3D cell culture through magnetic levitation

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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

«Perfect» 3D System

Scaffold
- purified ECM
- synthetic polymers
- composites

Cells
- stem/progenitor
- differentiated
- mixed cell types

Structure
- porosity
- topography
- stiffness

Spatial/Temporal Patterning
- cytokine gradients
- controlled release

Perfusion
- embedded channels
- vascularization

Bioreactors
- optimized culture conditions
- biomechanics

Computational Framework
- systems integration
- multi-scale modeling
- simulation
- feedback

Functional Readout
- real-time, label-free, non-destructive sensing
- imaging

Host Response
- generalized inflammation
- specific immunity

Innervation
- signal propagation
- coordinated response

## Scaffolds

<table>
<thead>
<tr>
<th></th>
<th>Materials</th>
<th>Advantages</th>
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</thead>
</table>
| **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 |

magnetic levitation method (MLM)

«Perfect» 3D System

magnetic levitation method (MLM)

magnetic nanoparticle (Nanoshuttle)

2D-culture

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

> self-assemble into hydrogels

Souza et al., Nature Nanotech 2010
magnetic levitation method (MLM)

magnetic nanoparticle (Nanoshuttle)

2D-culture

Haisler et al., Nature 2013
magnetic levitation method (MLM)

magnetic nanoparticle (Nanoshuttle)

2D-culture

incubation

Haisler et.al., Nature 2013

http://www.n3dbio.com/about/our-mission/
magnetic levitation method (MLM)

magnetic nanoparticle (Nanoshuttle)

2D-culture

3D-culture

Haisler et al., Nature 2013
Comparison of three-dimensional cell growth with standard two-dimensional tissue culture

Souza et al., Nature Nanotech 2010

~1 mm
Shape control of magnetically levitated culture

Calculation of magnetic fields

\[
\vec{B}(\vec{r}) = \frac{\mu_0}{4\pi} \int_{\mathcal{S}} \frac{\vec{K}((\vec{r}')) \times (\vec{r} - \vec{r}')}{|\vec{r} - \vec{r}'|^3} d\vec{a}'
\]

\( \vec{r} \) of...
Applications

- 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)

<table>
<thead>
<tr>
<th>Name</th>
<th>Animal</th>
<th>Type</th>
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</thead>
<tbody>
<tr>
<td>HEK293</td>
<td>Human</td>
<td>Embryonic kidney</td>
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<tr>
<td>MDA-231</td>
<td>Human</td>
<td>Mammary epithelial</td>
</tr>
<tr>
<td>MCF-10A</td>
<td>Human</td>
<td>Mammary epithelial</td>
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<tr>
<td>LNCaP</td>
<td>Human</td>
<td>Prostate epithelial</td>
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<td>A549</td>
<td>Human</td>
<td>Alveolar epithelial</td>
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<tr>
<td>HepG2</td>
<td>Human</td>
<td>Hepatocyte</td>
</tr>
<tr>
<td>3T3-L1</td>
<td>Mouse</td>
<td>Fibroblast</td>
</tr>
<tr>
<td>bEnd.3</td>
<td>Mouse</td>
<td>Brain endothelial</td>
</tr>
<tr>
<td>H-4-II-E</td>
<td>Rat</td>
<td>Hepatoma</td>
</tr>
<tr>
<td>U251-MG</td>
<td>Human</td>
<td>Glioblastoma</td>
</tr>
</tbody>
</table>

Primary cells

- Human Astrocyte
- Human Pulmonary endothelial
- Human Type II alveolar epithelial
- Human Bronchial epithelial
- Human Tracheal smooth muscle
- Human Pulmonary fibroblasts
- Human Umbilical vein endothelial
- Human Chondrocytes
- Human Aortic vascular smooth muscle
- Porcine Aortic valvular interstitial
- Porcine Aortic valvular endothelial

Stem cells

- Human Neural stem cells
- Human Mesenchymal stem cells
- Human Dental pulp stem cells
- Human Adipose stem cells

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

Alexes C. Daquinag, PhD, Glauco R. Souza, PhD, and Mikhail G. Kolonin, PhD
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

12h

+ Nanoshuttle Solution

multiwell ultralow-attachment plate

Self-assemble into spheroid

< 24 Hours

Levitated Cells

3D Multicellular Structures
Adipogenesis induction

3T3-L1 preadipocytes (levitated for 1 day)

+ 

Adipogenenic differentiation medium for 72h
(0.5mM isobutylmethylxanthine, 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

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

Hamsa Jaganathan¹*, Jacob Gage²*, Fransisca Leonard¹*, Srimenakshi Srinivasan¹, Glauco R. Souza², Bhuvanesh Dave³ & Biana Godin¹

¹Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030 USA, ²n3D Biosciences Inc, Houston, TX, 77030 USA, ³Cancer 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
breast tumor stroma consists of:
- fibroblasts
- adipocytes
- endothelial cells
- inflammatory cells with different enzymes and growth factors
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

Diameter (µm)

Number of Cells

Optical Density

Number of Cells

3D in vitro - day 10

In vivo – day 14
Comparison of 2D co-culture with the 3D in vitro breast tumor model

Breast cancer cell lines
- SUM159
- MDA-MB231

fibroblast cell lines
- 293T
- Hs578b

2D and 3D images of cell lines with different colors indicating breast cancer cells, fibroblasts, and DNA.
Comparison of 2D co-culture with the 3D in vitro breast tumor model

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

Hs578bst and CAF grow in sync with the cancer cells
Characterization of in vitro 3D co-cultures

- 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.
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 affect viability in 3D/2D systems
Effect of doxorubicin treatment on 3D in vitro tumors
Effect of doxorubicin treatment on 3D in vitro tumors

Tumor Growth

Day 1

Day 5

Day 7

100% F 50% F/50% B 100% B

No Treatment

Day 8

Day 9

Day 12

100% F 50% F/50% B 100% B

DXR Treatment

Day 8

Day 9

Day 12

100% F 50% F/50% B 100% B

↓ decrease of tumor area and density after doxorubicin treatment
- 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 breast tumor model to test drug efficiency
Progression of in vitro Cell-Based Models

Caicedo-Carvajal et al., Translational Medicine 2012
Thanks for your attention