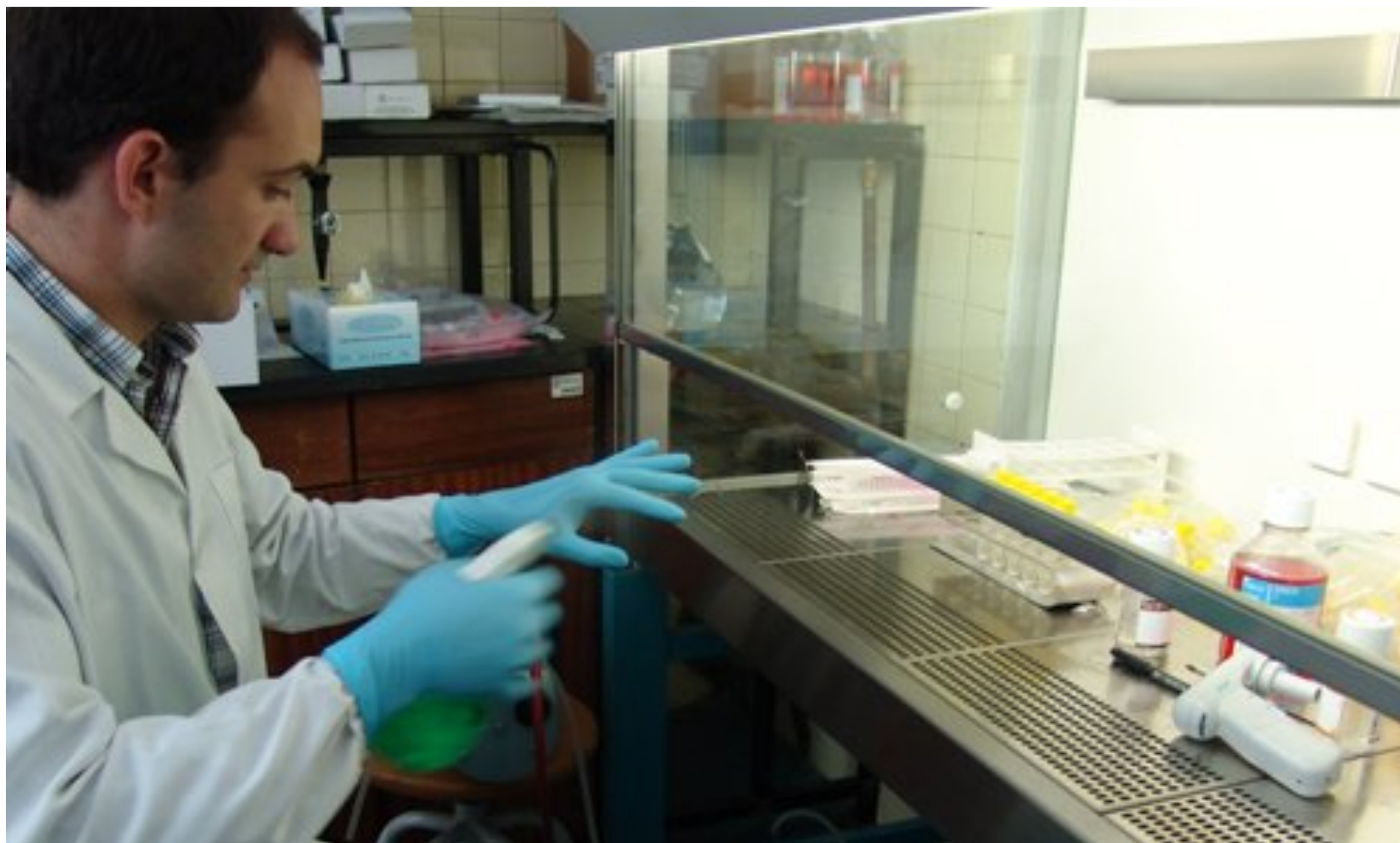
- Monocamada de células aderidas ao fundo da garrafa.
- Troca de meio.
- Viabilidade.



Repique das células - confluência

- Lavagem com PBS (*Phosphate Buffer Saline*) para remover excesso de soro.
- EDTA / tripsina (~2 minutos): descolamento das células da garrafa.

- *Bleach*: lise de células, modificação do meio.
- Descarte do meio e da garrafa/placa.



Métodos de assepsia, manipulação em câmara de fluxo laminar

Estufa de CO_2
(umidade)

Microscópio invertido

Classificação

- Cultura primária: cultura preparada diretamente de tecidos de um organismo, com ou sem passo inicial de fracionamento das células.

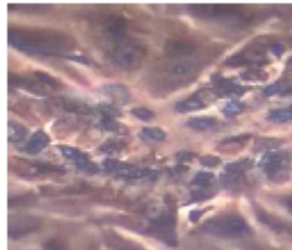
- Cultura secundária: as células cultivadas foram retiradas de uma cultura primária, elas podem ser repetidamente subcultivadas desta forma, por semanas ou meses.

- Manutenção das linhagens celulares: podem ser congeladas (solução de criopreservação) por longos períodos.

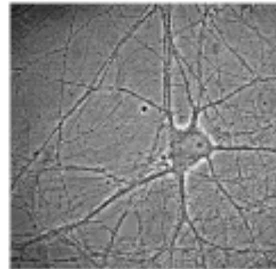
Tipos de culturas celulares

Culturas primárias

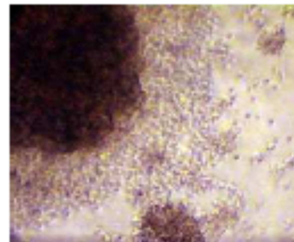
- Obtidas diretamente dos tecidos
- Culturas heterogêneas
- Mantém-se pouco tempo em cultura
- Mais propícias ao desenvolvimento de contaminações



Células ciliadas do epitélio nasal



Neurónios de rato (hipocampo)

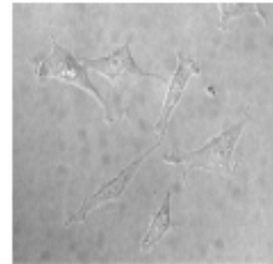


Linfócitos humanos

Linhagens celulares

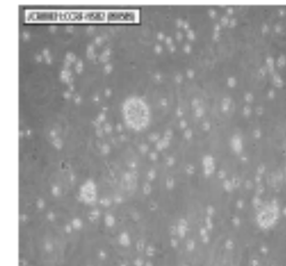
- Obtidas de culturas primárias
- Células imortalizadas (alteração genética)
- Crescimento rápido e contínuo
- Proliferação ilimitada ou limitada a um elevado número de passagens

COS-7



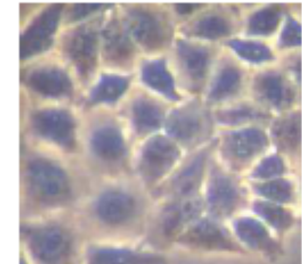
Células do epitélio renal de símios transformadas pelo vírus SV-40

HSB-2



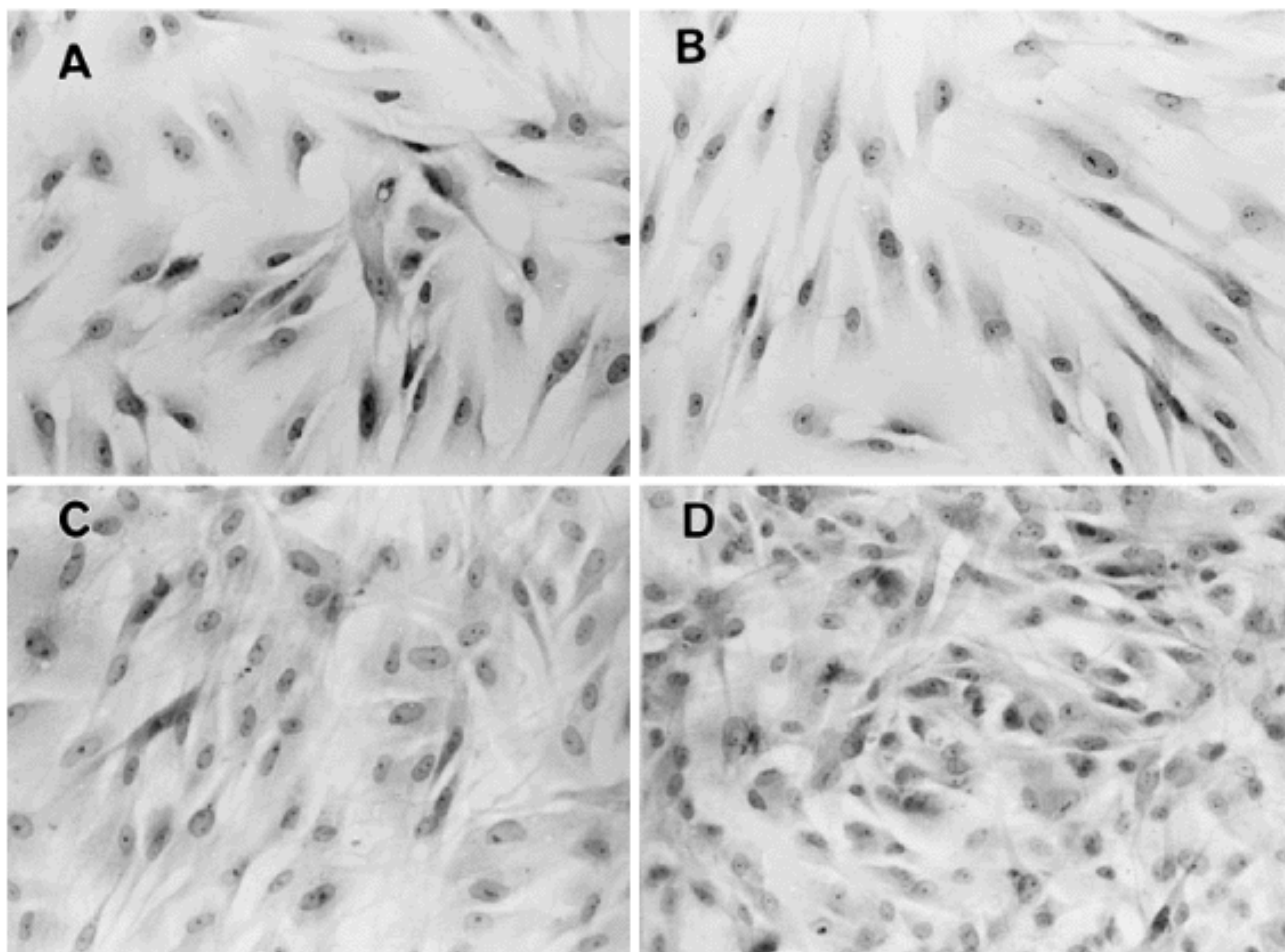
Leucemia linfoblástica aguda T

HeLa



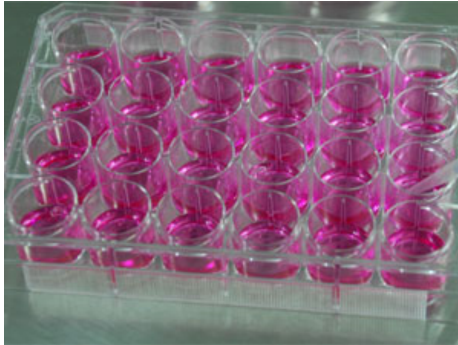
Células epiteliais de carcinoma cervical humano

FIGURA 3 - Características morfológicas dos fibroblastos de GN (A e C) e de FGH (B e D). Note o típico formato fusiforme de ambas as células em condições de subconfluência celular. Em condições de confluência celular, fibroblastos de GN demonstraram uma relação núcleo/ citoplasma maior que fibroblastos de FGH. Painel superior (A e B) – fibroblastos em condições de subconfluência celular. Painel inferior (C e D) – células em confluência. (H. E., 320 X.)



Exemplos de estudos

- Investigação de processos metabólicos celulares.
- Investigação de mecanismos patológicos e do envelhecimento (morfologia, bioquímica, genética).
- Investigação de mecanismos de infecção.
- Teste de fármacos: toxicidade, metabolismo, localização celular, cinética.
- Estudo de outras terapias: radiação, variação de temperatura, de atmosfera, nutricional.



Estudo do efeito da radiação laser em odontoblastos

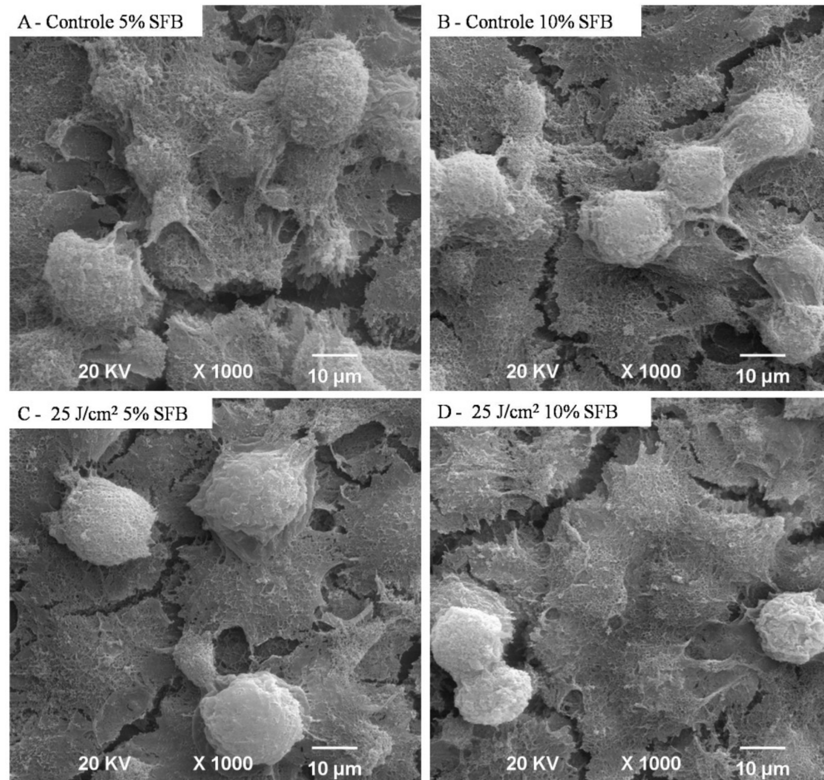
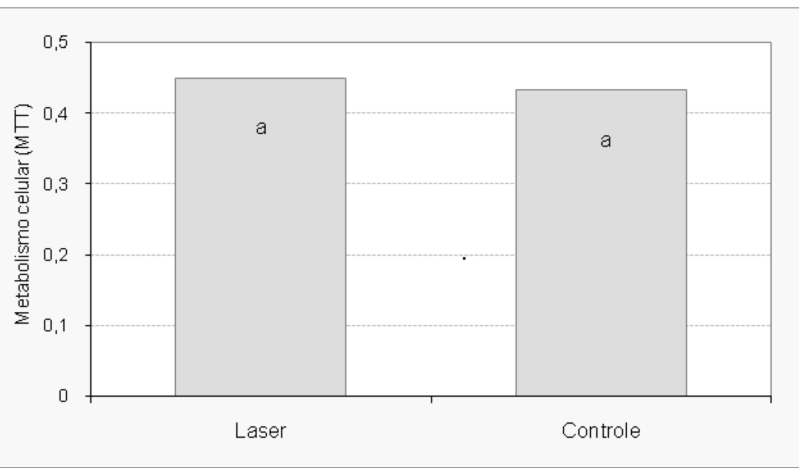
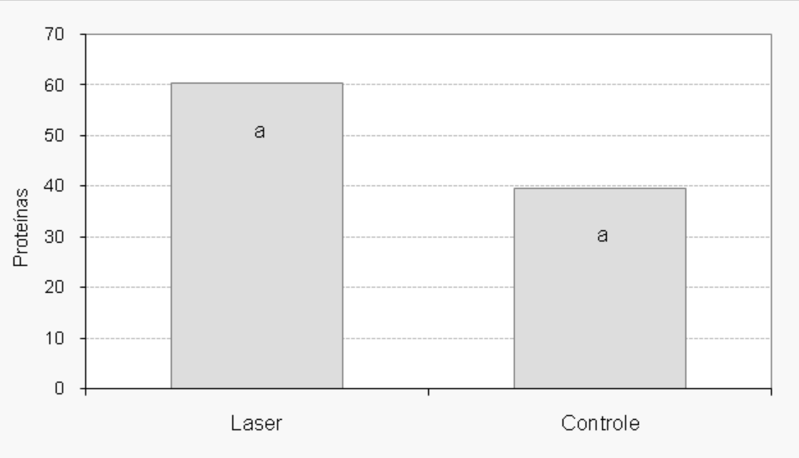


Figure 3. Panel of SEM micrographs representative of cell morphology in each group. A: (control – 5% FBS) The MDPC-23 cells exhibit normal morphology and are covering almost the entire surface of the glass substrate. B: (control – 10%FBS) The MDPC-23 cells present a wide cytoplasm from which numerous thin cytoplasmic processes are originating. C: In the same way as observed for the other control groups, great part of the glass substrate is covered by MDPC-23 cells, which present normal morphology. A larger number of cytoplasmic processes are observed in the groups in which the cells were irradiated. SEM, magnification original x1000.

