POLITECNICO MILANO 1863

MiMic Lab
Microfluidics and biomimetic Microsystems

BiomimX
THE BEATING ORGANS-ON-CHIP

ESTIV

ASCCT
American Society for Cellular and Computational Toxicology

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

March, 24th 2023

Erika Ferrari

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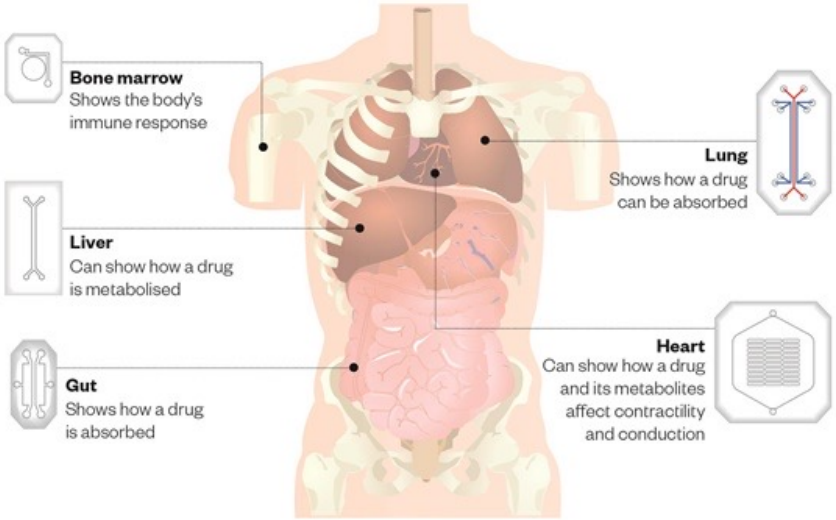
erika.ferrari@biomimx.com

Organs-on-chip

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

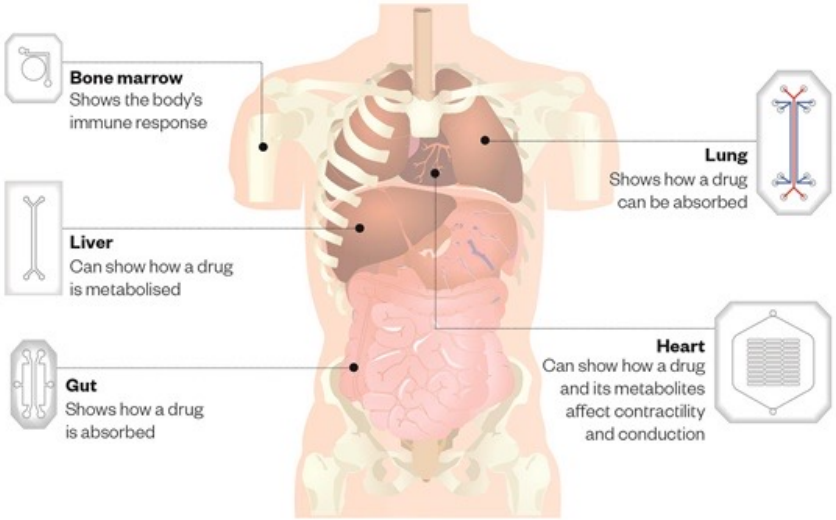


www.pharmaceutical-journal.com

Organs-on-chip

APPLICATION in the Drug Development Pipeline

- Efficacy
- Safety
- Complement/Replace animal models



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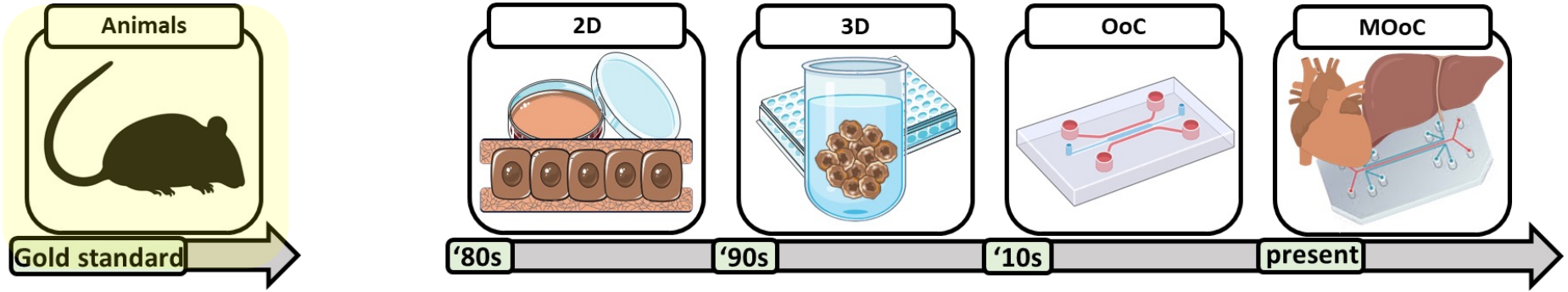
Drug Development Process - *preclinical*



