Assaying Organotypic 3D Microtissue Models

Tour of the Prom’s
Dr. Jens M. Kelm, CSO and co-founder
Lack of efficacy and toxicity are still the main reason’s of high attrition rates.
'Why 3D?' Such sessions are a thing of the past now because there is an accumulating body of evidence - joined by the recent article from Leslie and colleagues - demonstrating the importance and utility of 3D culture systems to discover and model biological process with in vivo relevance.

Editorial “3D culture reveals a signaling network”
Senthil K Muthuswamy
Breast Cancer Research, 2011
Better in vitro Biology

Drug-Target interactions

Drug – Cell response

Drug – Tissue response

Drug – Organism response

High failure rate

Reducing failure rate
Advancing cell-based assays

Resembling native-like cell functionality to improve predictive power of cell-based assays.
3D Microtissue culture in hanging drops

Seeding  
Medium/air interface

After 1 hour  
Cells

After 2-4 days  
Medium

Tumor tissue
Microtissue Formation
Size Reproducibility

HCT116-colon cancer MTs

± 1.48 μm ± 7.44 μm ± 8.79 μm
Microtissue analysis in specific spheroid assay plates (GravityTRAP™)
Microtissue Models: From Discovery to Safety

- Tumor
- Islets
- Vasculature
- Cartilage
- Liver
- Brain
- Bone
- Epithelial Barrier
- Heart
- Kidney
- Skin
- Ganglia
Drug discovery

TUMOR MICROTISSUES
Spheroid cell heterogeneity

Native tumor

- Secondary necrosis

Spheroid model

- Heterogeneous cell population
- Diffusion required
- High levels of ECM

Adapted from Friedrich J. Int J Radiat Biol. 2007 Nov-Dec;83(11-12):849-71.
Tumor spheroid expression profile closer to in vivo

Hierarchical cluster analysis of OV-90 (ovarian cancer cell line) expression data grown in different conditions

- **L**: monolayer culture
- **S**: spheroid cultures
- **TSC**: tumors from subcutaneous xenografts
- **TIP**: tumors from intraperitoneal xenografts
- **LSC** and **LIP**: monolayer cultures derived from these tumors

A 3D Filter can significantly reduce the number of candidates

Screening for apoptosis inducer

10’000 cpds → HCT116, colon cancer
382 cpds → 2D screen 25 uM, 72h
40 cpds → 3D screen 25 uM, 72h
11 cpds → 3D screen 12.5 uM, 72h

Fayad et al. 2011
Example of a 3D filter

Screening for an apoptosis inducer

Fayad et al. 2011
**Biochemical assays evaluated with InSphero’s microtissues:**

<table>
<thead>
<tr>
<th>Lytic Assays</th>
<th>Non-Lytic Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Viability</td>
<td>Redox State</td>
</tr>
<tr>
<td>CellTiter-Glo*</td>
<td>GSH/GSSG-Glo*</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Metabolism</td>
</tr>
<tr>
<td>Caspase-3/7 Glo*</td>
<td>Cell Death/Growth</td>
</tr>
<tr>
<td></td>
<td>CytoTox-ONE*</td>
</tr>
<tr>
<td></td>
<td>Cell Death</td>
</tr>
<tr>
<td></td>
<td>NanoLuc</td>
</tr>
</tbody>
</table>

*CellTiter-Glo*, *GSH/GSSG-Glo*, *Caspase-3/7 Glo*,
The holy grail of lytic assays for microtissues: Tissue Disruption

Fluorescence activity (RFU)

- human liver MTs
- rat liver MTs

Buffers:
- Buffer 1
- Buffer 2
- Buffer 3
- Buffer 4
- Buffer 5
- PBS

www.insphero.com | Tour of the Prom’s - Lausanne | 13.03.2013
Cell number assessment in microtissues

Assay: PicoGreen
Model: NIH3T3

Calibration curve with single cell suspension (input cell population)

\[ y = 1.6265x \]
\[ R^2 = 0.9686 \]
Cell viability: ATP

Model: SNB-19 human glioblastoma cells

CellTiter-Glo protocol optimization
- 3X pipet mix at CTG Reagent add’n
- 20 min incubation prior to read