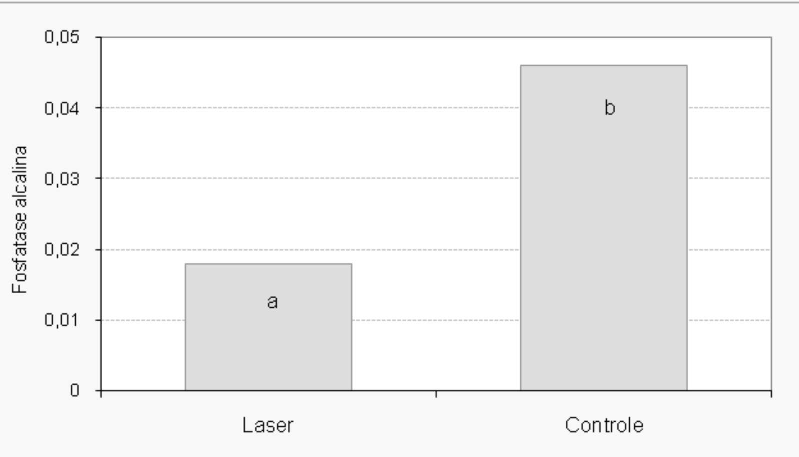
(A)



(B)

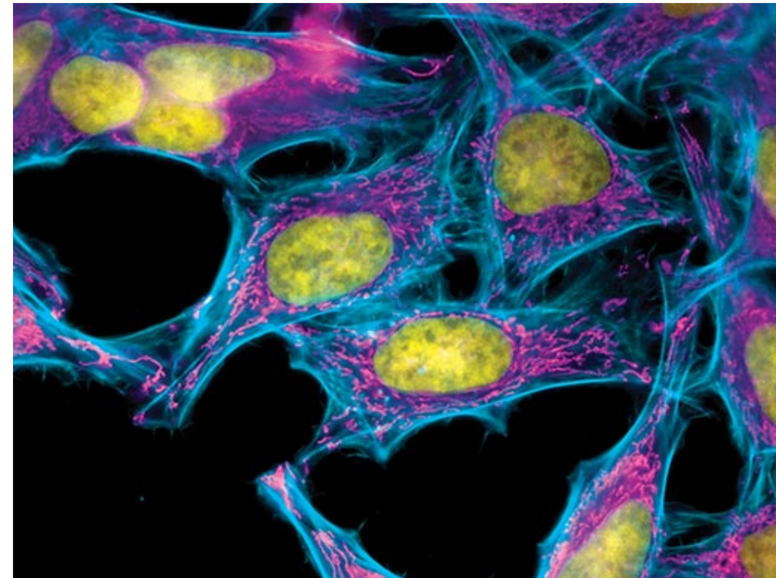


(C)



Células HeLa

- In 1951, a scientist at Johns Hopkins Hospital in Baltimore, Maryland, created the first immortal human cell line with a tissue sample taken from a young black woman with cervical cancer. Those cells, called HeLa cells, quickly became invaluable to medical research—though their donor remained a mystery for decades. In her new book, *The Immortal Life of Henrietta Lacks*, journalist Rebecca Skloot tracks down the story of the source of the amazing HeLa cells, Henrietta Lacks, and documents the cell line's impact on both modern medicine and the Lacks family.
  Read more: http://www.smithsonianmag.com/science-nature/Henrietta-Lacks-Immortal-Cells.html#ixzz1Gwt0BMi1



- **Who was Henrietta Lacks?**
  She was a black tobacco farmer from southern Virginia who got cervical cancer when she was 30. A doctor at Johns Hopkins took a piece of her tumor without telling her and sent it down the hall to scientists there who had been trying to grow tissues in culture for decades without success. No one knows why, but her cells never died.
  Read more: http://www.smithsonianmag.com/science-nature/Henrietta-Lacks-Immortal-Cells.html#ixzz1Gwt7ChKV

Vantagens do uso da cultura

- Número menor de animais usados nos estudos *in vivo*.
- Controle dos fatores externos.
- Investigação de atividades em células.
- As células são mais facilmente manipuladas e replicadas (mais barato).
- Experimento em células iguais (reprodutibilidade – resultados consistentes).

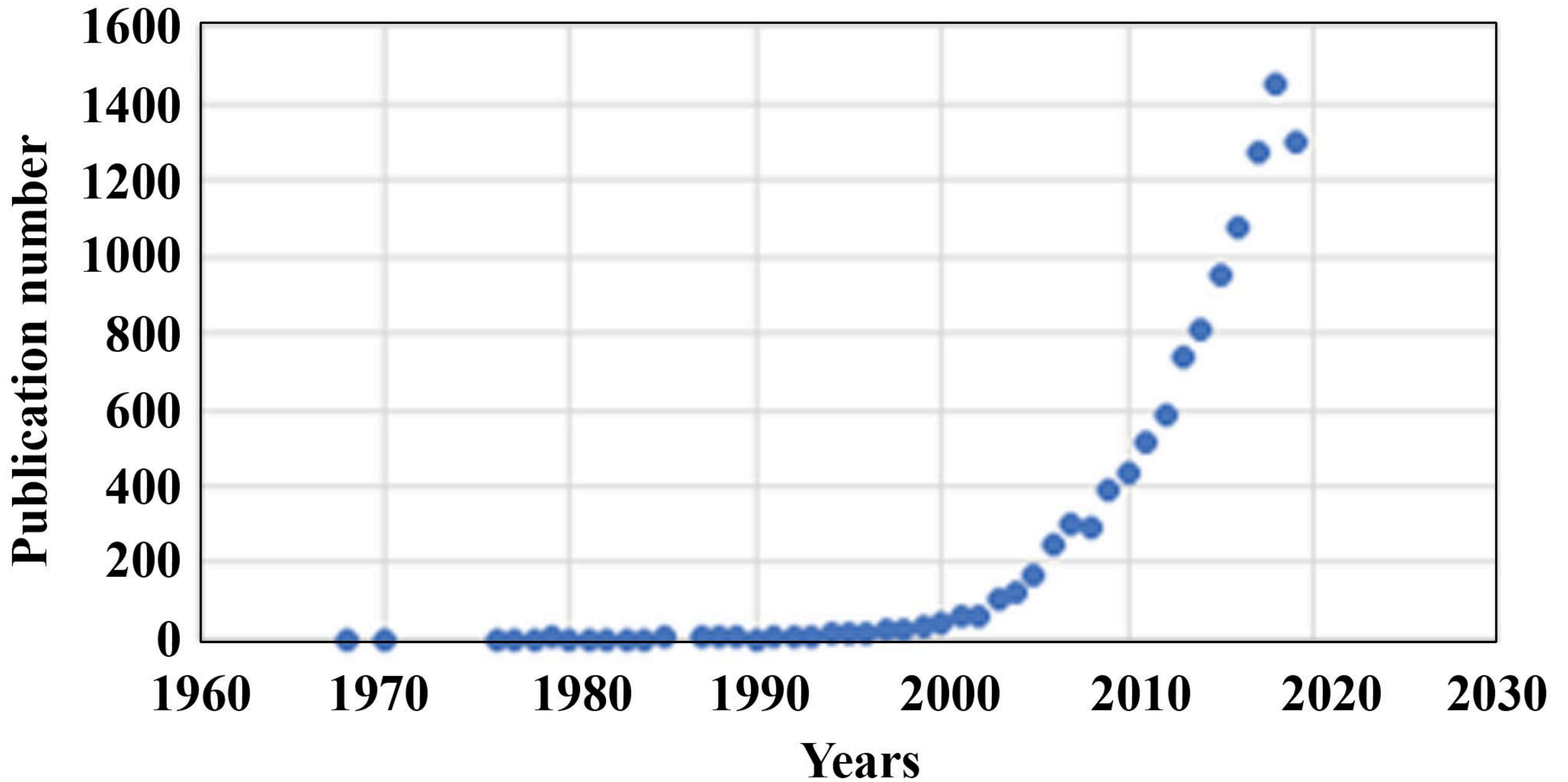
Desvantagens do uso da cultura

- Perda de características fenotípicas.
- Impossibilidade do estudo sistêmico (inter-relações com outros tipos celulares, reações em cadeia, atuação de proteínas e citocinas...).
- Investigação apenas em monocamada de células (comportamento celular pode ser diferente quando em organização tecidual).

Cultura celular – 3D

Important characteristics	2D cell culture	3D cell culture	References
Cell shape	• Cells shape is flat and elongated since the cells can only grow and expand two dimensionally • Cells grow into a monolayer on the plate	• Natural cell shape is preserved and cell growth • Cells grow into 3D aggregates/spheroids • Spheroids contain multiple layers	Costa et al., 2016; Langhans, 2018
Cell exposure to medium	• All cells in the culture receive the same amount of nutrients and growth factors from the medium in the plate • This causes more cells to be in the same stage of the cell cycle	• Nutrients does not have to be equally divided amongst all cells but can be if needed • The core cells often remain inactive since they receive less oxygen and growth factors from the medium • This process resembles the core cells in tumor cells, making it possible to mimic the behavior and structure of a tumor cell *in vivo*	Dhaliwal, 2012; Costa et al., 2016; Langhans, 2018
Cell junction	• Cell junctions are less common and less accurately represent real junctions	• Cell junctions are common and allow for cell-to-cell communication • Cells communicate through exchange ions, small molecules, and electrical currents	Pontes Soares et al., 2012; Ravi et al., 2015; Costa et al., 2016; Langhans, 2018; Lang et al., 2019
Cell differentiation	• Cell differentiation is poor	• Cells are well differentiated	Imamura et al., 2015; Costa et al., 2016; Langhans, 2018
Drug sensitivity	• Cells often have little resistance to drugs making it appear as though drugs administered to the cells were a successful treatment • Drugs are not well metabolized	• Cells often have more resistance to drug treatment • Drug metabolism is much better • Gives a more accurate representation of the drug's effects	Haisler et al., 2015; Imamura et al., 2015; Langhans, 2018
Cell proliferation	• Cells proliferate at an unnaturally rapid pace.	• Proliferation rates are realistic and can be high or low depending on technique and types of cells being studied.	Ravi et al., 2015, Langhans, 2018
Expression levels	• Gene and protein expression levels are often vastly different compared to *in vivo* models	• Gene and protein expression levels resemble levels found from cells *in vivo*	Ravi et al., 2015; Costa et al., 2016; Langhans, 2018
Cost	• For large-scale studies, it is much cheaper than using 3D culture	• Are typically more expensive than 2D cell culture techniques and require more time • 3D cell culturing reduces the differences between *in vitro* and *in vivo* drug screening, decreasing the likelihood of needing to use animal models	Ravi et al., 2015; Costa et al., 2016; Langhans, 2018
Apoptosis	• Drugs can easily induce apoptosis in cells	• Higher rates of resistance for drug-induced apoptosis	Costa et al., 2016
Response to stimuli	• Inaccurate representation of response to mechanical stimuli of cells • Cells cannot experience gravity since they are unable to expand into the third dimension	• Accurate representation of response to mechanical stimuli of cells • Cells can experience gravity giving a more accurate representation of a cell *in vivo*	Ravi et al., 2015; Costa et al., 2016
Usage and analysis	• Highly replicable and easily interpretable • Better for long-term cultures	• Can be difficult to replicate experiments • Can be difficult to interpret data	Kapałczyńska et al., 2018

https://www.frontiersin.org/files/Articles/513823/fmolb-07-00033-HTML/image_m/fmolb-07-00033-t001.jpg



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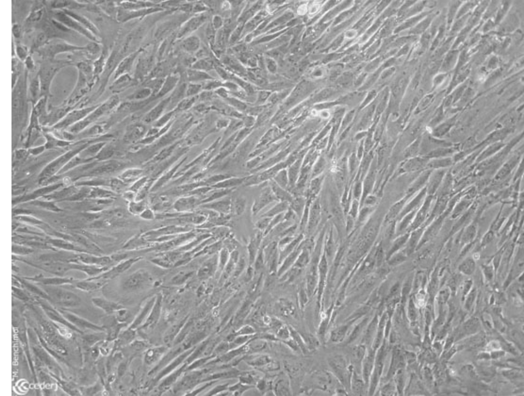
Cultura 3D

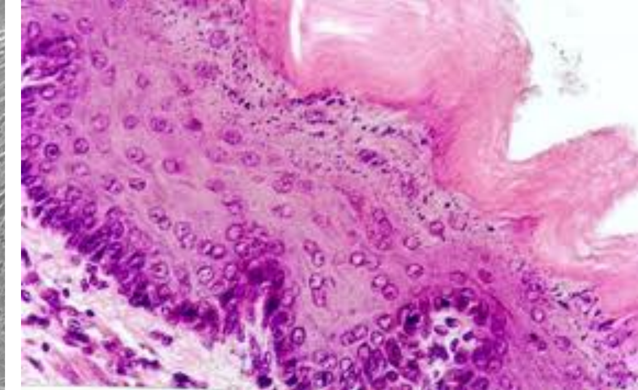
- Organização das células em estrutura tecidual.
  - Tecido: conjunto de células especializadas que desempenha uma função.
  - Interação entre células: reconhecimento, adesão, comunicação.

Célula X Tecido

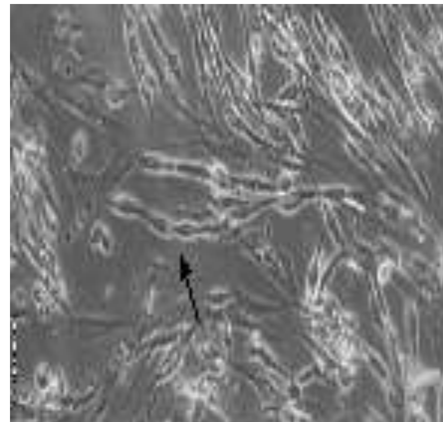
- Morfologia
- Metabolismo
- Função



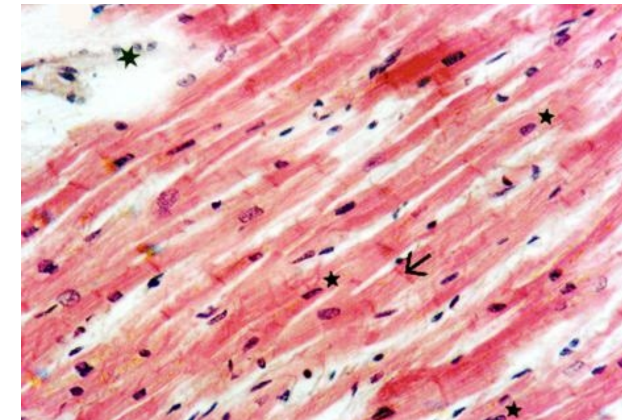
Células epiteliais



Tecido epitelial (pele)



Mioblastos



Tecido muscular (coração)

Por que cultura 3D?

- A adição da 3ª dimensão em uma cultura celular cria diferenças significativas nas características e no comportamento celular.
    - Maior similaridade entre as células em cultura e o organismo vivo.

Efeitos distintos observados em culturas 2D e 3D:

- Diferenciação
- Metabolismo de fármacos
- Expressão de proteínas
- Função celular geral
- Morfologia
- Proliferação
- Resposta a estímulos
- Viabilidade

- *The Big Picture:*

  Simply put, cells in 3D environments are much more similar to cells in a living organism (in vivo) than flat, unnaturally thin, single layer cells grown on 2D plastic.
  Shape: Typical cells in 3D are ellipsoids with dimensions of 10-30 µm. Cells in 2D are flat with typical thickness of 3 µm.
- **Environment:** Typical cells in 3D have nearly 100% of their surface area exposed to other cells or matrix. Cells in 2D have approximately 50% of their surface area exposed to fluid, approximately 50% exposed to the flat culture surface or intermediate, and a very small percent exposed to other cells.
- **Behavior:** Cells in 3D, as compared to 2D, show differences.
-

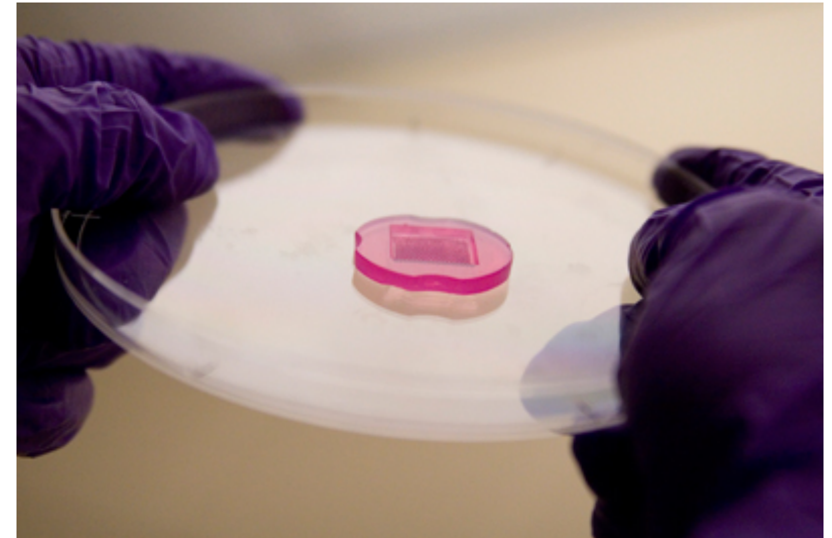
  Intuitively one can appreciate that with cells, form affects function. Unnaturally shaped cells in unnatural environments would be expected to function … unnaturally. In the mid-late 20th century, the unnatural shape of cultured cells was actually a plus – when viewed through a common microscope it's quite convenient to view flat, thin shapes in single layers. Good, if you want to identify and study what mitochondria are. Not so good, if you want to predict the complex behavior of a biological system of many different interacting cell types functioning in three dimensional environments, for example the response of a human being to a potential drug.