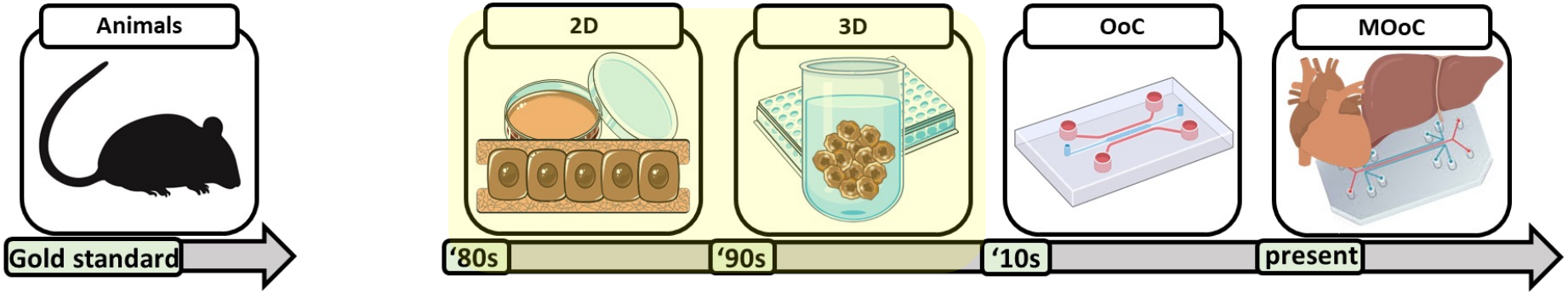
Reproduce organ complexity ✓

Differences between animals and humans ✗

High animal-to-animal variability ✗

Ethical problem ✗

Drug Development Process - *preclinical*



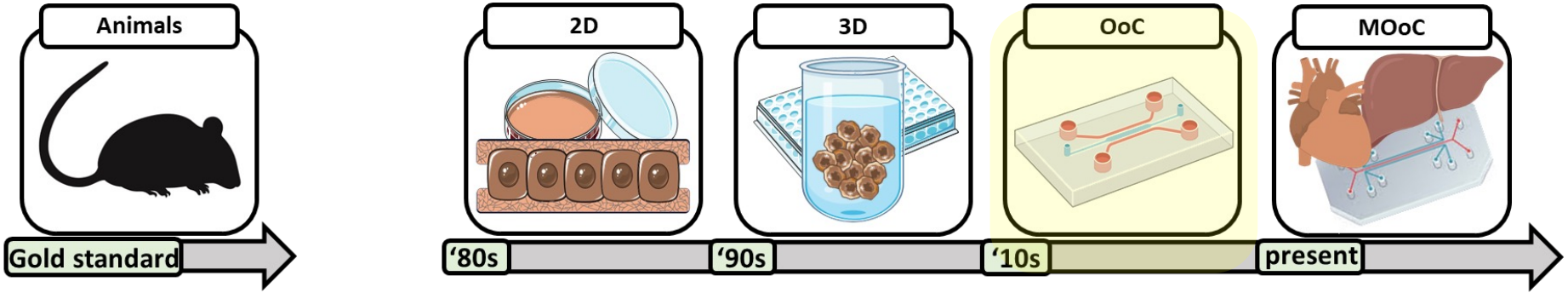
Cheap ✓

Human models ✓

Static conditions ✗

No organ complexity ✗

Drug Development Process - *preclinical*



Low costs and volume samples ✓

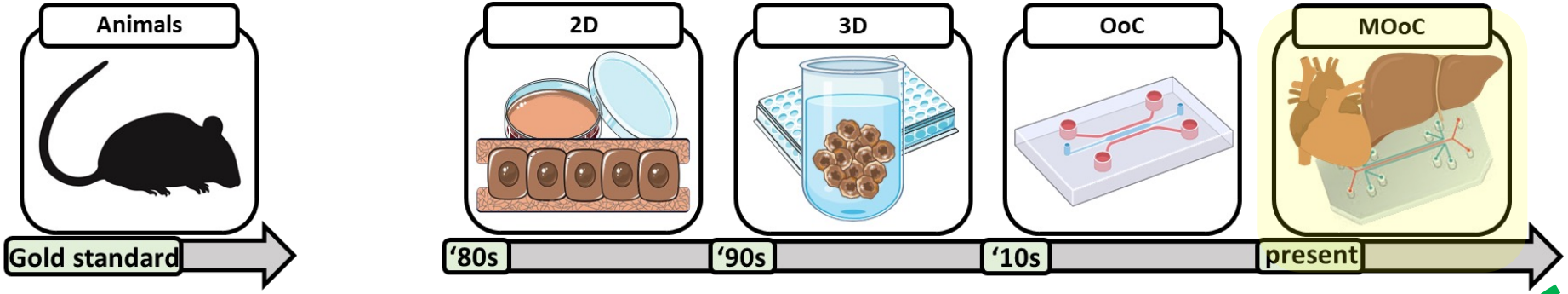
3D human models in controlled environment ✓

