LivHeart: A Multi Organ-on-Chip Platform to Study Off-Target Cardiotoxicity of Drugs Upon Liver Metabolism

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Organs-on-chip

“Microfluidic devices able to mimic activities, mechanics, interactions and physiological responses of human organs *in vitro* “

www.pharmaceutical-journal.com
Organs-on-chip

APPLICATION in the Drug Development Pipeline

• Efficacy
• Safety
• Complement/Replace animal models
Drug Development Process - preclinical

Reproduce organ complexity ✓
Differences between animals and humans ×
High animal-to-animal variability ×
Ethical problem ×
Drug Development Process - preclinical

Characters:
- Animals
- 2D: '80s
- 3D: '90s
- OoC: ‘10s
- MOoC: present

Characteristics:
- Cheap ✓
- Human models ✓
- Static conditions ✗
- No organ complexity ✗
Drug Development Process - *preclinical*

- **Animals**
- **2D**
- **3D**
- **OoC**
- **MOoC**

**Gold standard**

- ‘80s
- ‘90s
- ‘10s
- Present

- Low costs and volume samples ✓
- 3D human models in controlled environment ✓
- Recapitulation of organ architecture ✓
- Stimuli to mimic organ complexity ✓
- NO organ-organ communication ✗
Drug Development Process - preclinical

- Low costs and volume samples
- 3D human models in controlled environment
- Recapitulation of organ architecture
- Stimuli to mimic organ complexity
- Organ-organ communication
- Earlier elimination of problematic drugs
Drug safety process

APPLICATION in the Drug Development Pipeline

- Efficacy
- Safety
- Complement/Replace animal models

“Almost 95% of lead candidates identified by current in vivo screens do not become successful drugs due to unforeseen toxicity”
Liver-Heart models - limitations

**ECHO platform**
- High priming volume
- Monocultures

**HESPEROS platform**
- 2D hepato-cardiac model $\rightarrow$ low functionality
- No control of organ models communication


Our Liver and Heart models

2D hepatic model → HepG2 + 3T3 fibroblasts
- Optimized for microfluidic platforms
- Validated for metabolism

3D cardiac model → nRCM embedded in a fibrin gel
Mechanical stimulation & Electrical recording
- Enhanced cardiac viability
- Improved maturation and functionality
Our Liver and Heart models

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Beating heart on chip

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Ferrari E et al. Biomed Mater. 2021 Jun 7;16(4)
μPCC on chip

STARTING POINT

Micropatterned co-cultures (MPCCs) of hepatocytes and 3T3 fibroblasts

→ highest production of albumin;

AIM: Build MPCCs inside microfluidic devices to generate a liver-on-chip model

PATTERNING

MICROFLUIDICS

Collagen coated glass slide ‘+’ alignment signs

PDMS channel layer

bonding

Cell seeding and medium perfusion

250µm


Ferrari E et al. Biomed Mater. 2021 Jun 7;16(4)
μPCC on chip

- HepG2 seeding and attachment
- 3T3 fibroblasts seeding to generate μPCCs
- Viability > 83% after 7 days of culture
- Good albumin production

Ferrari E et al. Biomed Mater. 2021 Jun 7;16(4)
µPCC on chip

- HepG2 seeding and attachment
- 3T3 fibroblasts seeding to generate µPCCs
- Viability > 83% after 7 days of culture
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Beating heart on chip

uBeat® Technology (EP3289065B1)

Mechanical stimulation
(uniaxial stretching 10-15% strain)

• Functional 3D constructs

Synchronously beating construct

\[ \text{cTnI, Phalloidin, DAPI} \]

\[ \text{cTnI, DAPI} \]

10µm

Mann Whitney Test (non-normal distributions; *P < 0.05; **P < 0.01; ***P < 0.001)

Beating heart on chip

μECG Technology (EP3620508A1 – Granted UIBM, EPO)

Positioning of electrodes for:
- Electrical activity recording (field potential)
- Electrical stimulation (uniform electric field)