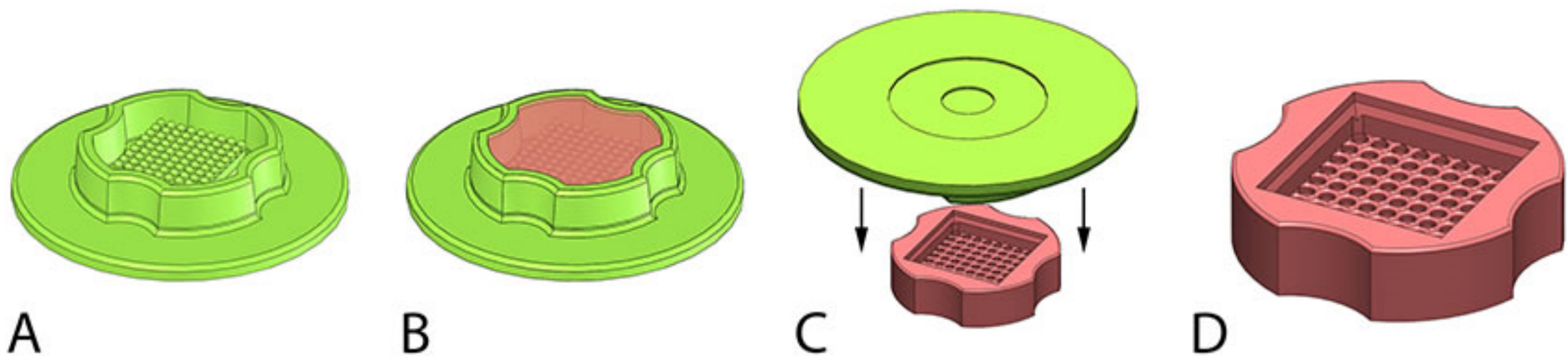
  Today, there is increasing awareness of the drawbacks of 2D cell culture and the related effect on the value of the research being performed. Not surprisingly, scientists are shifting their focus to cells cultured in 3D, as illustrated by the publications graph below. This website is dedicated to providing a single information source for cell culture researchers undertaking the challenge of implementing 3D techniques to create more relevant in vitro models of complex biological systems.

Introducing the 3D Petri Dish™

The 3D Petri Dish™ is the natural 3D cell culture environment that is scaffold-free and that maximizes cell-to-cell interactions.
Useful for spheroids, mammospheres, gliomaspheres, hepatospheres, chondrospheres, osteospheres, cell aggregates, neurospheres, cardiospheres, embryoid bodies and myoballs, we sell you the micro-molds to cast your own 3D Petri Dish™ out of agarose and these gels fit in standard 12 and 24 well dishes. The micro-molds are autoclavable and reusable, so you save thousands of dollars on your 3D cell culture experiments. You also realize significant new technological advantages:

- Make hundreds of spheroids of uniform size in a single pipeting step.
- Control spheroid size.
- Image an array of spheroids on the same optical plane.
- Form spheroids of two different cell types.
- Grow spheroids from single cell clones.
- Harvest spheroids for RT-PCR and Western Blots without enzymes.
- Form microtissues in complex shapes for novel applications.





Micro-mold

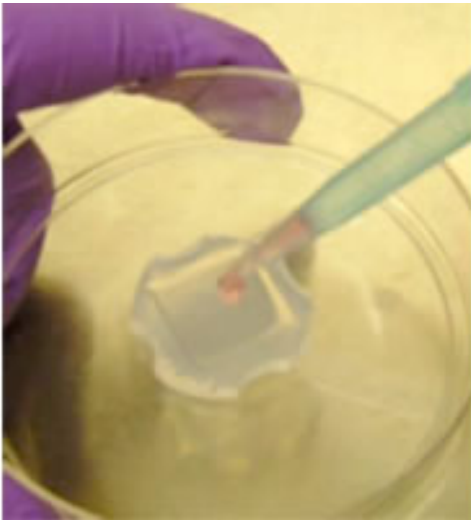
Micro-mold filled with molten agarose

3D Petri Dish™ of gelled agarose released from the micro-mold

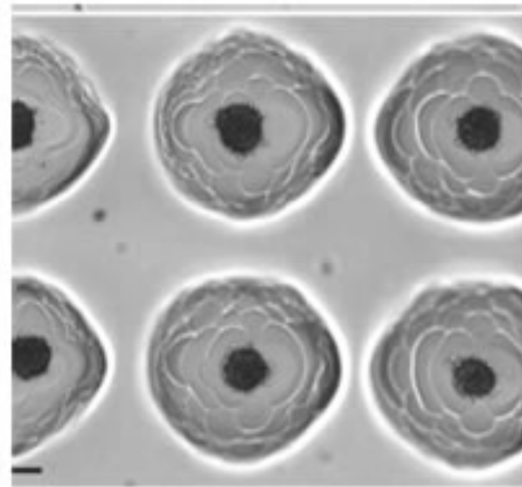
Final 3D Petri Dish™

Cells seeded on the 3D Petri Dish™ used for 3D Cell Culture

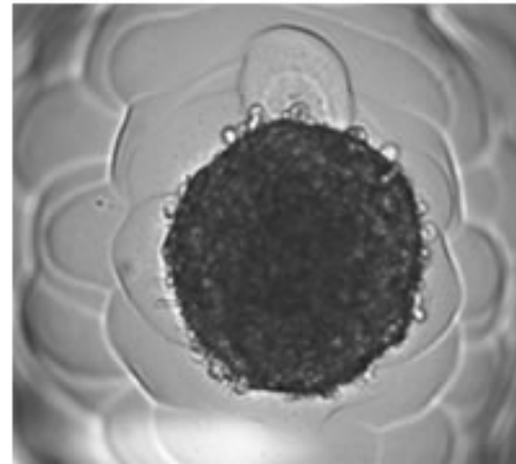
When cells are seeded onto the 3D Petri Dish™, cell-to-cell adhesion drives the self assembly of the 3D microtissue, typically in 1-2 days. No synthetic scaffold to alter cell physiology, no variability due to ill defined gels, no animal products. 3D cell culture in a format suitable for high content microscopy that has worked with over twenty different cell types both primary cells and cell lines.



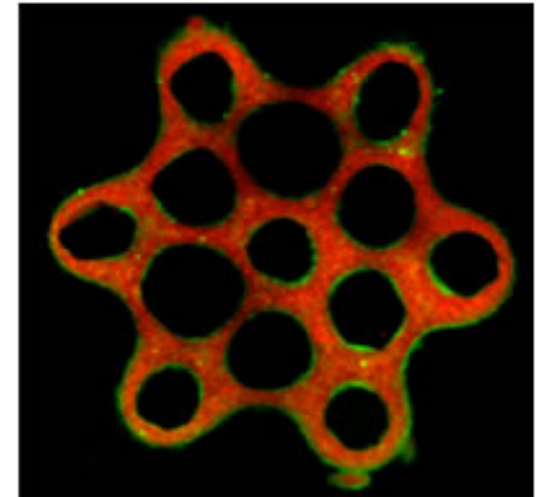
3D Petri Dish™ being seeded with cells



Spheroids formed in 3D Petri Dish™



Spheroid formed in 3D Petri Dish™



Honeycomb formed in 3D Petri Dish™

Sigma® offers a broad portfolio of three-dimensional cell culture systems including MaxGel™ human Extracellular Matrix (ECM), HydroMatrix™ synthetic peptide, and mouse ECM, to support stem cell and other cell cultures. These products provide three-dimensional environments in which cells are better able to mimic their *in vivo* counterparts.

Culture cells in a way that resembles their *in vivo* environments

Promote cell growth and migration to support the proliferation of stem cell and other mammalian cells

Reduced level of growth factors promotes lot-to-lot consistency

3D Matrices

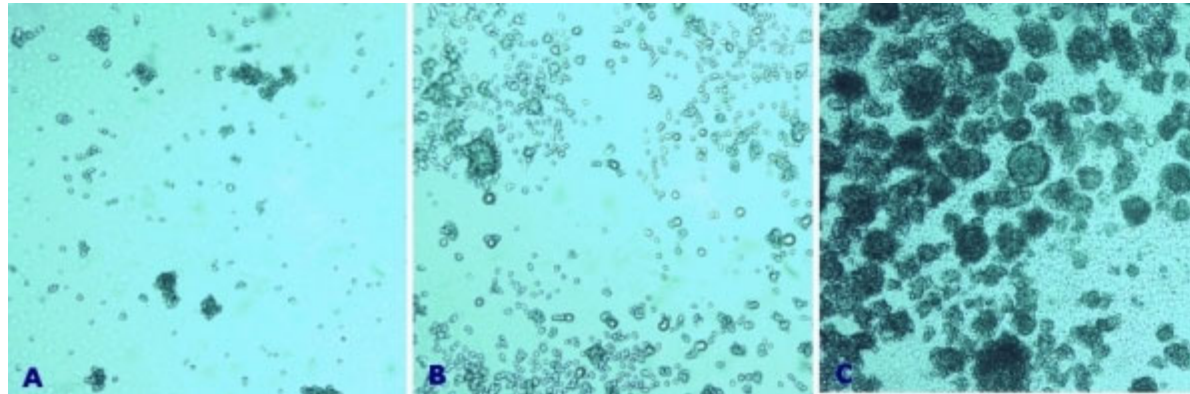
HydroMatrix™ synthetic peptide

MaxGel™ human ECM

Mouse ECM

HydroMatrix™ Peptide Hydrogel

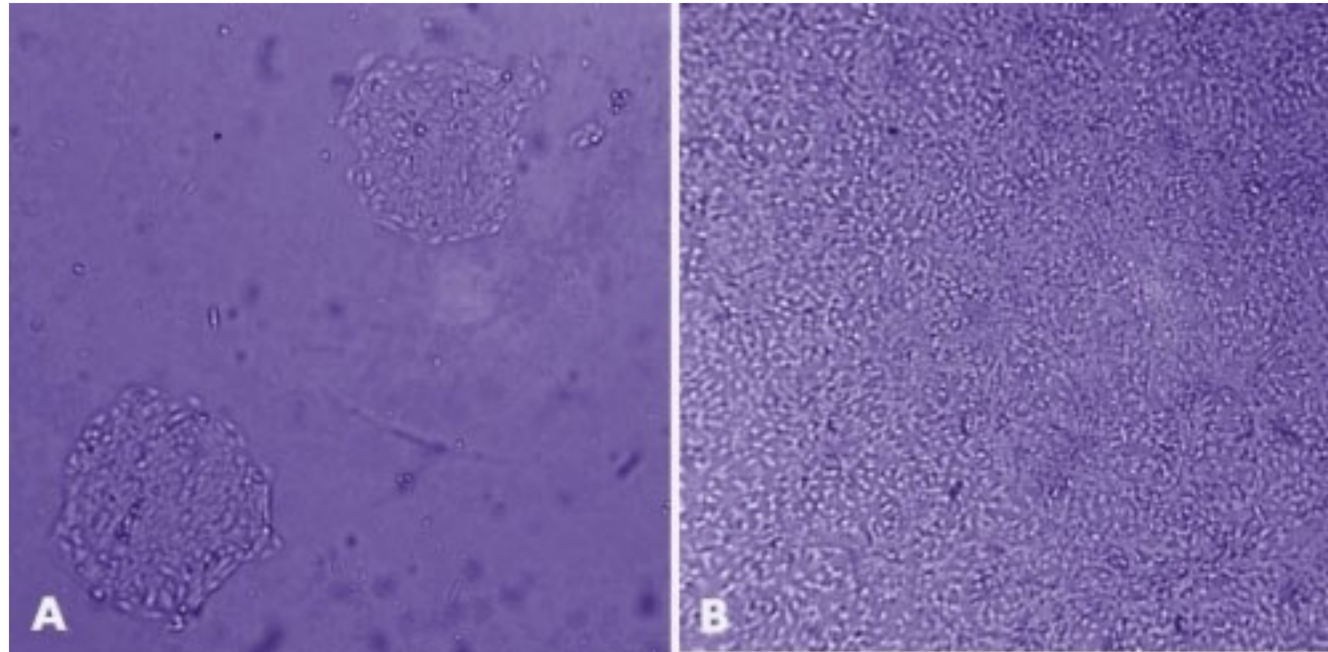
A synthetic peptide nanofiber scaffold, HydroMatrix offers the precision and control of a synthesized matrix with the natural three-dimensional architecture of highly crosslinked peptide hydrogel. The HydroMatrix scaffold self-assembles from fluid precursors into a highly cross-linked peptide 3-dimensional hydrogel in response to changes in temperature or ionic strength. By adjusting the concentration of the HydroMatrix solution, researchers are able to control the flexibility of the 3-D architecture, and tailor the structure to meet their individual needs. HydroMatrix promotes cell growth and migration and has been shown to support the proliferation of many cell types, including neural stem cells, neurons, glia, astrocytes, fibroblasts, and keratinocytes.



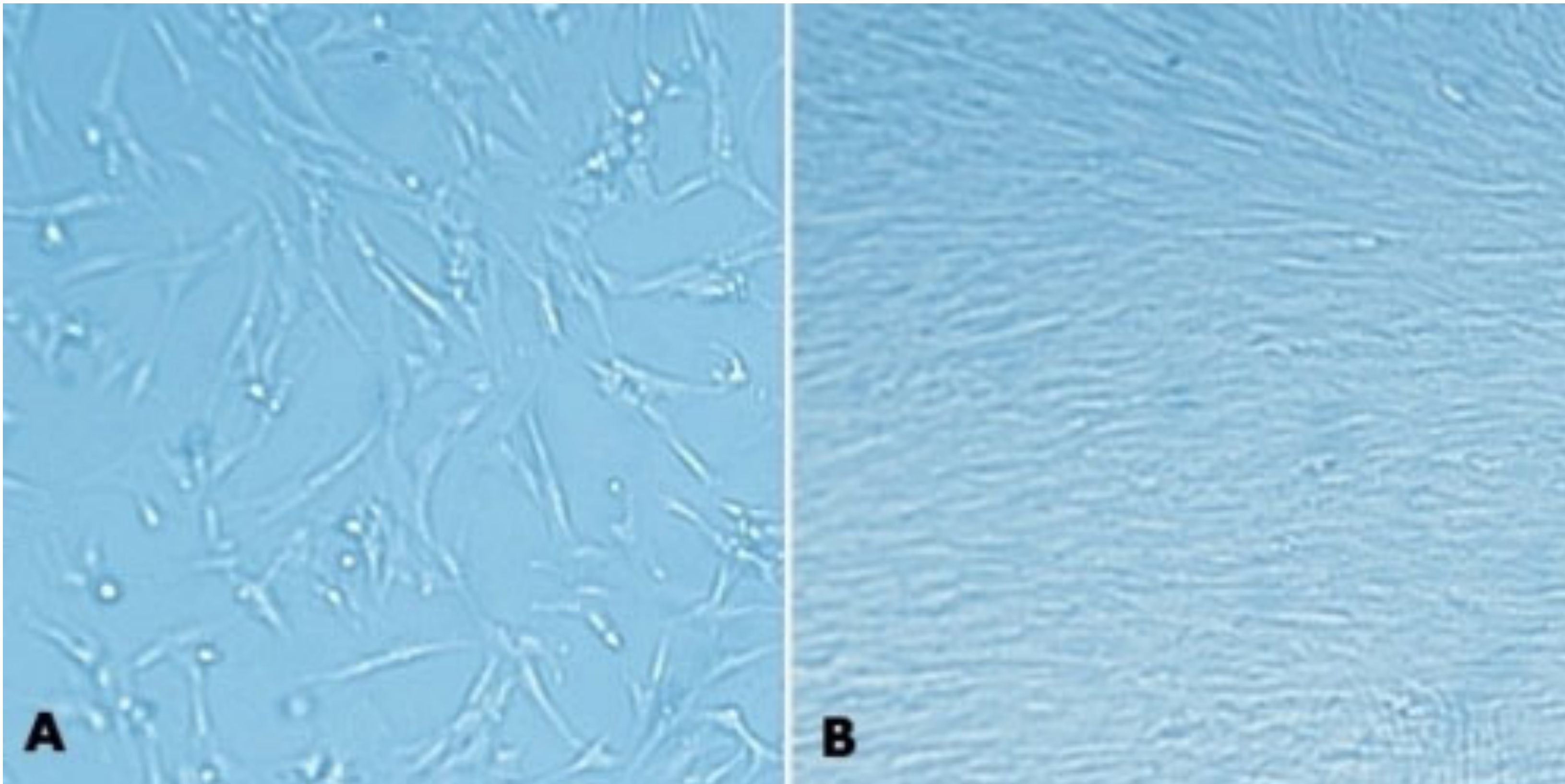
HydroMatrix Peptide Cell Culture Scaffold enables excellent cell growth. Rat neural stem cells (NSC’s) were cultured on three surfaces. NSC's grew poorly on tissue culture plastic (A) and slightly better in poly-L-lysine/laminin-coated plates (B). NSC's demonstrated excellent growth on HydroMatrix peptide hydrogel 0.5% (w/v) (C).

MaxGel™ Human ECM

Produced *in vitro*, MaxGel human ECM provides a rich three dimensional environment to promote cellular proliferation. MaxGel ECM contains extracellular matrix components including collagens, laminin, fibronectin, tenascin, elastin, a number of proteoglycans and glycosaminoglycans. The cell-cultured derived ECM effectively reproduces the cooperative interaction of epithelia and mesenchyme during development and in organotypic cell culture of skin. The human MaxGel ECM promotes cell growth and migration and has been shown to support the proliferation of many cell types, including neural stem cells, neurons, glia, astrocytes, fibroblasts, hepatocytes and keratinocytes.



MaxGel ECM Matrix enables improved expansion of Adult Keratinocytes. HaCaT cells (derived from human adult skin keratinocytes) were grown for 24 hours after plating on tissue culture plastic (A) and on 1% human ECM (B), which demonstrate that HaCaT cells proliferate better on ECM.