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

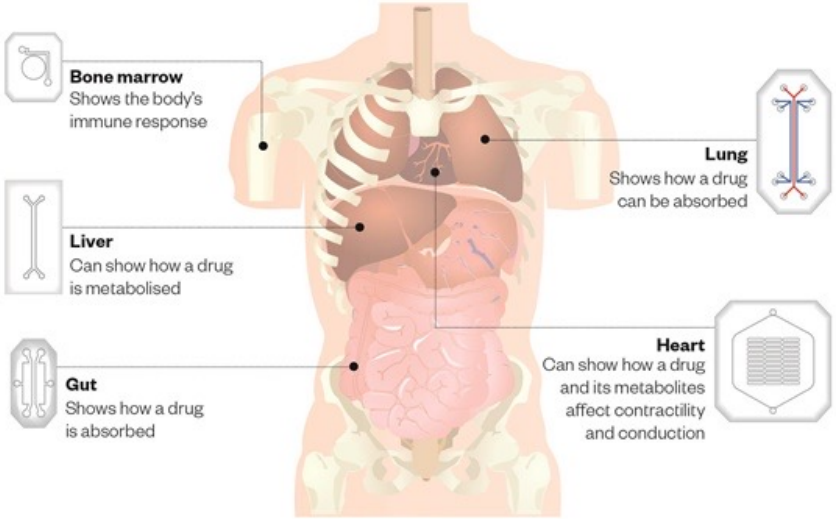
Drug safety process

APPLICATION in the Drug Development Pipeline

- Efficacy
- **Safety**
- Complement/Replace animal models

Liver

Heart

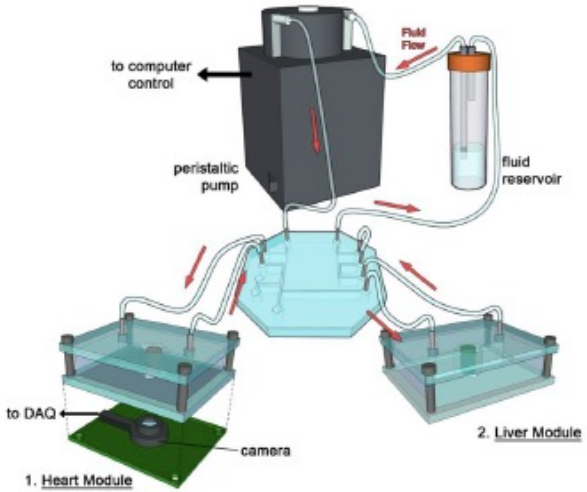


www.pharmaceutical-journal.com

" 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

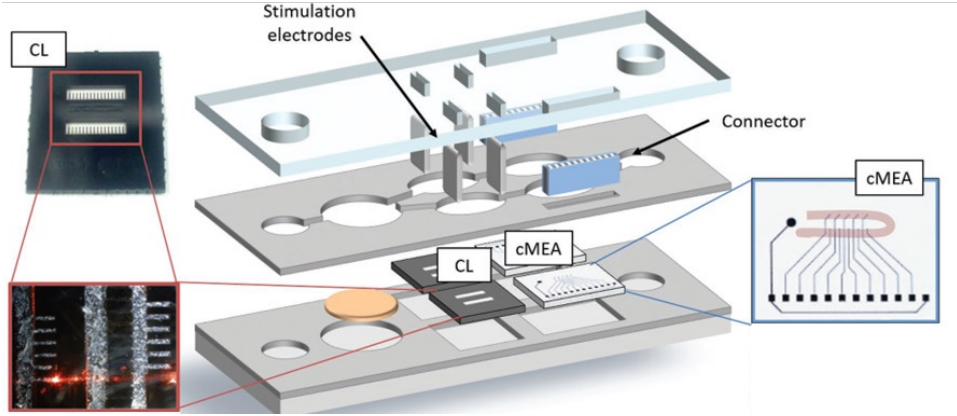


Skardal, A. *et al*. *Sci. Rep.* **7**, 1–16 (2017).

High priming volume

Monocultures

HESPEROS platform



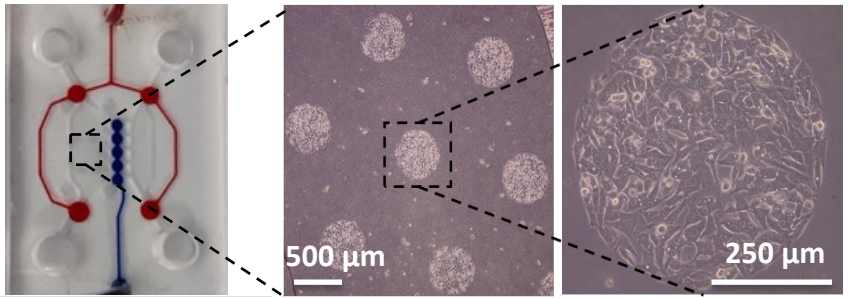
Oleaga, C. *et al*. *Biomaterials* **182**, 176–190 (2018)

2D hepato-cardiac model → low functionality

No control of organ models communication

Our Liver and Heart models

µPCC on chip



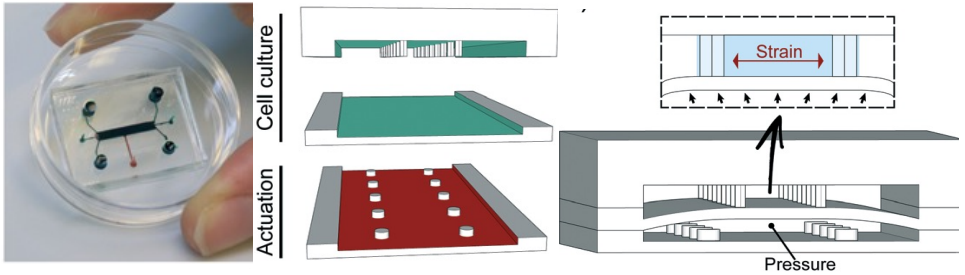
Ferrari E et al. Biomed Mater. 2021 Jun 7;16(4)

2D hepatic model → HepG2 + 3T3 fibroblasts

→ Optimized for microfluidic platforms

→ Validated for **metabolism**

Beating heart on chip



Marsano, A. *et al*. *Lab Chip* **16**, 599–610 (2016)
Visone, R. et al. *Biofabrication* (2021)

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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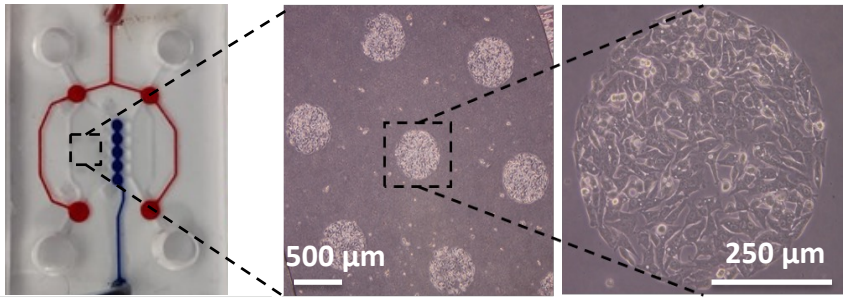

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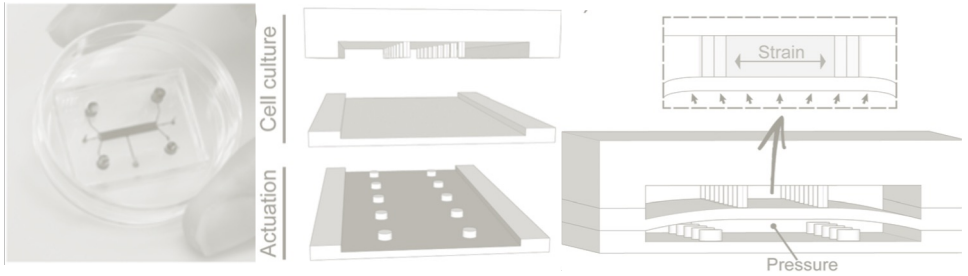