Visone R. et al. Biofabrication 2021
Liver-Heart on chip

**AIM:** Develop a **multiorgan-on-chip** platform capable to detect the cardiotoxicity of drugs upon liver metabolization

LivHeart platform

In collaboration with BiomimX

µECG (patented EP3620508A1)

Produced via PHOTO (@ PoliFAB) and SOFT (@ MiMic Lab) Lithographic techniques

LivHeart platform

Valve Layer

Reservoir valve system

Communication valve system

In collaboration with BiomimX

Produced via PHOTO (@ PoliFAB) and SOFT (@ MiMic Lab) Lithographic techniques

LivHeart platform

Produced via PHOTO (@ PoliFAB) and SOFT (@ MiMic Lab) Lithographic techniques

Valve operating pressure

- Colored PBS $\rightarrow$ communication and reservoirs valves
- Application of decreasing pressure values

**Communication Valves**

**Reservoirs Valves**

LivHeart platform - technical results

Ferrari E, Visone R, et al. *Adv Mat Tech (under review)*
LivHeart platform - technical results

Actuation operating pressure - stretching

\[ \varepsilon_{yy} = \frac{\Delta y(P500) - \Delta y(P0)}{\Delta y(P0)} = 0.11 \]

10-15\% physiological uniaxial strain

\[ \varepsilon_{xx} = \frac{\Delta x(P500) - \Delta x(P0)}{\Delta x(P0)} = 0.03 \]

Marsano et al., 2016

P= 0 mmHg - P= 500 mmHg

Scale bars=100µm

Diffusion characterization - numerical

- Transport of Diluted Species
- Medium → Phosphate buffered saline (PBS)
- Drug → C0 = 10 μM & D = 6 × 10^{-6} cm^2/s
  - r Terfenadine → MW=472g/mol
  - η DMEM w/10% FBS: 9.4e10-4 Pa*s
- t = [0, 48] h & time step = 1 h
Rhodamine MW=479 g/mol
Terfenadine MW=472 g/mol
Fexofenadine MW=502 g/mol

**LivHeart platform - technical results**

**Diffusion characterization - experimental**

**Controlled diffusion with NO convection (i.e., no beads movement)**

**Rhodamine diffusion**

- Liver chamber
- Heart chamber

- Normalized Intensity (%)
- Time [h]

N=4

LivHeart platform - biological results

Model validation with Terfenadine

"Terfenadine (TER), a multichannel blocker (i.e., K+ and Ca2+ ion channels) is a drug able to cause a prolongation of the QT interval, which may lead to cardiotoxic effects."

LivHeart platform - biological results

<table>
<thead>
<tr>
<th>Seeding</th>
<th>Tissue maturation</th>
<th>Drug treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day -7</td>
<td>Day 0</td>
<td>Day 6</td>
</tr>
<tr>
<td>Coverslip functionalization</td>
<td>HepG2 seeding</td>
<td>Open valve Drug/DMSO diffusion</td>
</tr>
<tr>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 7</td>
</tr>
<tr>
<td>NIH-3T3 seeding</td>
<td>NRCM seeding</td>
<td>Analysis</td>
</tr>
<tr>
<td>Day 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug/DMSO administration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mass Spectrometry

MRM chromatograms of TER→FEX condition (N=5)

- **Fexofenadine detected at 2 min**
- **Residual Terfenadine detected at 2.48 min**

Liver metabolized Terfenadine (TER→FEX) condition follows the trend of the control compared to when Terfenadine (TER) is directly administered on the heart.
The LivHeart allowed the testing of a non-cardiotoxic metabolite generated from a cardiotoxic drug.
Conclusions
Design and development of a reliable micropatterned Liver-Heart platform encompassing a continuously monitored mechanically active 3D cardiac model to undertake drug toxicity studies upon hepatic metabolism (demonstrated on the case study Terfenadine)

Limitations
• 2D cultures
• User-dependent
• PDMS
• Low-mid throughput

Future Developments
Adopt human-derived cardiomyocytes/hepatocytes as more relevant cell type in the 3D cardiac/liver models
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Thank You!
Questions?