MaxGel ECM Matrix enables improved expansion of Fetal Lung Fibroblasts. MRC-5 cells (derived from human fetal lung fibroblasts) were grown for 24 hours after plating on tissue culture plastic (A) and on 1% human ECM (B). As seen with other cells, MRC-5 cells propagate better on ECM.

Original article

Extracellular matrix gel is necessary for *in vitro* cultivation of insulin producing cells from human umbilical cord blood derived mesenchymal stem cells

GAO Feng, WU De-quan, HU Yan-hua and JIN Guang-xin

Keywords: *umbilical cord blood; mesenchymal stem cells; induction; insulin producing cells; extracellular matrix*

Background Pancreatic islet cell transplantation is an effective approach to treat type 1 diabetes. However, this therapy is not widely used because of the severe shortage of transplantable donor islets. This study investigated whether mesenchymal stem cells (MSCs) derived from human umbilical cord blood (UCB) could be transdifferentiated into insulin producing cells *in vitro* and the role of extracellular matrix (ECM) gel in this procedure.

Methods Human UCB samples were collected and MSCs were isolated. MSCs specific marker proteins were analyzed by a flow cytometer. The capacities of osteoblast and adipocyte to differentiate were tested. Differentiation into islet like cell was induced by a 15-day protocol with or without ECM gel. Pancreatic characteristics were evaluated with immunofluorescence, reverse transcription polymerase chain reaction (RT-PCR) and flow cytometry. Insulin content and release in response to glucose stimulation were detected with chemiluminescent immunoassay system.

Results Sixteen MSCs were isolated from 42 term human UCB units (38%). Human UCB-MSCs expressed MSCs specific markers and could be induced *in vitro* into osteoblast and adipocyte. Islet like cell clusters appeared about 9 days after pancreatic differentiation in the inducing system with ECM gel. The insulin positive cells accounted for (25.2±3.4)% of the induced cells. The induced cells expressed islet related genes and hormones, but were not very responsive to glucose challenge. When MSCs were induced without ECM gel, clusters formation and secretion of functional islet proteins could not be observed.

Conclusions Human UCB-MSCs can differentiate into islet like cells *in vitro* and ECM gel plays an important role in pancreatic endocrine cell maturation and formation of three dimensional structures.

Chin Med J 2008;121(9):811-818

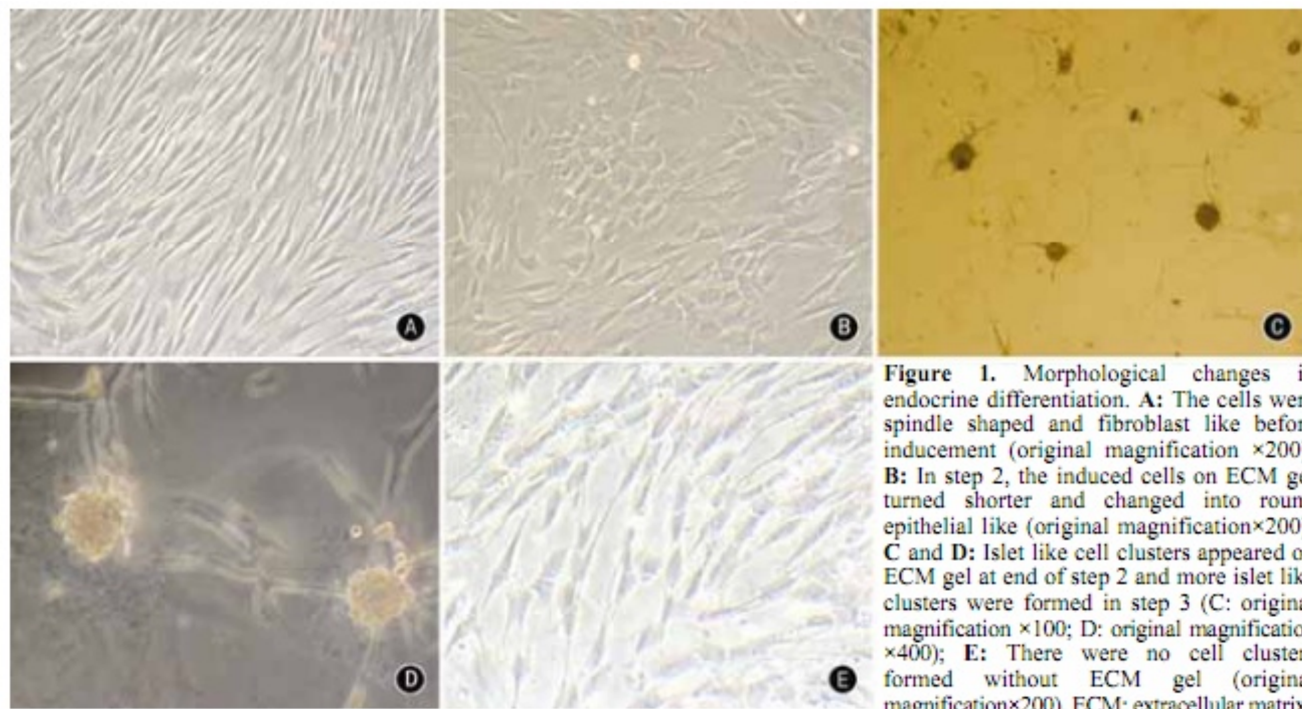


Figure 1. Morphological changes in endocrine differentiation. **A:** The cells were spindle shaped and fibroblast like before inducement (original magnification ×200); **B:** In step 2, the induced cells on ECM gel turned shorter and changed into round epithelial like (original magnification×200); **C** and **D:** Islet like cell clusters appeared on ECM gel at end of step 2 and more islet like clusters were formed in step 3 (C: original magnification ×100; D: original magnification ×400); **E:** There were no cell clusters formed without ECM gel (original magnification×200). ECM: extracellular matrix.

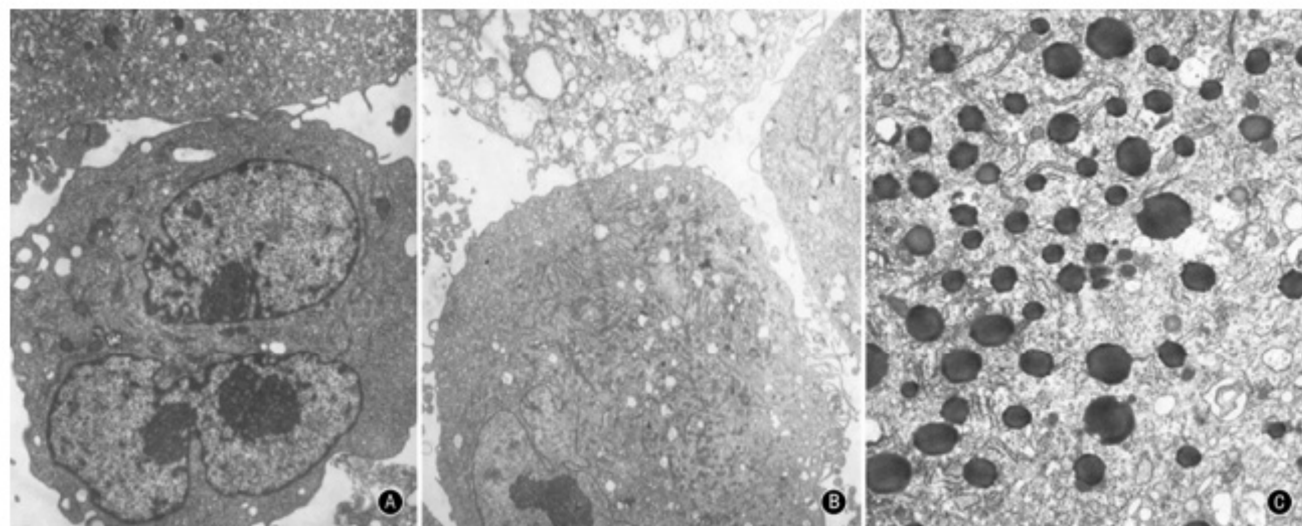
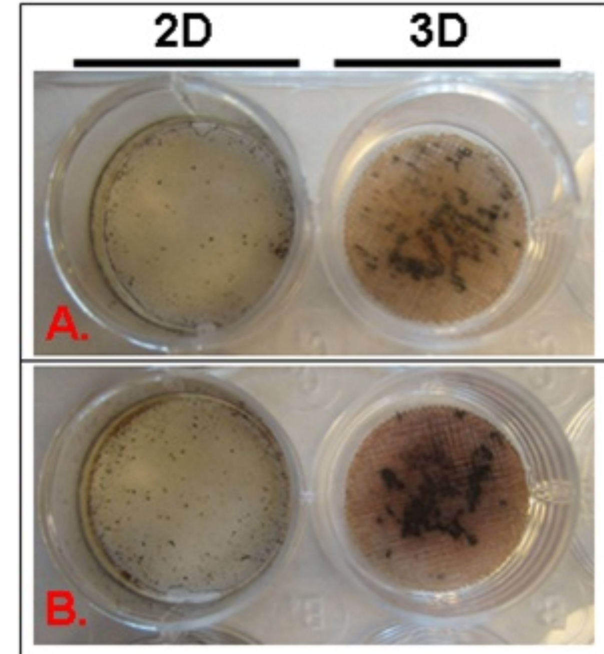


Figure 2. Electron microscopic analysis of islet like cells. **A:** Few secretory granule was found in pre-induced human UCB-MSCs (original magnification×6000); **B:** The secretory granules were few also in differentiated cells without ECM gel coated (original magnification×6000); **C:** However, within the cytoplasm of induced cells on ECM gel, small secretory granules were often found (original magnification×8000). ECM: extracellular matrix.

Currently, cells are routinely cultured in vitro using 2 dimensional (2D) cell culture techniques. It has been well documented that cell culture in tissue culture plates and flasks does not mimic the in vivo cell growth. Therefore, the cell expansion and cell-drug interactions under 2D are not appropriate in vitro models. 3D cell culture technique, on the other hand, offers a better cell culture environment because it is one step closer to the in vivo cell growth environment. However, because 2D culture is easy to carry out and there are no satisfactory 3D cell culture devices available, 2D cell culture is still the predominately used cell culture technique. As a publication in the journal Nature pointed out, “Awareness of the potential of 3D tissue culture among scientists is far too low. But the benefits of the technique are so self-evident that little marketing will be needed to persuade the uninitiated to move up one dimension, just as soon as the issues of convenience are resolved” [Nature, Vol. 424, 21, August 2003].

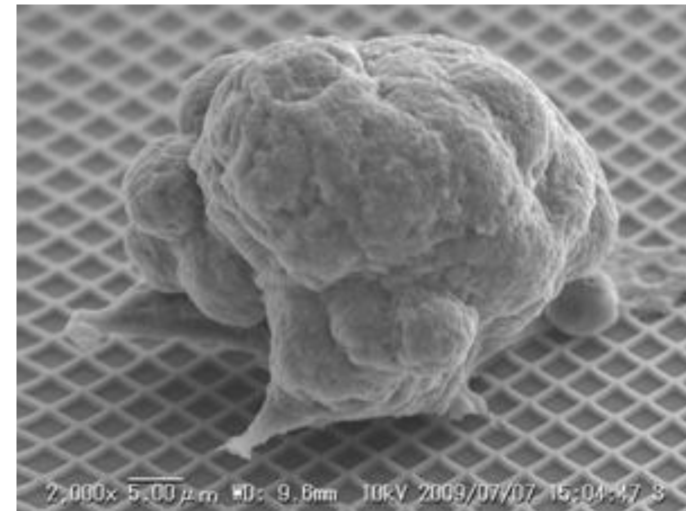


Having realized the importance of conducting cell culture under 3D, 3D Biotek has been working on developing easy to use 3D cell culture devices. The initial application of these 3D cell culture devices will be primarily in the stem cell research, tissue engineering, and drug discovery and will expand to other cell culture related fields gradually.

Nanotechnology Based Cell Culture Plates for Matrix Free 3D Cell Culture

Achieve 3D Cell Culturing with conventional 2D Techniques

- Ideal for 3D culturing of cancer cell lines, primary tumors, mesenchymal stem cells, adipocytes, osteoblasts, hepatocytes, and more
- Synthetic, animal-free product with superb lot-to-lot consistency
- Easy handling using conventional 2D cell culture technique
- Spheroids attach to the plate, medium change is possible
- Uniform and reproducible spheroid formation
- Spheroids preserve differentiation characteristics
- Spheroids can be easily harvested
- Optical properties allow high performance imaging



3D Cell Culturing by Magnetic Levitation

Glauco R. Souza, Ph.D.
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gsouza@n3dbio.com

THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER
Making Cancer History®

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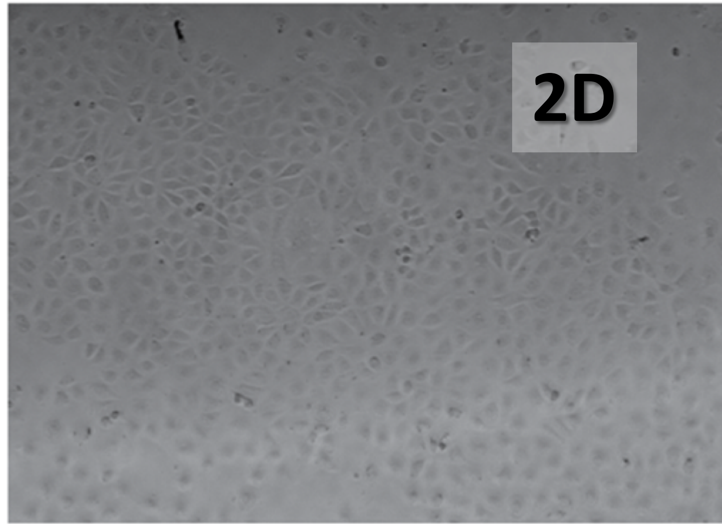
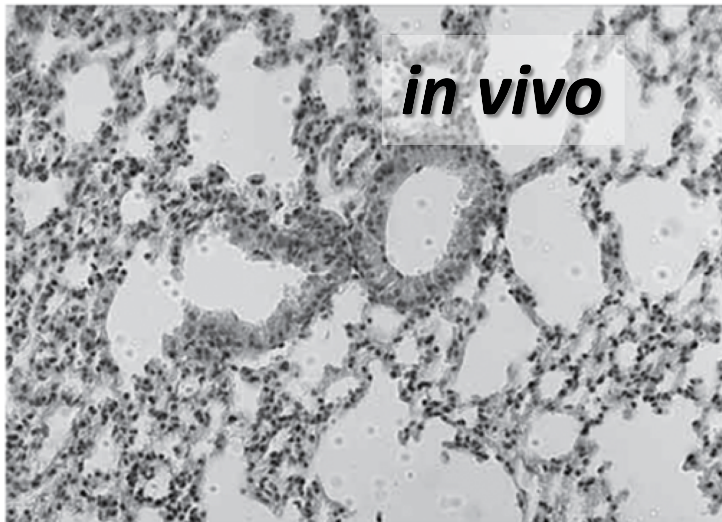
Why 3D Cell Culturing?

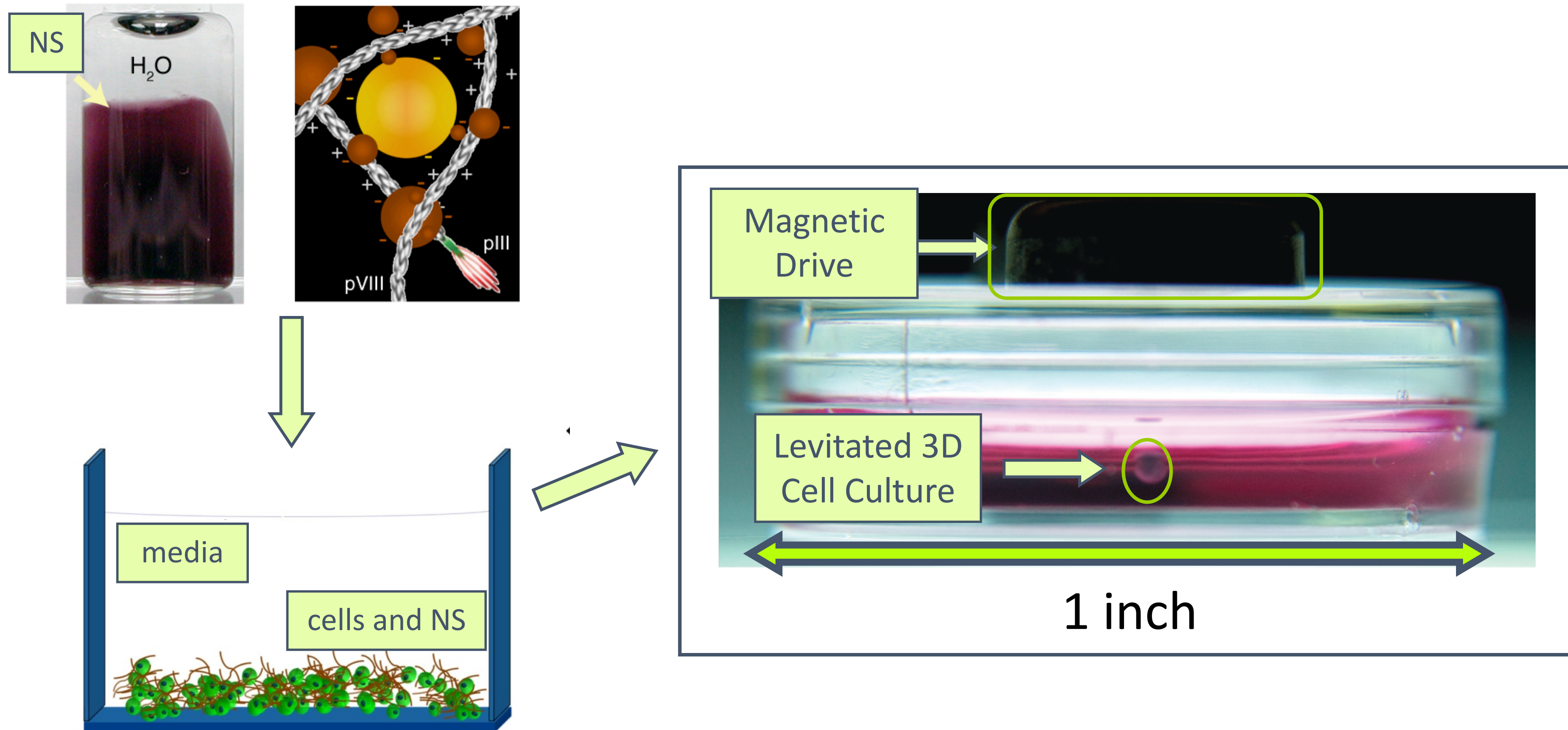
Paradigm Shift





- Culturing cells remains essential to all work in life sciences.
- Now widely recognized that cells grown in 2D inaccurately represents real tissue.