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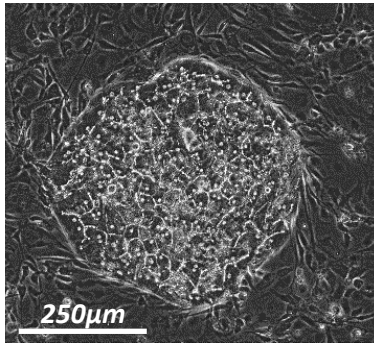
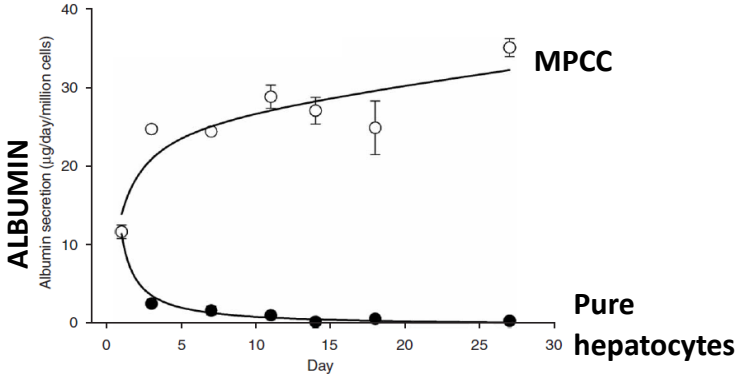


MTM lab, Ferrari E. Thesis (2018)

STARTING POINT

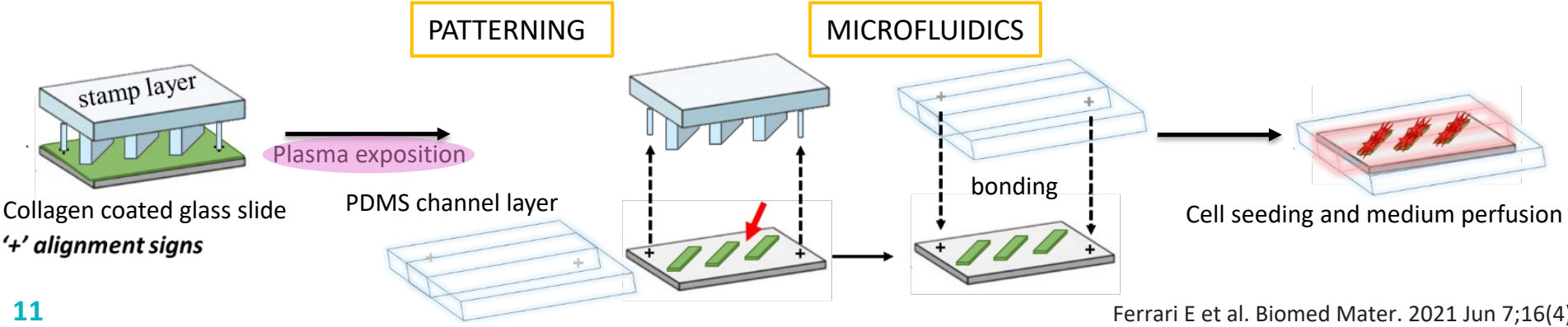
Micropatterned co-cultures (MPCCs) of hepatocytes and 3T3 fibroblasts

→ highest production of albumin;



Khetani, S.R. & Bhatia, S.N. *Nat. Biotechnol.* **26**, 120–126 (2008)

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



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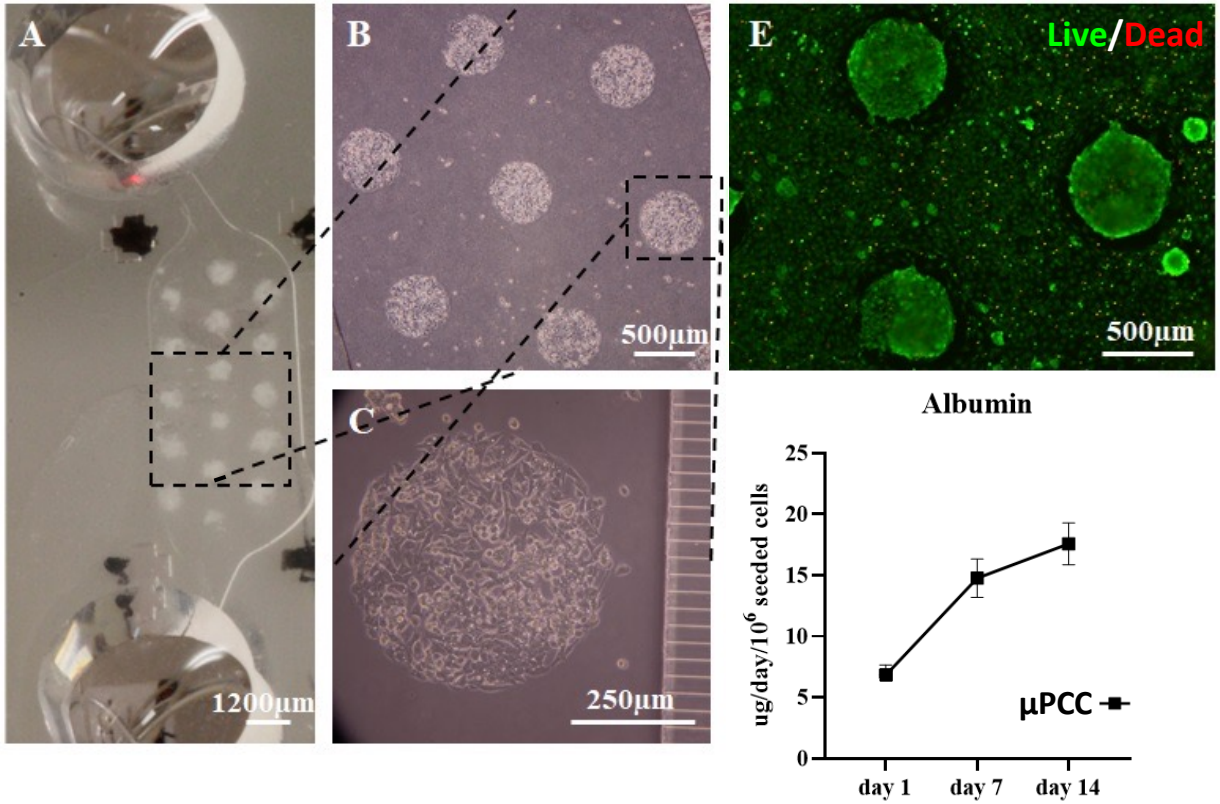
µPCC on chip