- Varied cell number at inoculation
- Day 4 → MT transferred for study

Graph showing ATP per microtissue vs cell number at inoculation.
Caspase 3/7: Critical Time Window

IC50: 0.31uM
Apoptosis: Caspase 3/7 activity

Model: 3D Insight rat liver microtissue

Caspase 3/7 activity

RLU/microtissue vs. Staurosporin treatment (4h, 24h, 48h)
Drug discovery

DISCRIMINATING DRUG EFFECTS IN MULTI-CELL TYPE MICROTISSUES

In collaboration with:

Pomega Ltd., Madison US and Dübendorf CH
Sirion Biotech GmbH, Munich
Tecan Austria GmbH, Grödig
Colon cancer co-culture

NIH3T3-tGFP : HCT116-NanoLuc
Monitoring distinct cell populations in a co-culture model

HCT116Luc

NIH3T3RFP

NIH3T3RFP:HCT116Luc

NIH3T3RFP:HCT116Luc
IC50 determination in co-culture systems

<table>
<thead>
<tr>
<th>Compound</th>
<th>ATP [µM]</th>
<th>nanoLuc [µM]</th>
<th>RFP [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>STS</td>
<td>1,25</td>
<td>1,23</td>
<td>n.d.</td>
</tr>
<tr>
<td>CIS</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>n.d.</td>
</tr>
<tr>
<td>TAX</td>
<td>1,08</td>
<td>0,50</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

### STS

- **DMSO**
  - 0,16 uM
  - 0,63 uM
  - 2,5 uM

### CIS

- **DMSO**
  - 15,6 uM
  - 62,5 uM
  - 250 uM

### TAX

- **DMSO**
  - 0,01 uM
  - 0,2 uM
  - 2,5 uM
Pre-clinical safety

LIVER MICROTISSUES
3D InSight™ rat liver microtissues

Immunofluorescence image of rat hepatocytes co-cultured with non parenchymal cells (NPC). Green: DPP-IV (bile canalicular marker), blue: DAPI, red: ICAM-1 (endothelial marker)

In collaboration with Jan Hengstler and Seddik Hammad
3D InSight™ human liver microtissues

Cryopreserved Hepatocytes

Production in GravityPLUS

Assaying in GTRAP

Cryopreserved NPCs
Acetaminophen-induced liver microtissue toxicity
Metabolic activity of human liver microtissues

CYP3A4-induction

- DMSO control
- 20 uM Rif, 48h

RLU

hLiMT - NPC

hLiMT + NPC
Correct Prediction of Toxicity Where Classical Methods Fail

Endpoint: ATP content

Colchicine

HepG2-monolayer

MEC: >10 μM

Hepatocyte monolayer

MEC: >10 μM

Human liver microtissue

MEC: 10 μM
Pre-clinical safety

INFLAMMATION-MEDIATED LIVER TOXICITY
Kupffer-Macrophages are incorporated in rLiMTs

In collaboration with Marianne Uteng, Pierre Moulin, and Francois Pognan, Novartis Pharma Basel

Nuclei: 405 Dapi
Kupffer macrophages: rat CD68
Bile canalicule: DPPIV
Morphological characterization of human liver microtissues

CK8

CD68

IL-6 secretion

48h LPS-treatment (10ug/ml)

IL-6 (pg/well)

hLiMT - NPC

hLiMT + NPC
Why did we develop InSphero’s 3D models?

Example: Trovan (Pfizer)

Introduction in 1997

2.5 million prescriptions
4 patients needed liver transplantation
6 patients died

→ Withdrawn 1998

Estimated loss for Pfizer: US$ 8.5 billion

Trovan toxicity was not detected in-vitro
Inflammation-mediated idiosyncratic trovafloxacin toxicity rLi^{MT}

Trovafoxacin/Levofloxacin treatment
Readout: Released LDH (Cytotox-Glo, Promega)
Inflammation-mediated idiosyncratic trovafloxacin toxicity hLi\textsuperscript{MT}

Trovaflroxacin/Levofoxacin treatment
Readout: Released LDH (Cytotox-Glo, Promega)
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Questions Welcome

Jens.kelm@insphero.com