The Bio-Assembler™

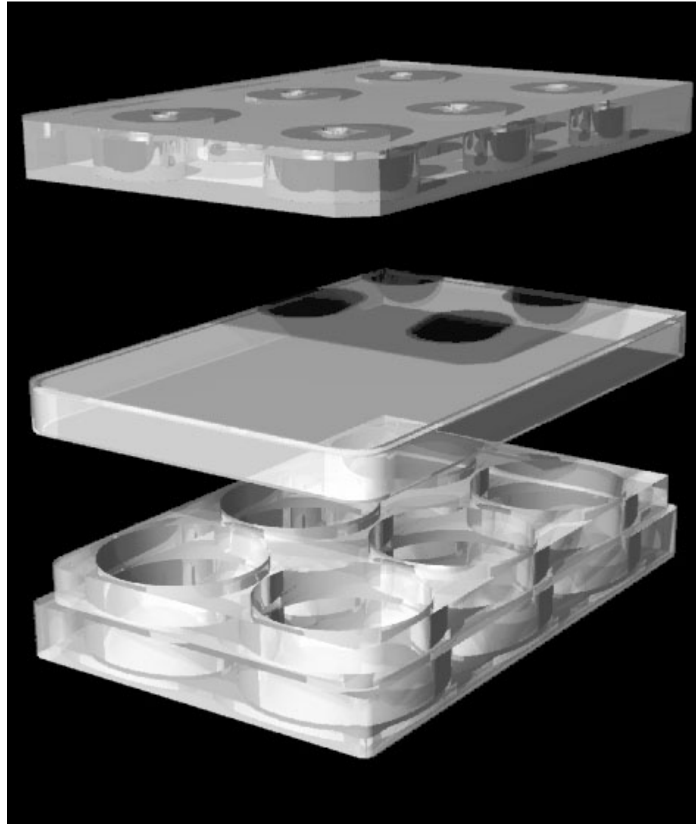


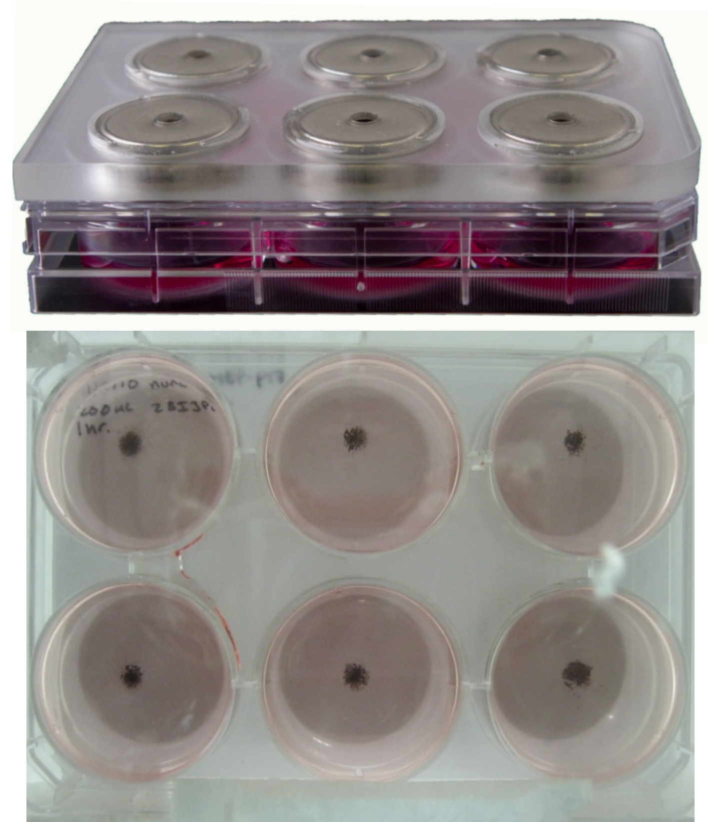
*NS = Nanoshuttles

Souza *et al.* Nature Nanotechnol. April 2010

Bio-Assembler™ Kit

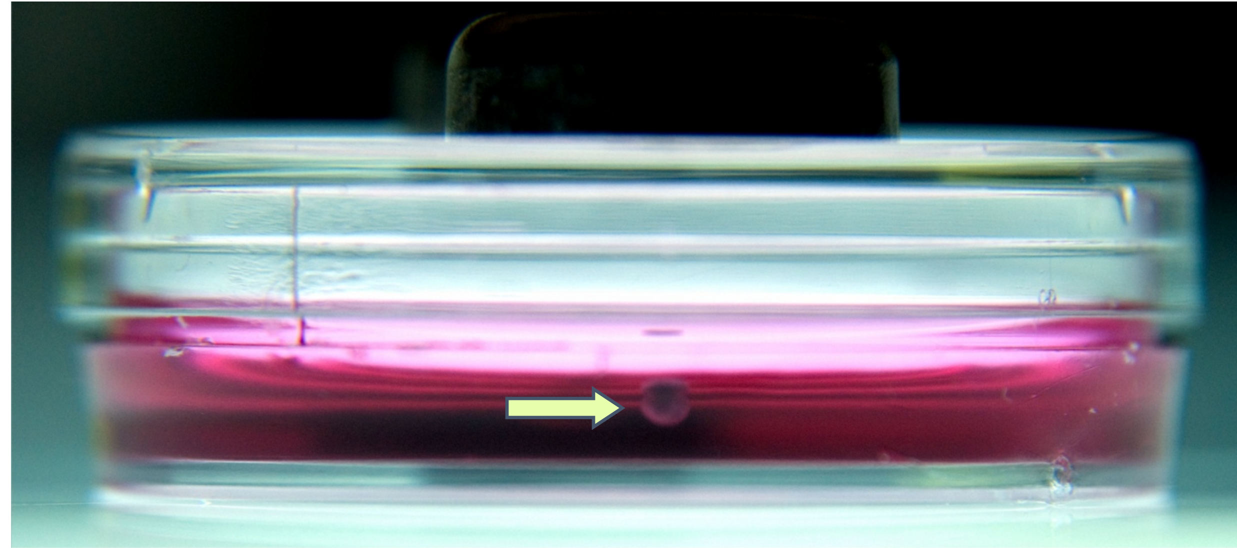
6-Well Bio-Assembler™

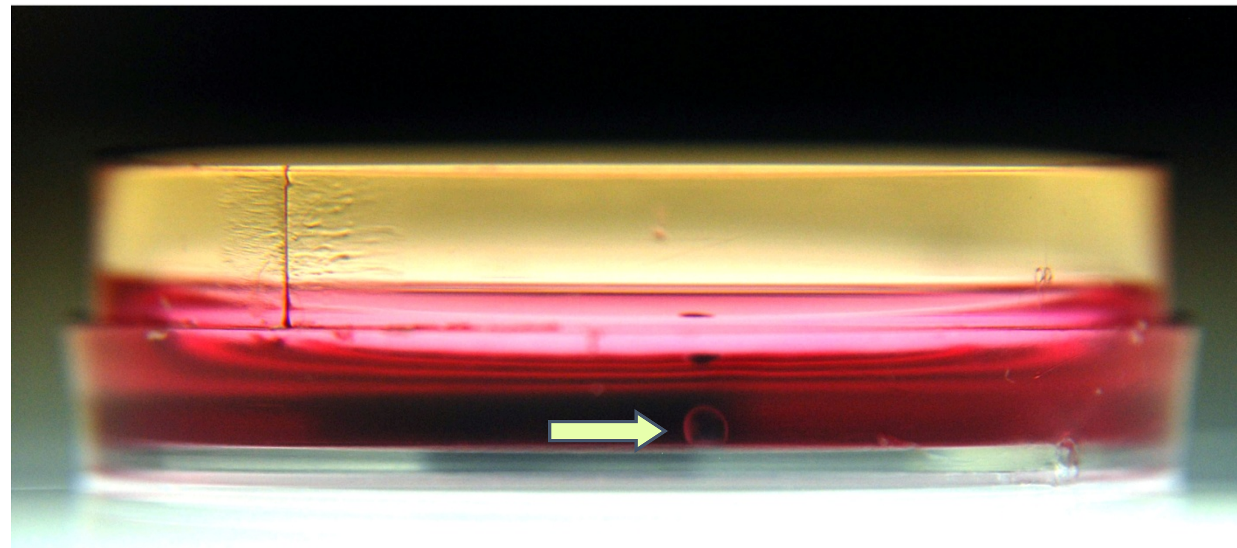




n3D

Magnetic Levitation

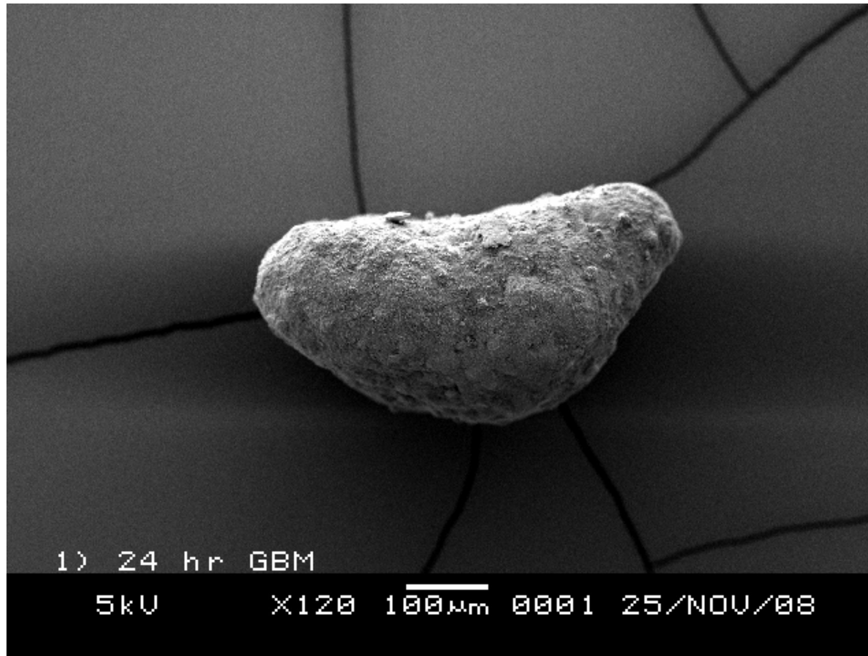




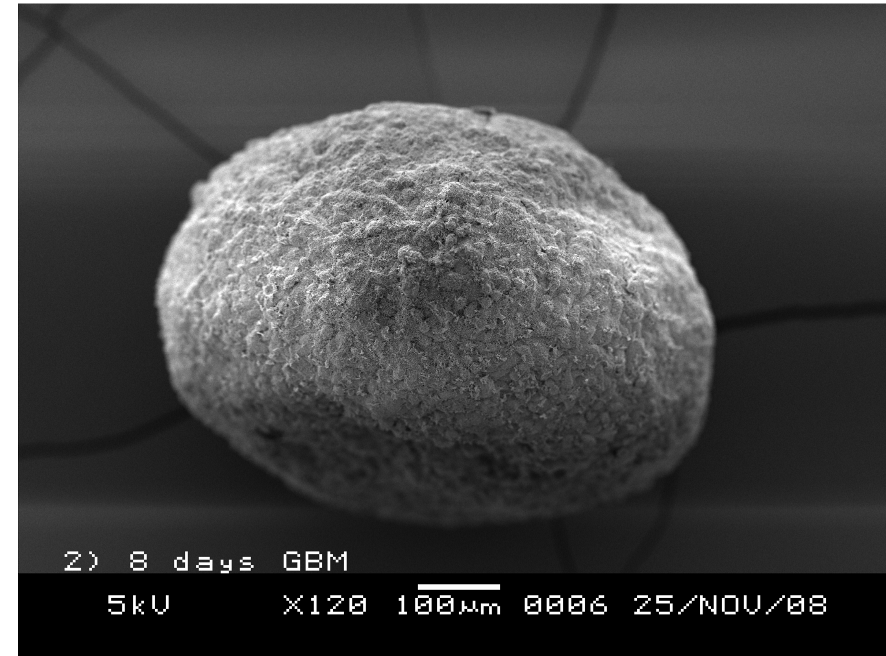
3D Structure

SEM – 24 hours vs. 8 days GBM Cell Cultures

24 hours

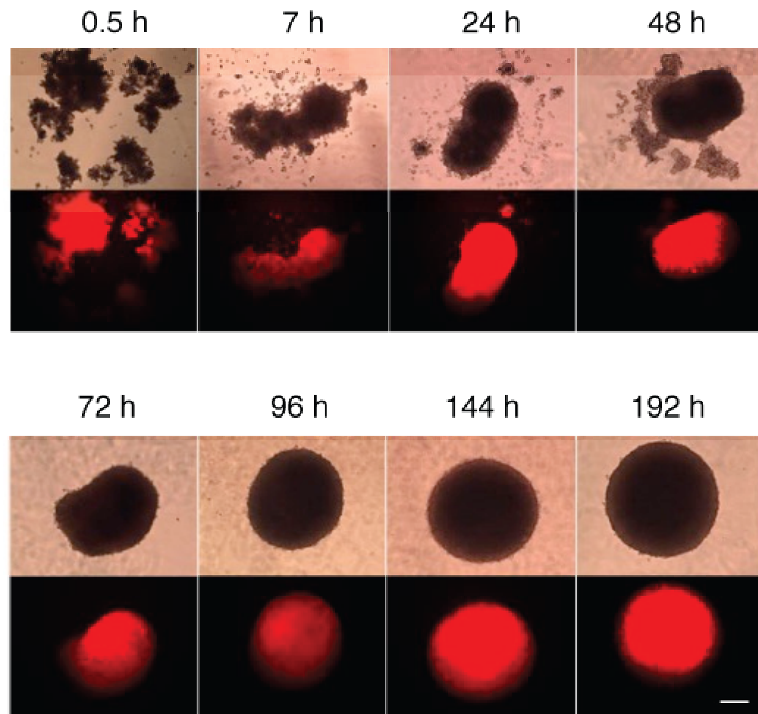


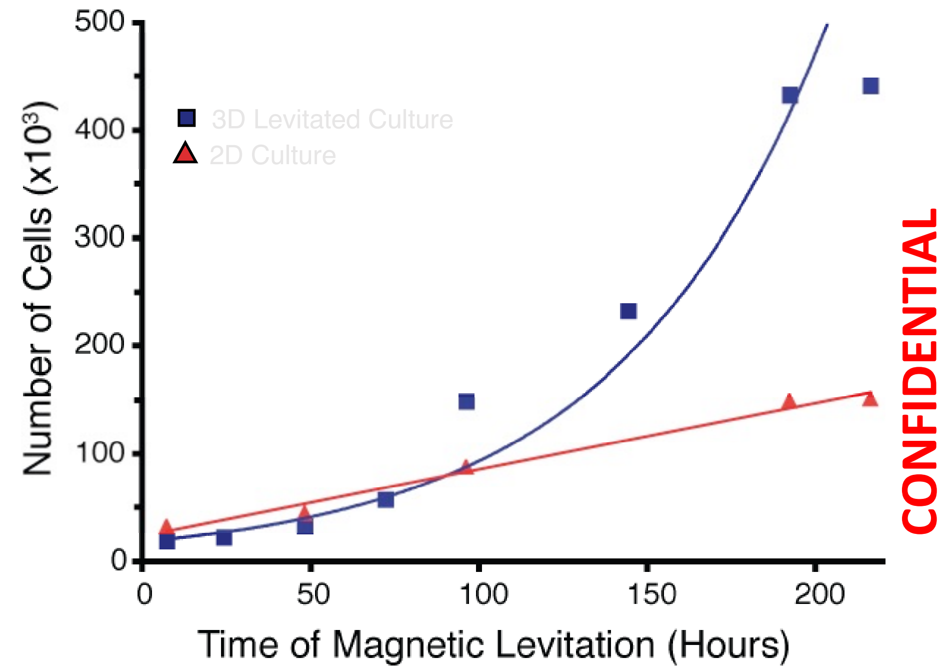
8 days



Souza *et al.* Nature Nanotechnol. April 2010

Cell Assembly and Growth
Glioblastoma (GBM) Culture and Growth Rate vs. 2D





CONFIDENTIAL

Data have been independently validated

Souza *et al.* Nature Nanotechnol. April 2010

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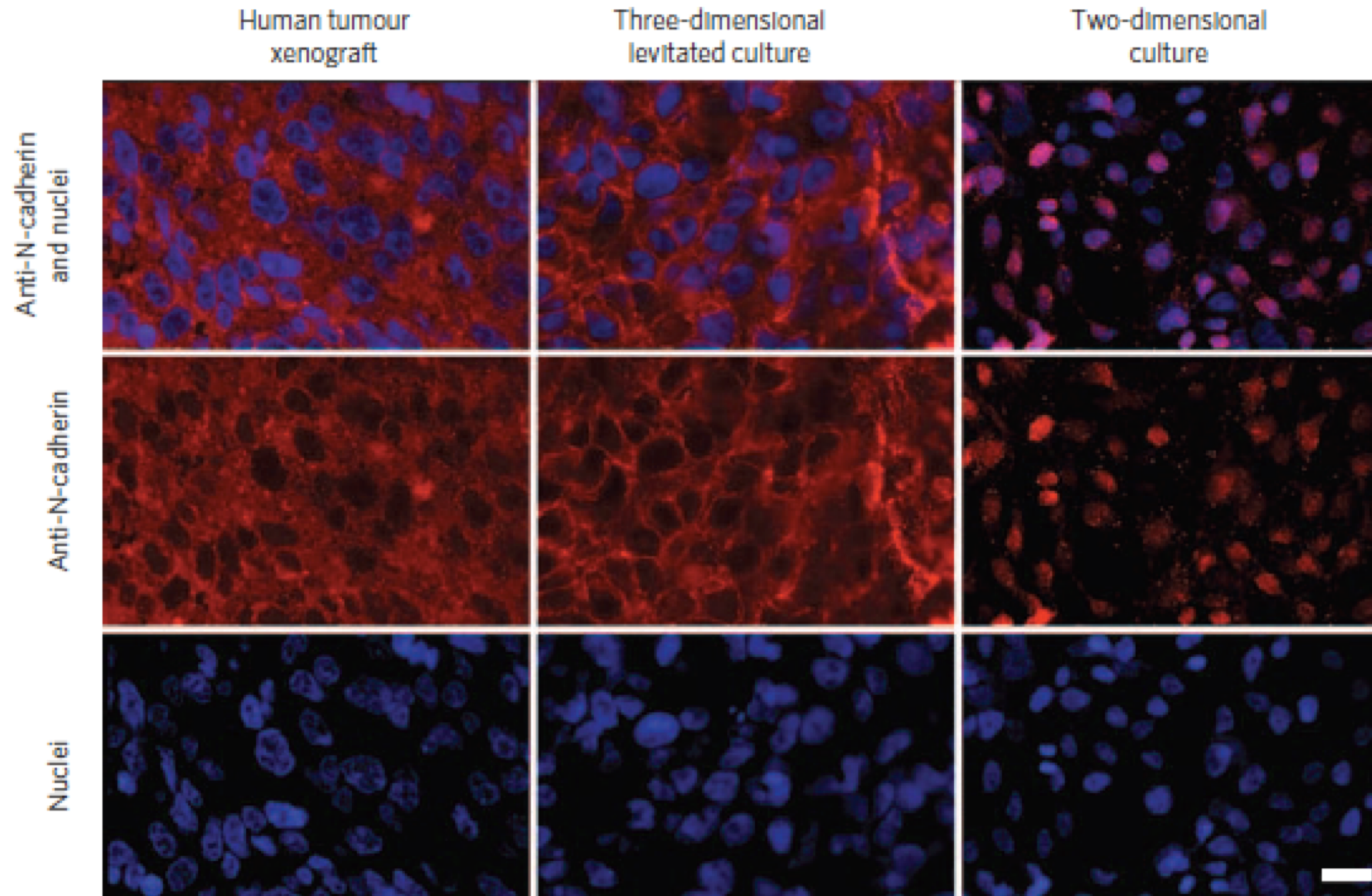
What about *in vivo* like?

In Vivo like *In Vitro*
Mouse Brain Xenograft Comparison - glioblastoma



Souza *et al.* Nature Nanotechnol. April 2010

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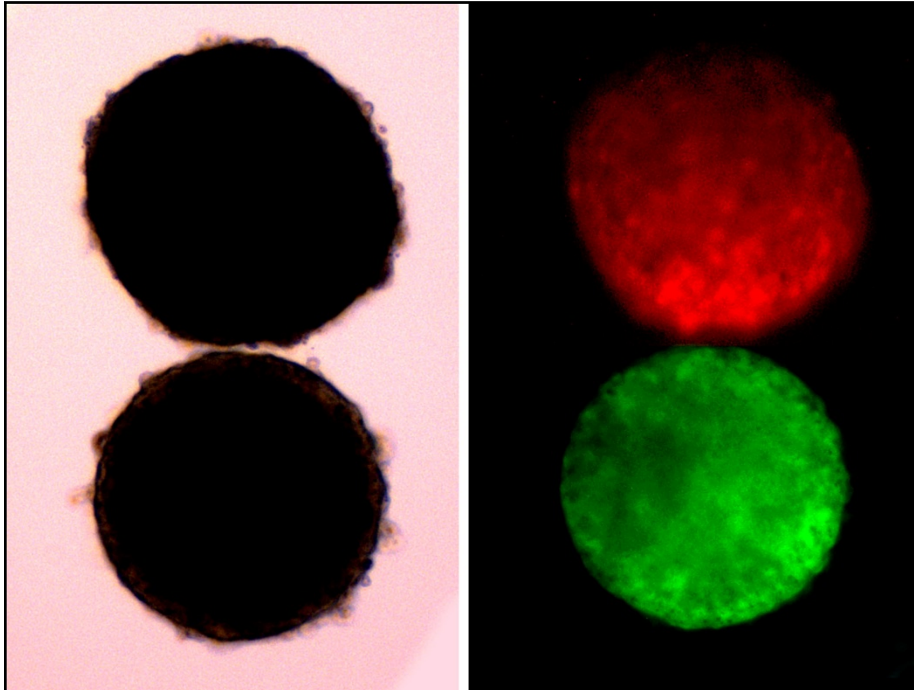