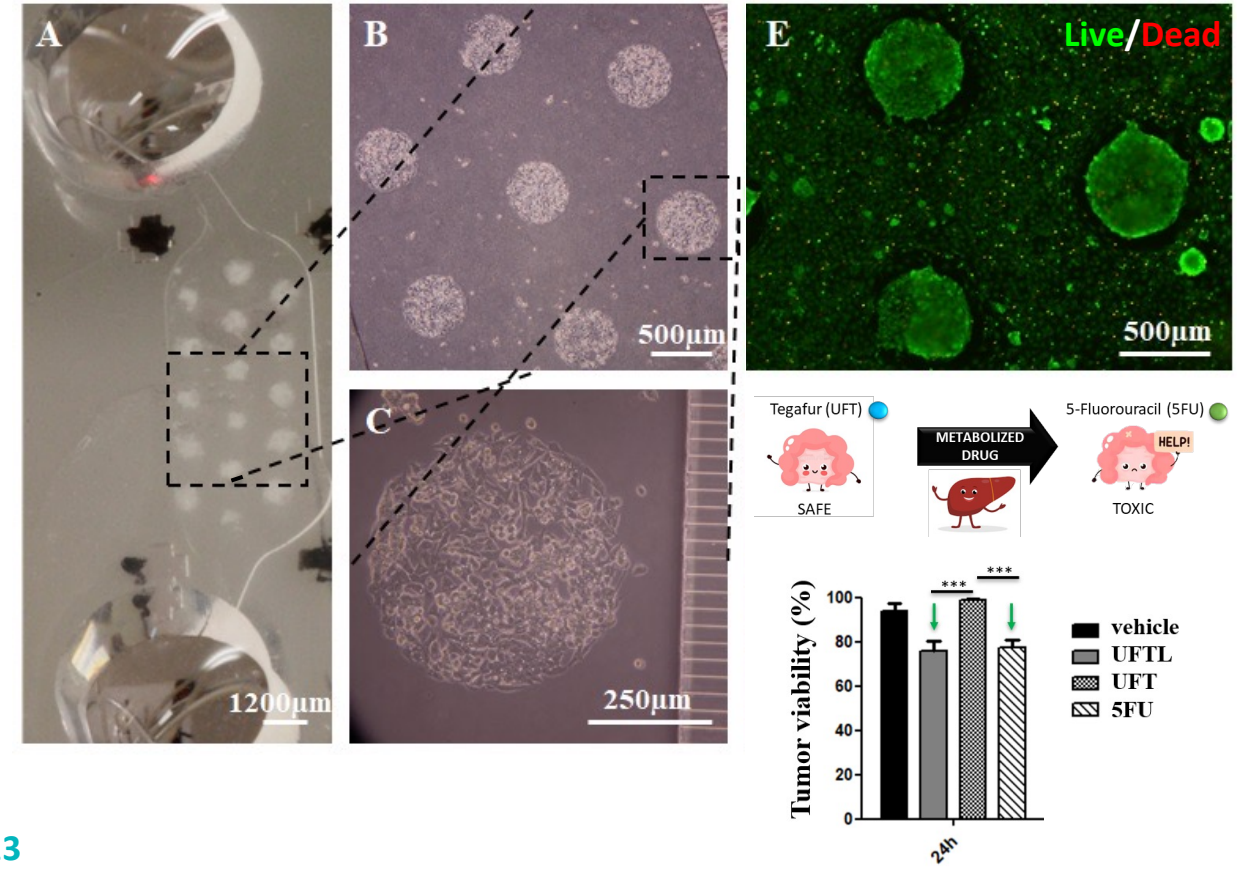


- HepG2 seeding and attachment
- 3T3 fibroblasts seeding to generate µPCCs
- Viability > 83% after 7 days of culture
- Good albumin production

ACCELERA
PART OF NMS GROUP

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

µPCC on chip



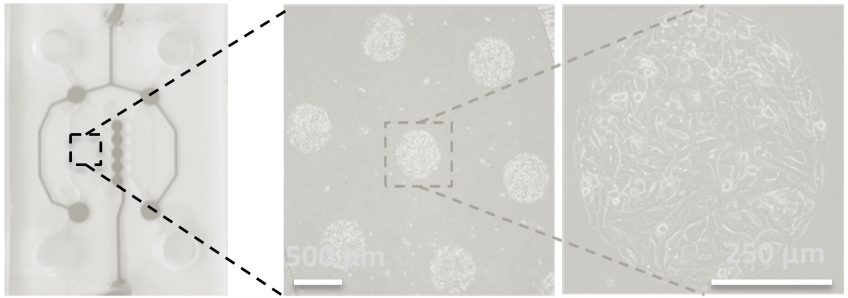
- HepG2 seeding and attachment
- 3T3 fibroblasts seeding to generate µPCCs
- Viability > 83% after 7 days of culture
- Good albumin production
- Capable of Tegafur metabolism

ACCELERA
PART OF NMS GROUP

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

Our Liver and Heart models

µPCC on chip



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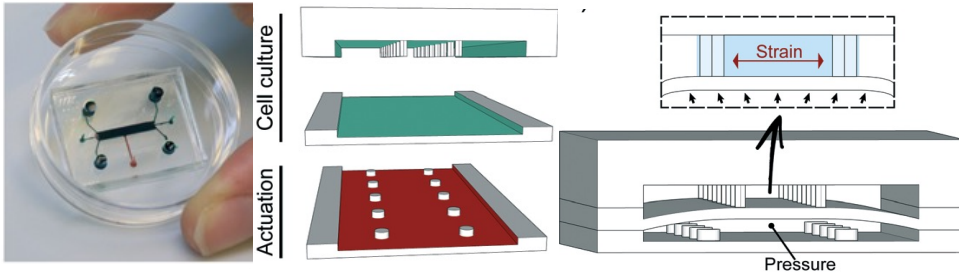

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3D cardiac model → nRCM embedded in a fibrin gel

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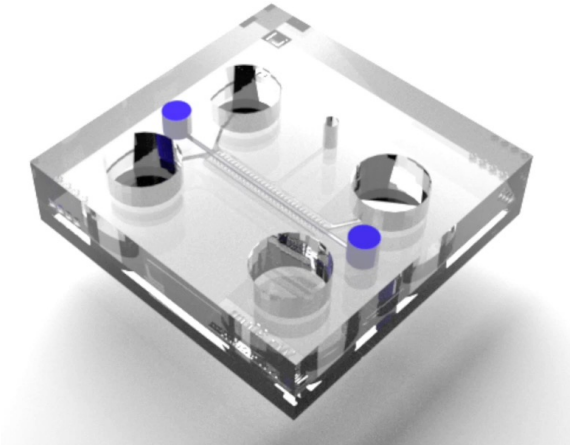
Beating heart on chip

uBeat® Technology (EP3289065B1)

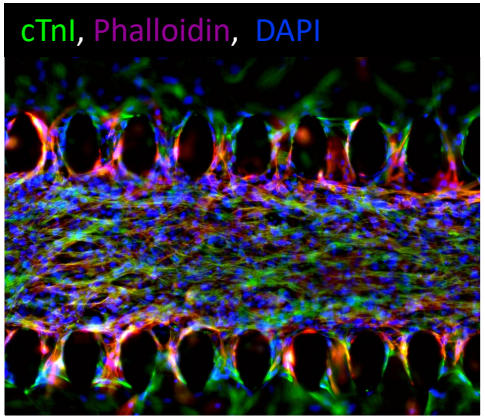


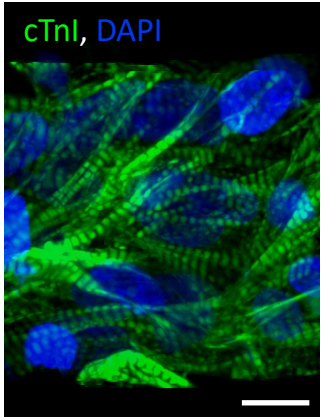
Mechanical stimulation

(uniaxial stretching 10-15% strain)

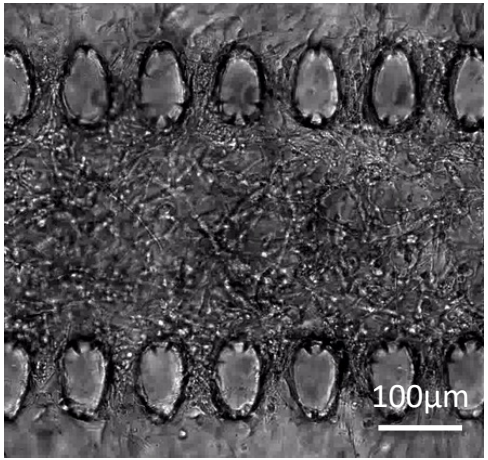
- Functional 3D constructs

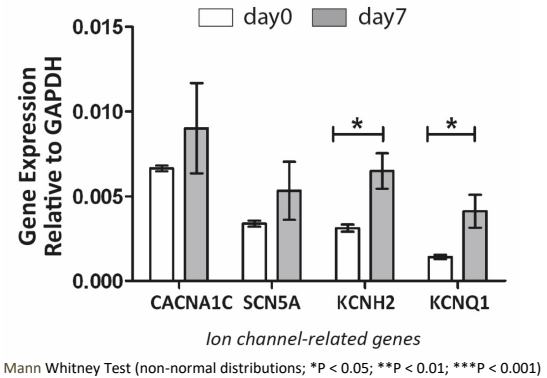
IF





Synchronously beating construct



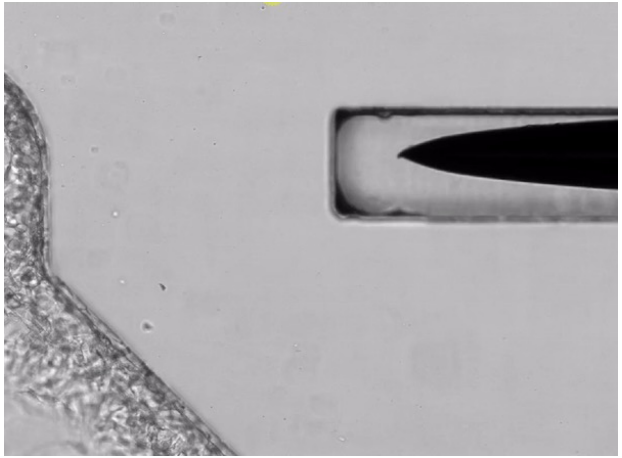


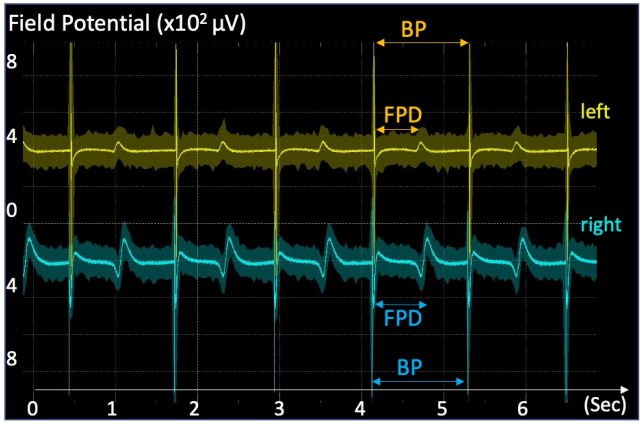
Mann Whitney Test (non-normal distributions; *P < 0.05; **P < 0.01; ***P < 0.001)