Co-Culture, Spatial Control, Invasion Assay

n3D

Invasion Assay & Co-Culture

Magnetic Guidance = Control

t = 0

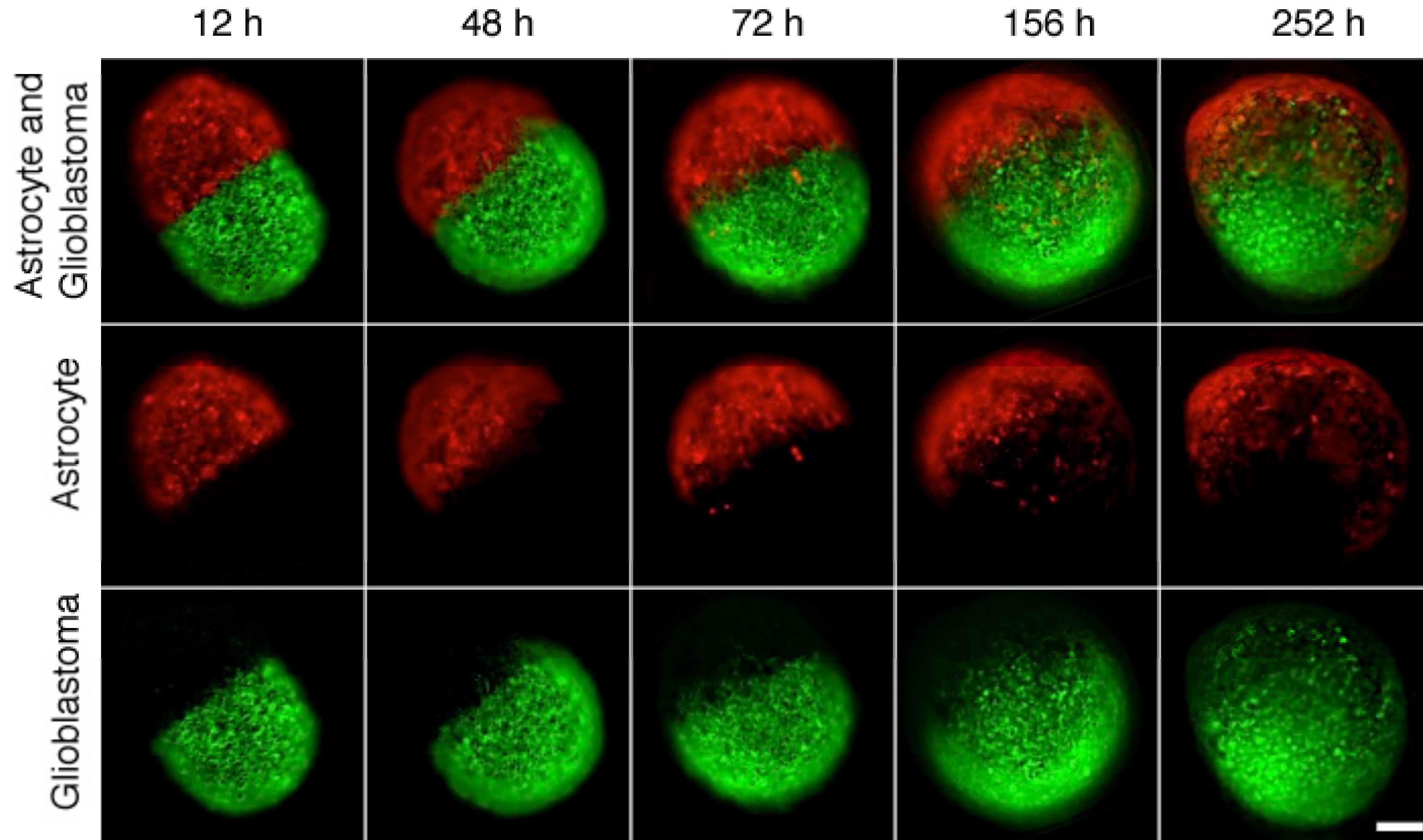


Normal Human Astrocyte
mCherry fluorescence

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

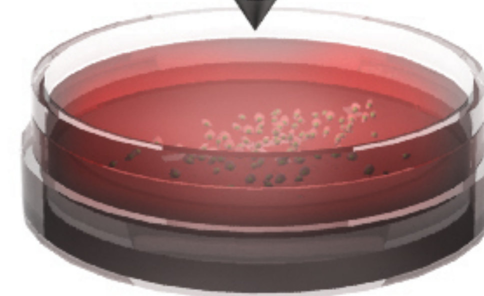
Step-by-Step

Simple as 2D Methods

add Nanoshuttle to media & cells

magnetic drive

lid



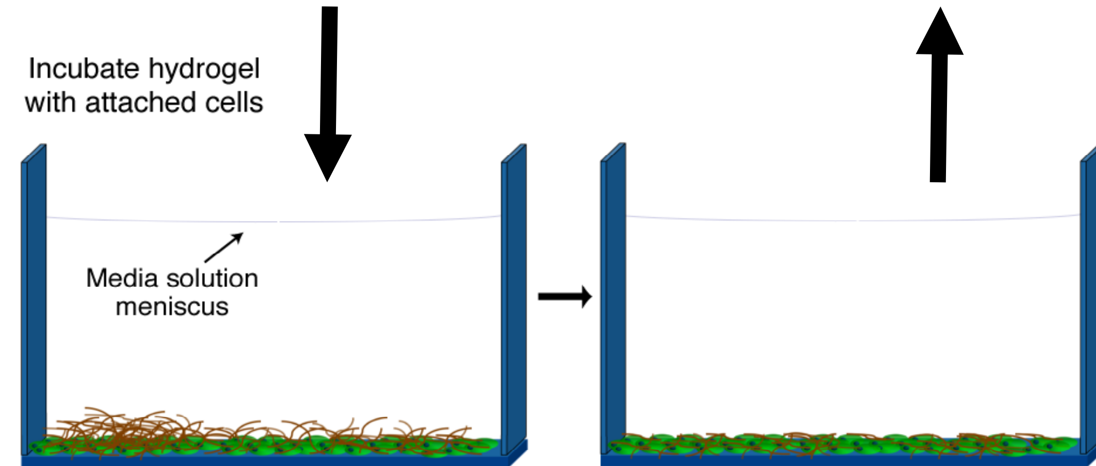
petri-dish

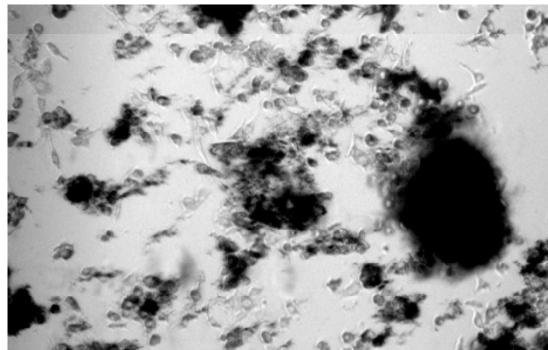
3D Cell Culture By Magnetic Levitation

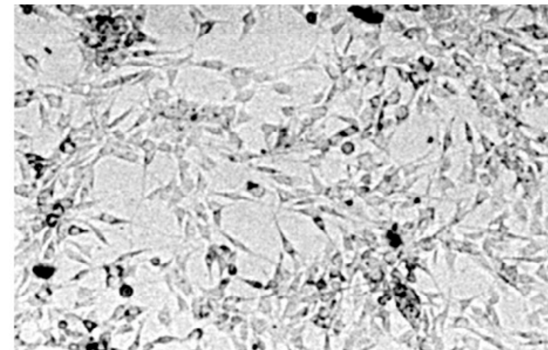
Neural Stem Cells

Add & Incubate
With Magnetic Nanoshuttle

Remove Excess
Magnetic Nanoshuttle

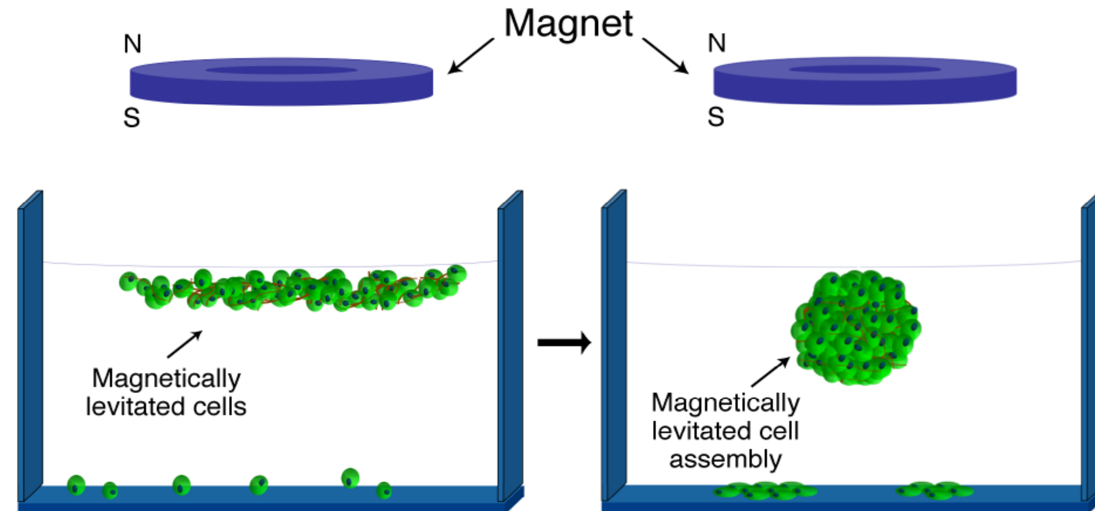


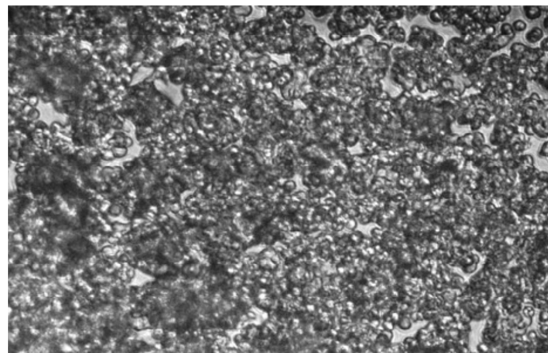


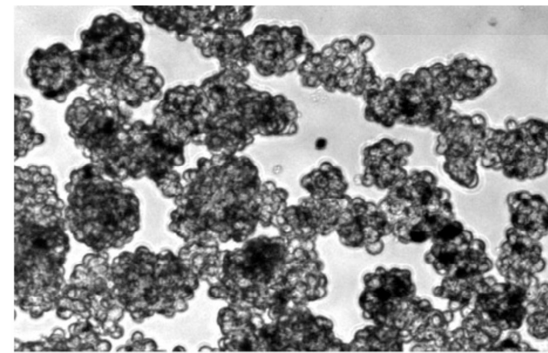


3D Cell Culture By Magnetic Levitation

Neural Stem Cells







Tuning the Culture

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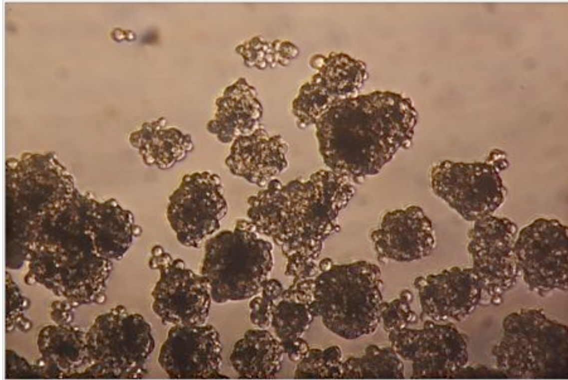
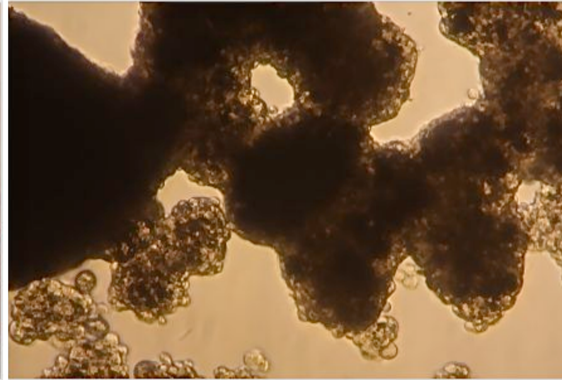
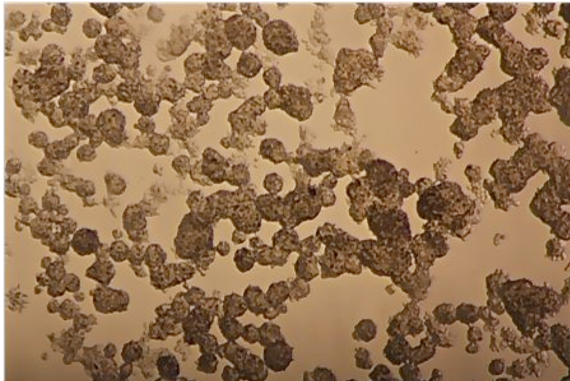
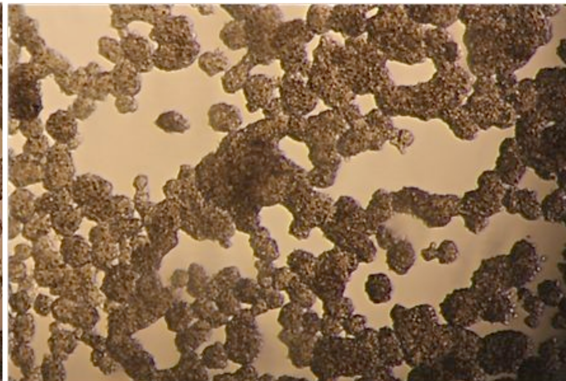
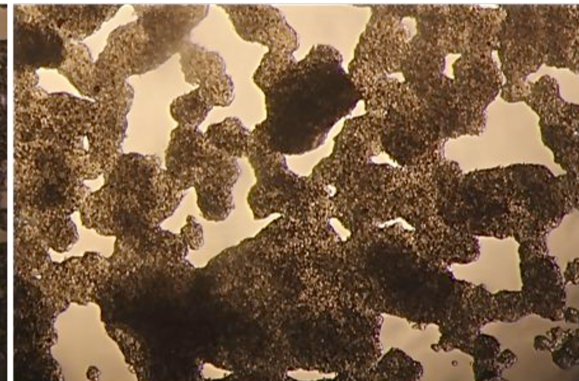
Other Cell Types