Marsano A. et al. Lab Chip. 2016; Visone R. et al. Biofabrication 2021

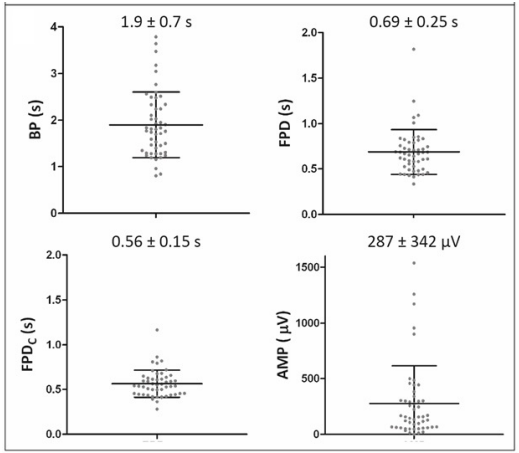
Beating heart on chip

µECG Technology (EP3620508A1 – Granted UIBM, EPO)





Electrophysiological parameters



Means ± SD
n=51

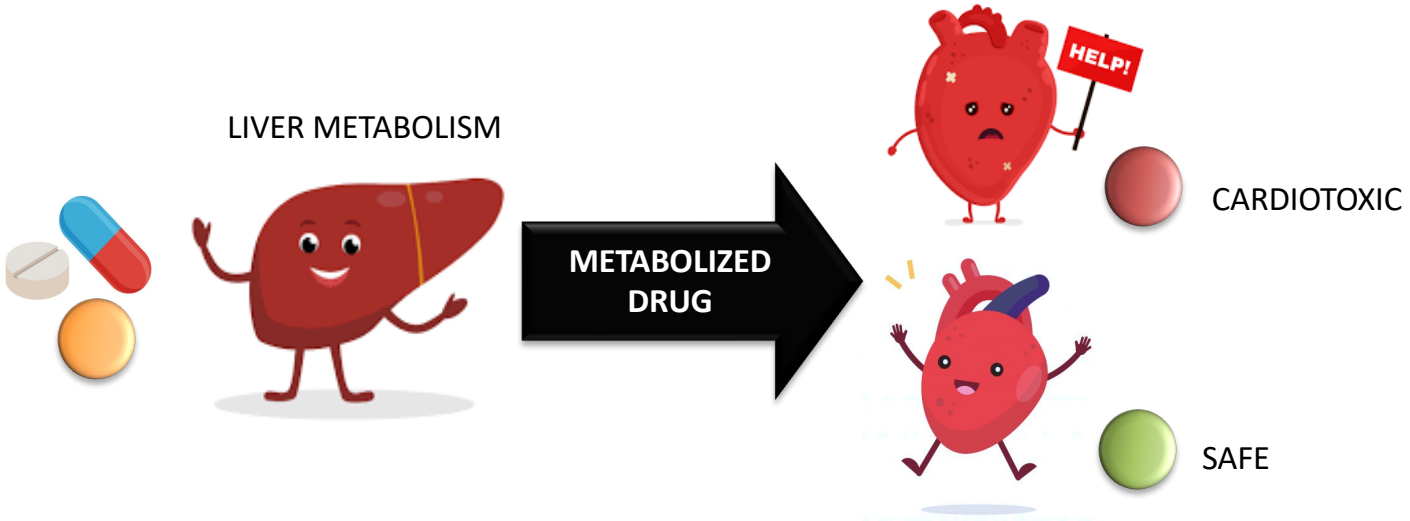
Positioning of electrodes for:

- Electrical activity recording (field potential)
- Electrical stimulation (uniform electric field)