Levitation Time

	24 hours	48 hours	10 Days
Condrocytes			

Number of Cells – 24 hours

	200k	400k	880k
Hepatoma			

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Lung Primary Cells

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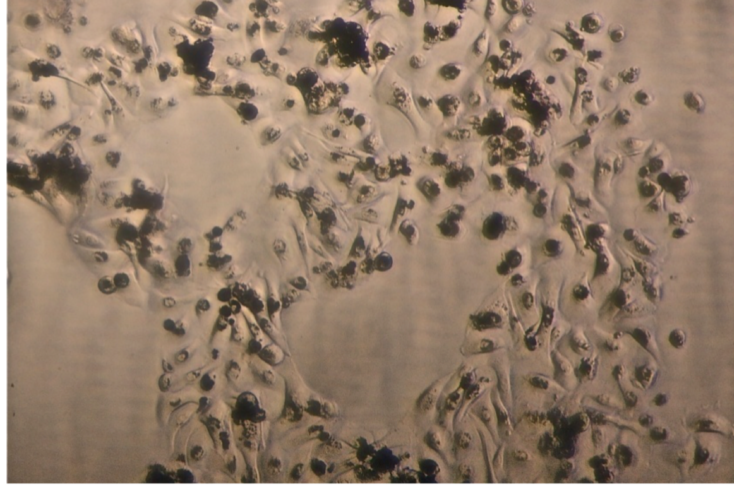
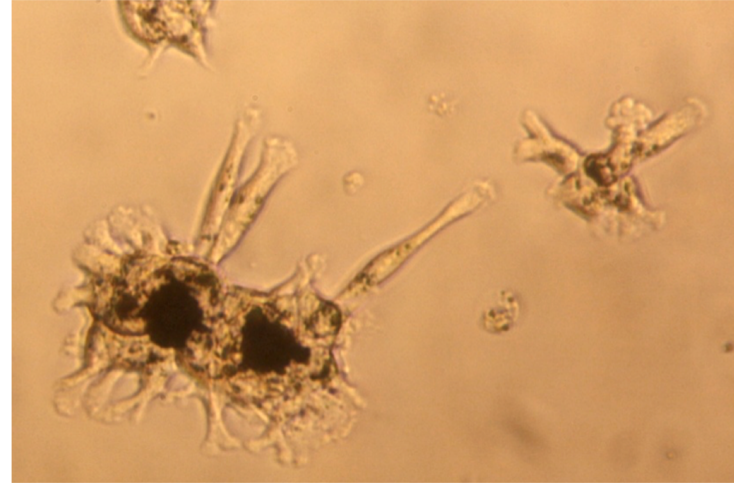
Human Lung Primary Cells

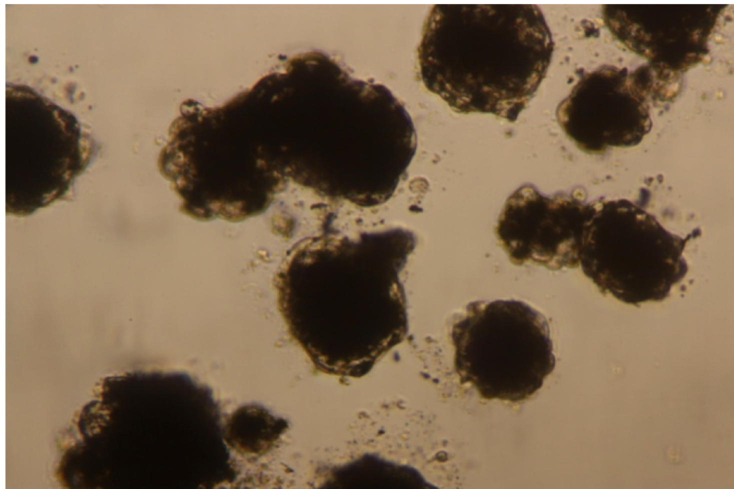
Epithelial



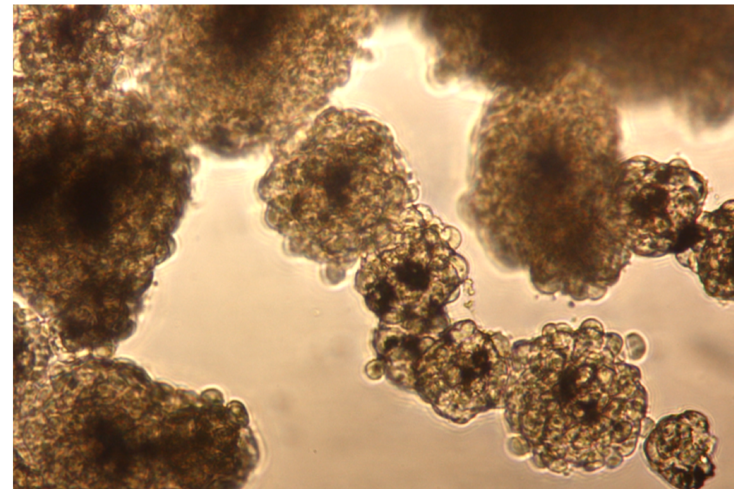
Smooth Muscle



Endothelial

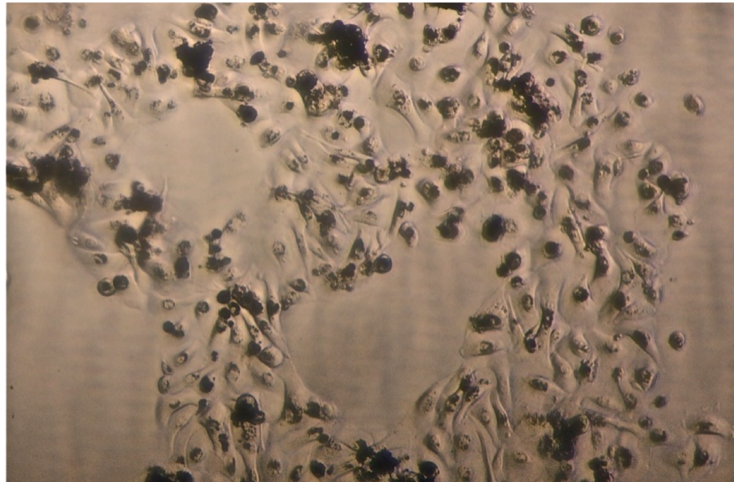


Fibroblast

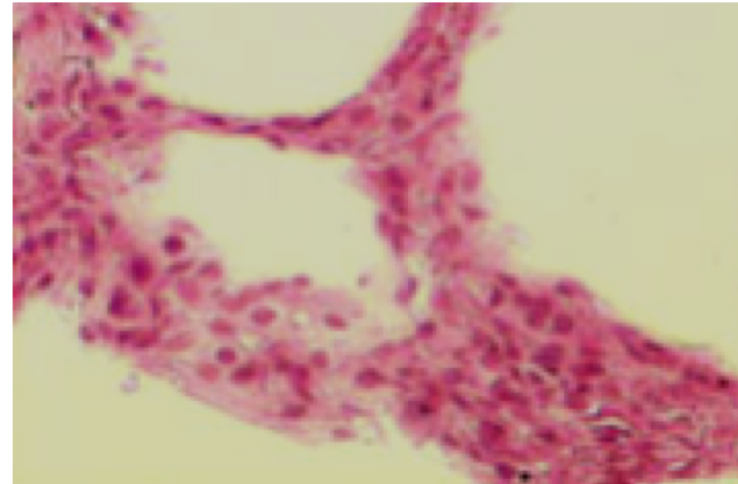


Primary Small Airway Epithelial Cells

Bio-Assembler™



Alveolar
Simple Squamous



http://biosc139drbloom.blogspot.com/2009/01/lab-4-histology.html

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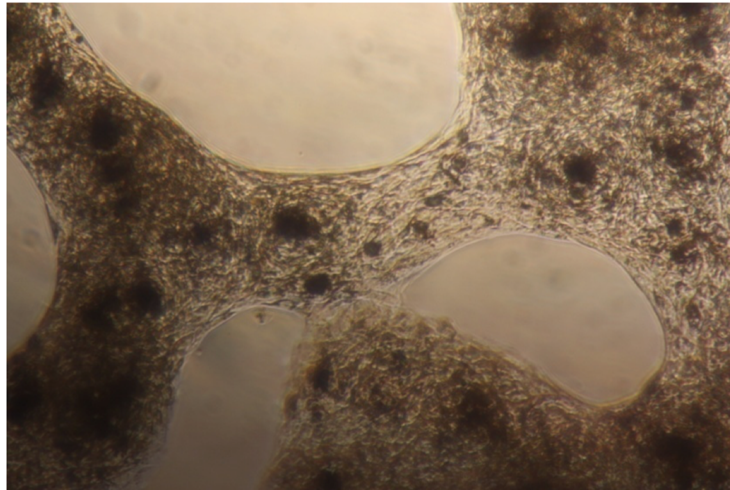
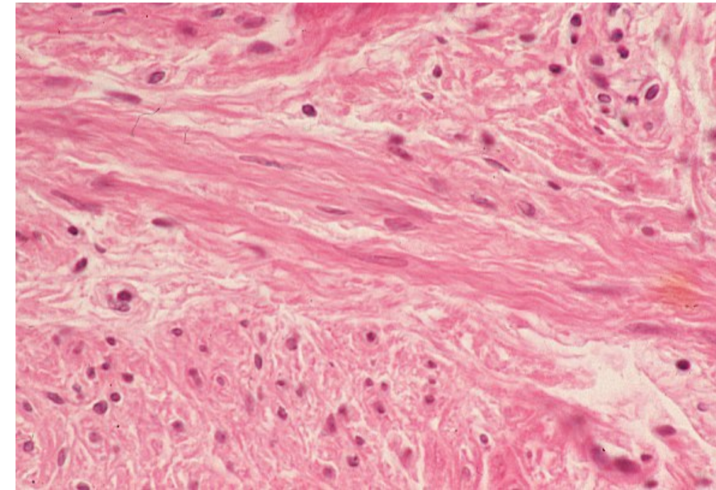
Primary Smooth Muscle Cells

Bio-Assembler

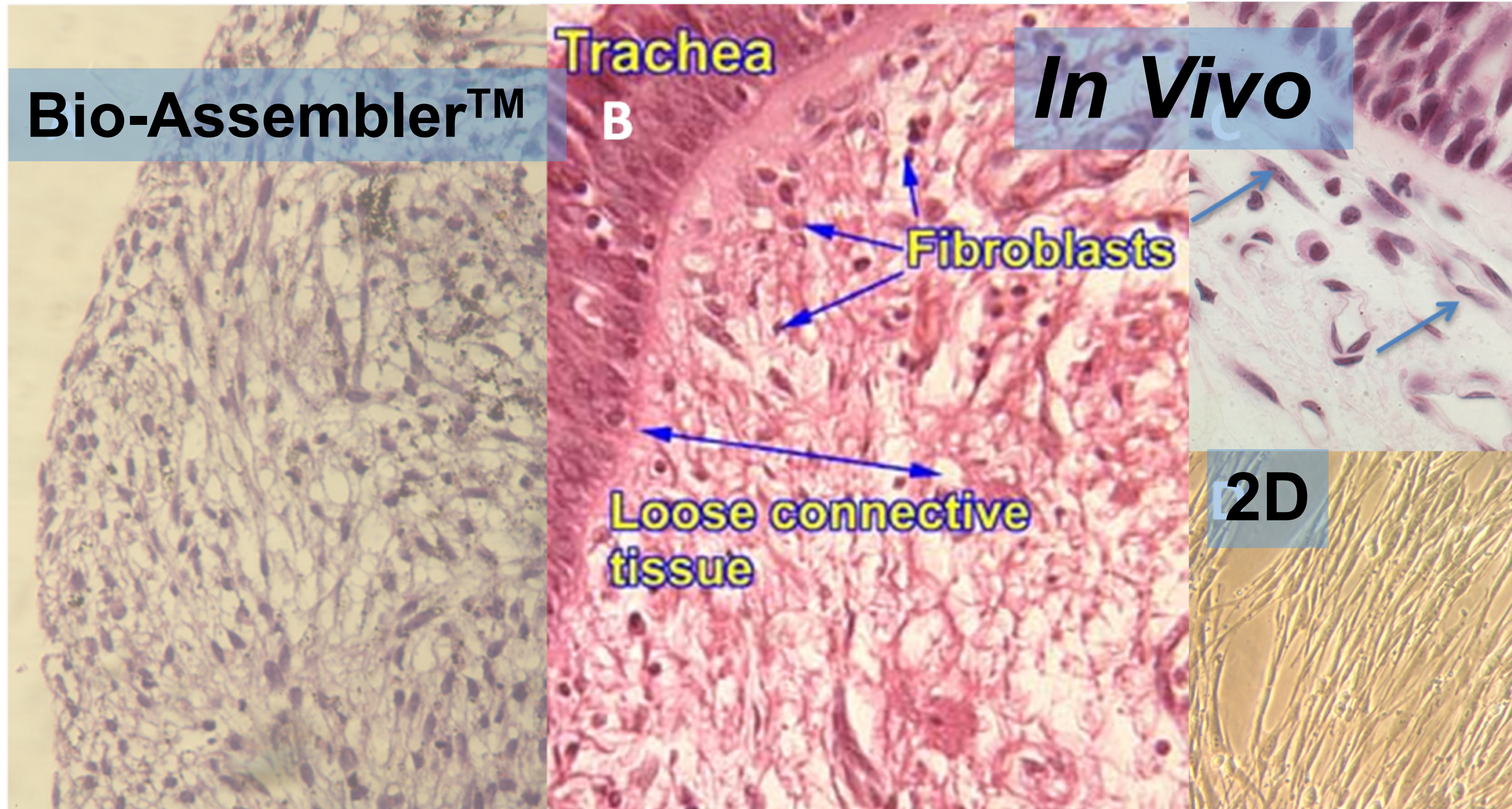


In Vivo

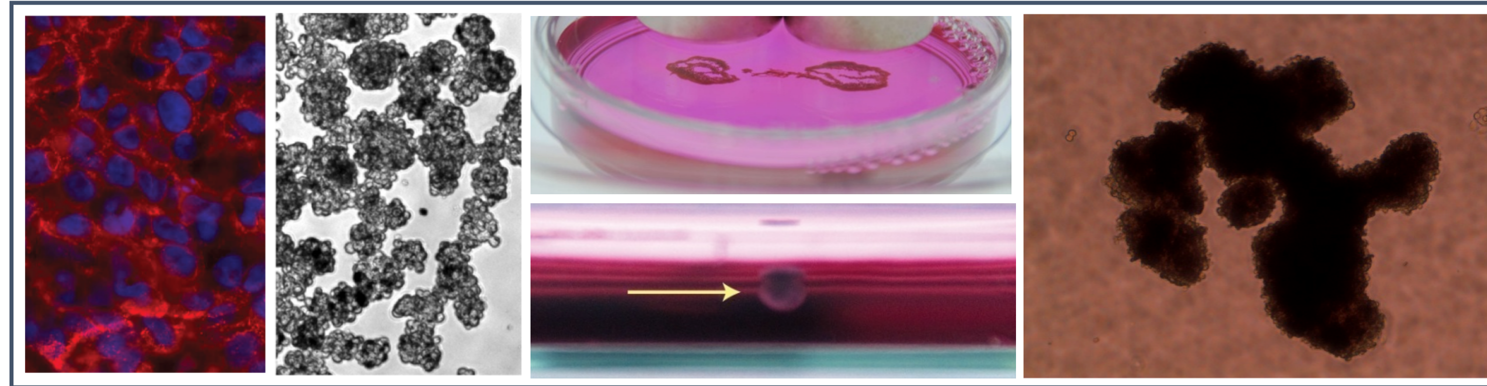


http://www.pathguy.com/histo/056.htm

Primary Pulmonary Fibroblasts



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**
- **Condrocytes**

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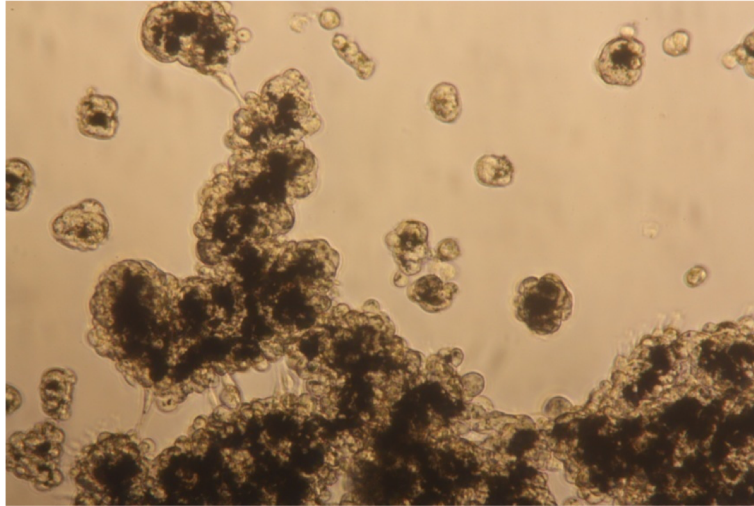
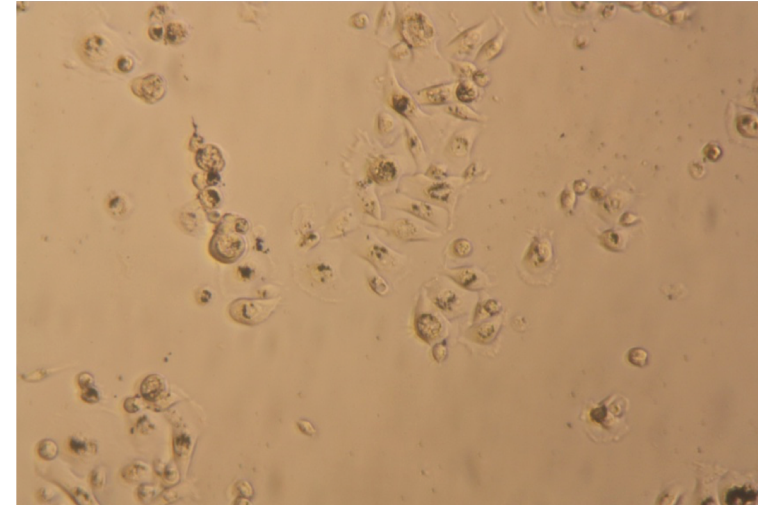
Rapid 3D Formation by Promoting Cell-Cell Interaction

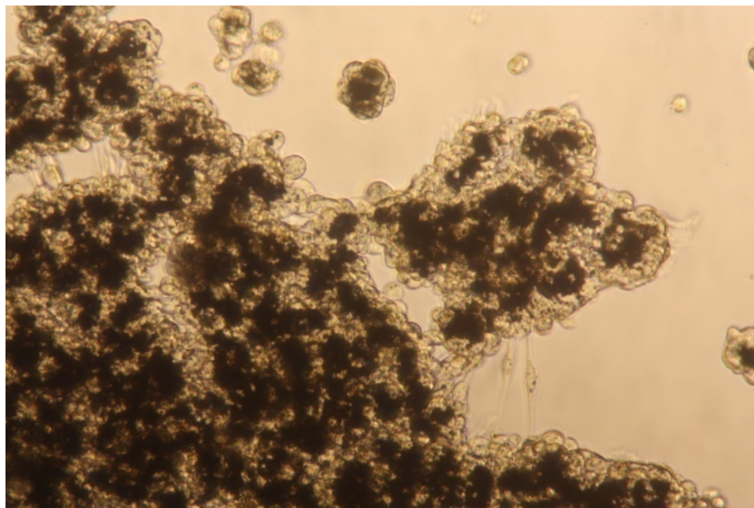
n3D

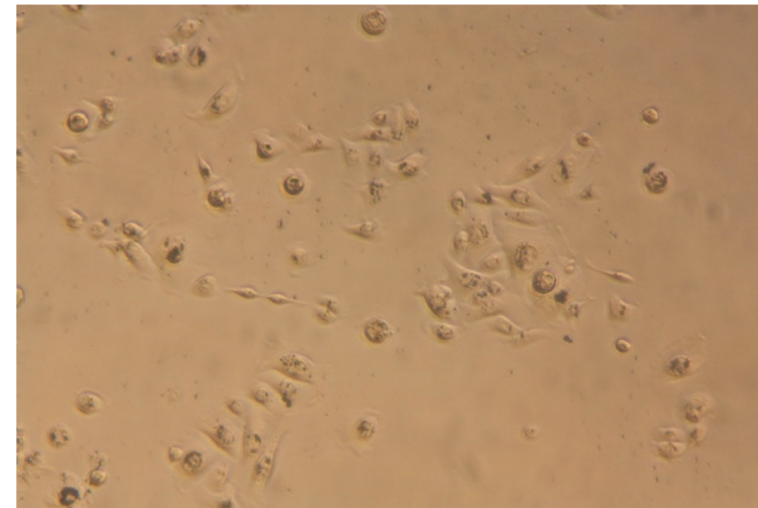
4 Hours of Levitation – Primary Cells

Fibroblast | Epithelial



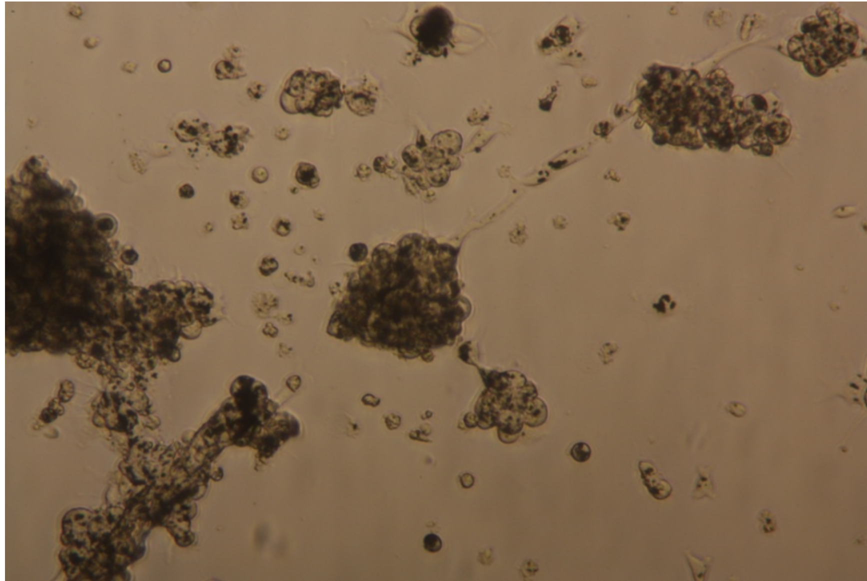


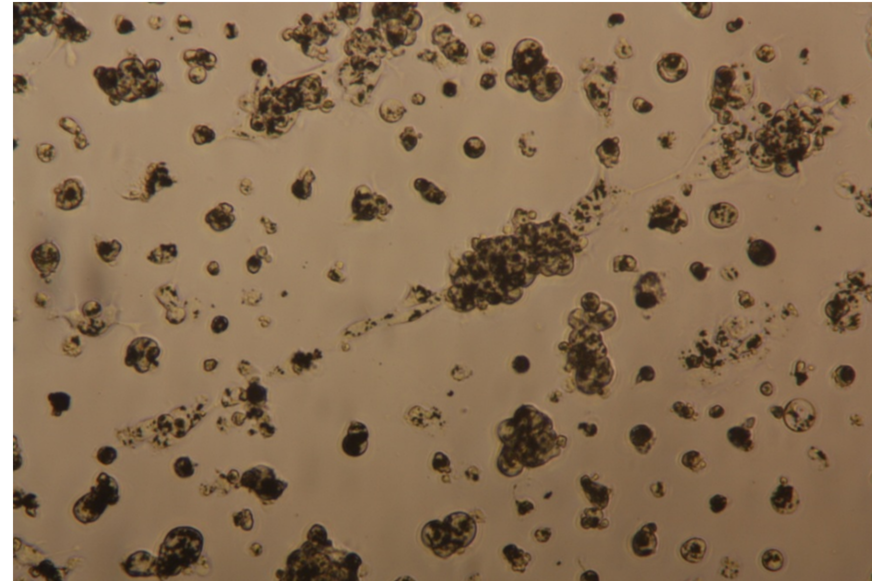




HUVEC – 6 Hours Culture

Human Umbilical Vein Endothelial Cells

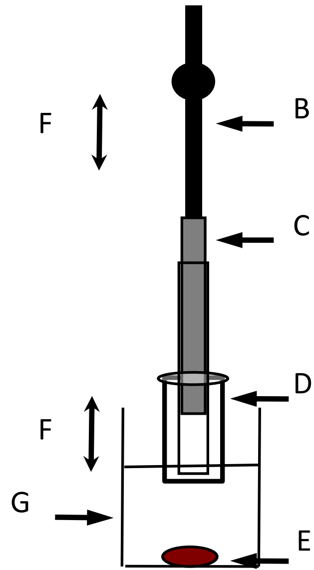


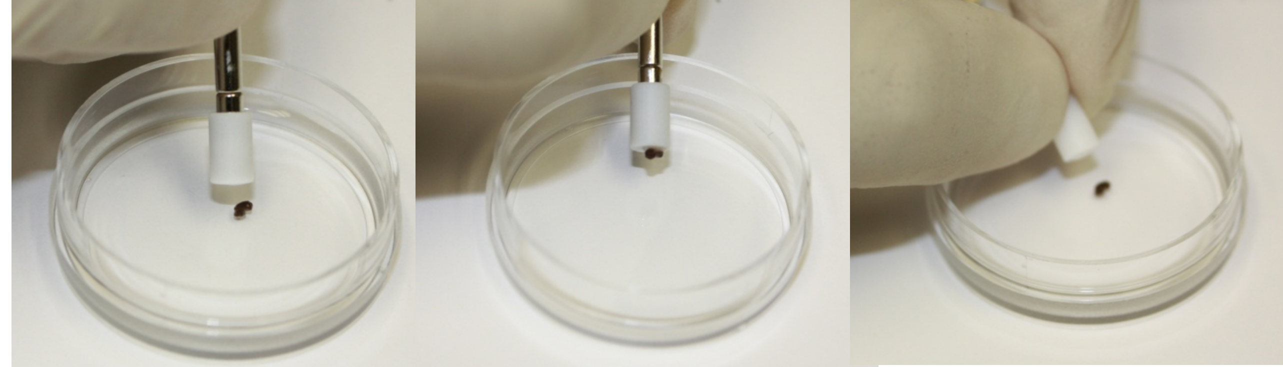


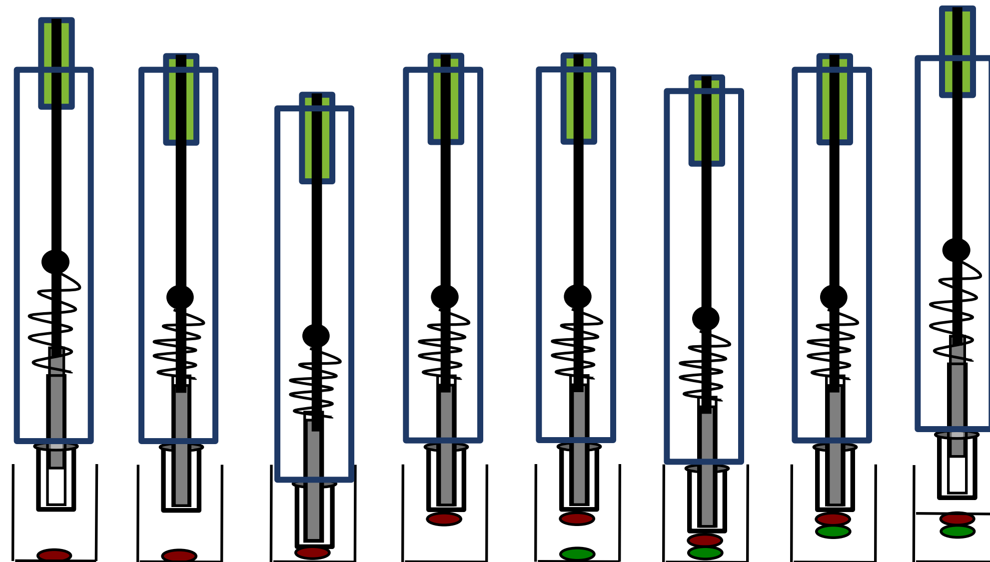
On Going R&D
New Tools

n3D

Magnetic Pen







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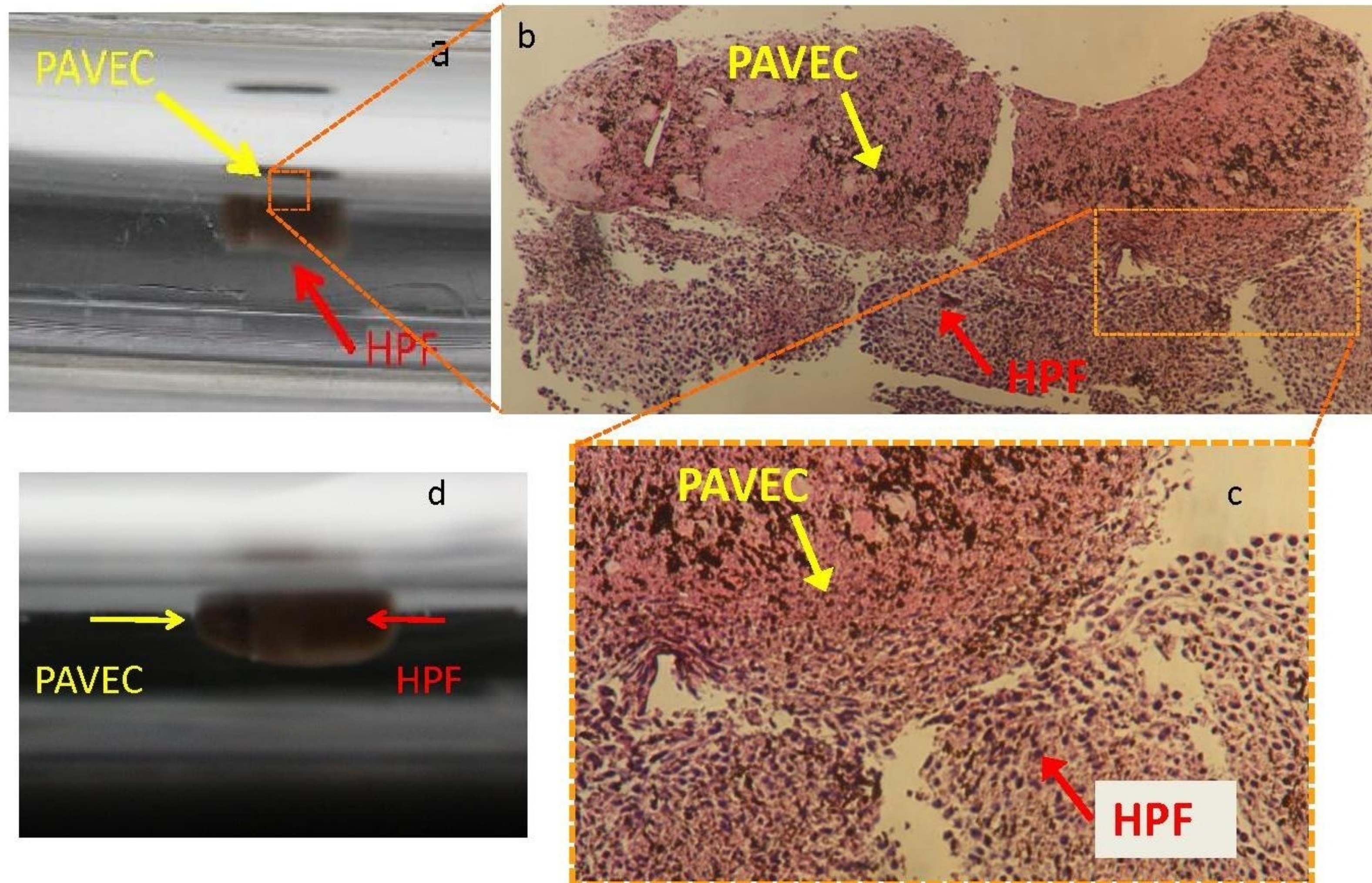
Layered Co-Culture

Human Pulmonary Fibroblasts

Porcine Aortic Valve Endothelial Cells



Vantagens

- Procedimento simples e rápido.
- Obtenção do tecido mais rápida.
- Ausência de compostos animais.

Desvantagens

- Limitação do uso em alguns tipos de experimentos (interferência).