Visone R. et al. Biofabrication 2021

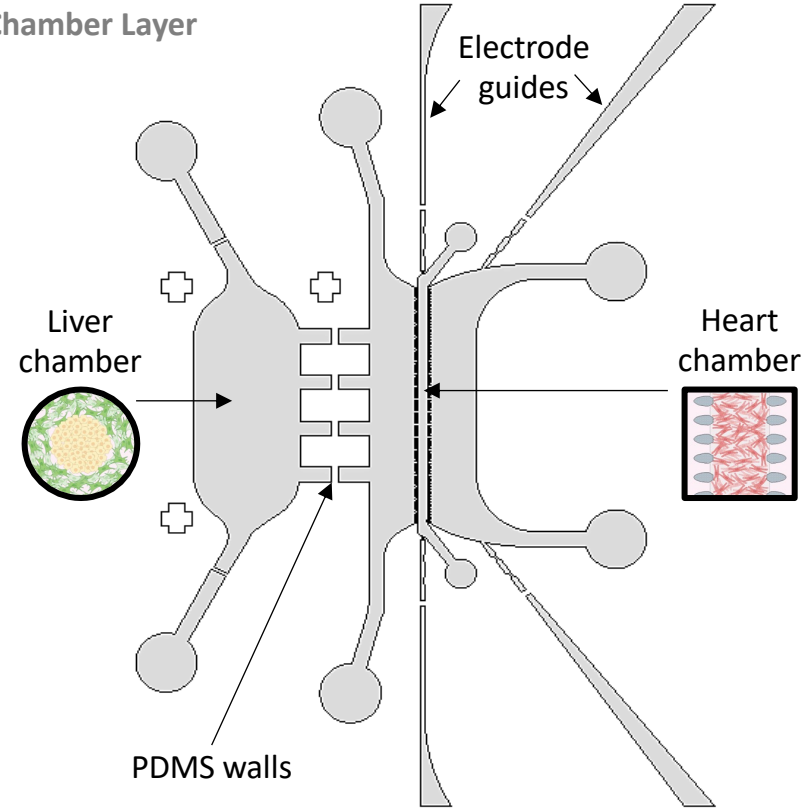
Liver-Heart on chip

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



Ferrari E, Visone R, et al. *Adv Mat Tech (2023)*

LivHeart platform



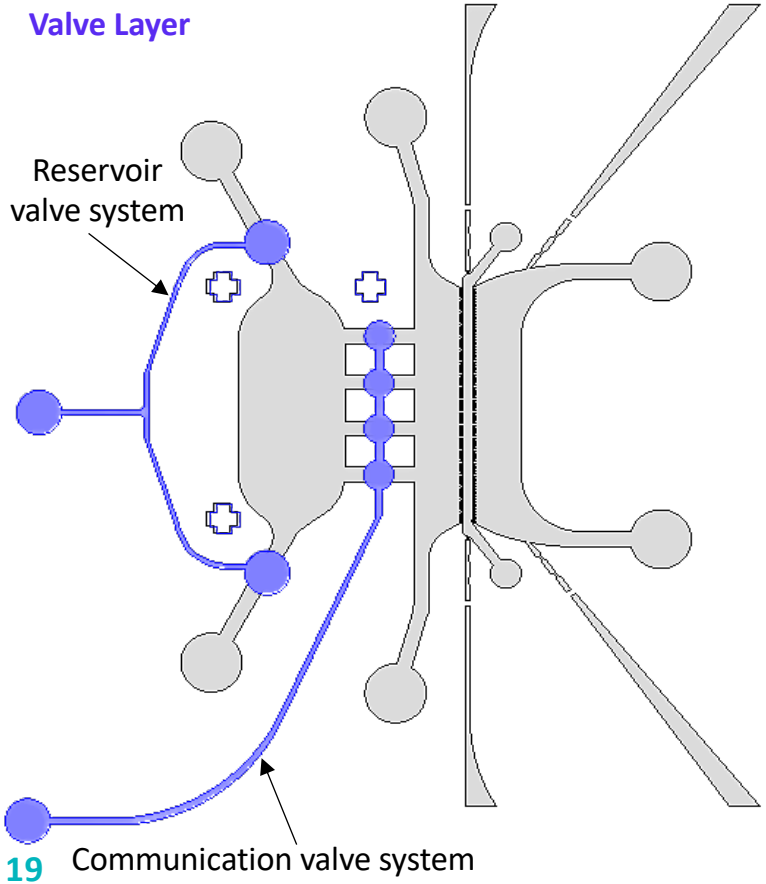
In collaboration with BiomimX
THE BEATING ORGANS-ON-CHIP
µECG *(patented EP3620508A1)*

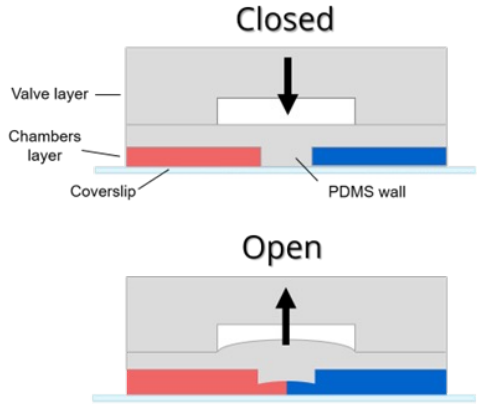
Produced via PHOTO (@ PoliFAB) and SOFT (@ MiMic Lab) Lithographic techniques

Ferrari E, Visone R, et al. *Adv Mat Tech (2023)*

LivHeart platform

In collaboration with BiomimX THE BEATING ORGANS-ON-CHIP





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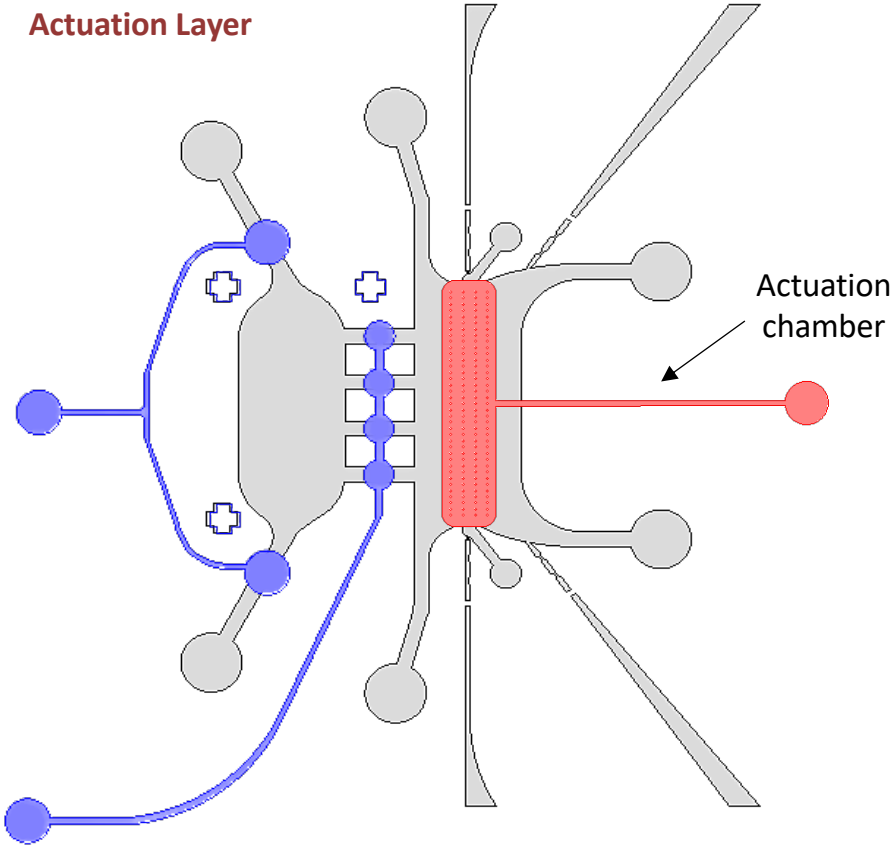
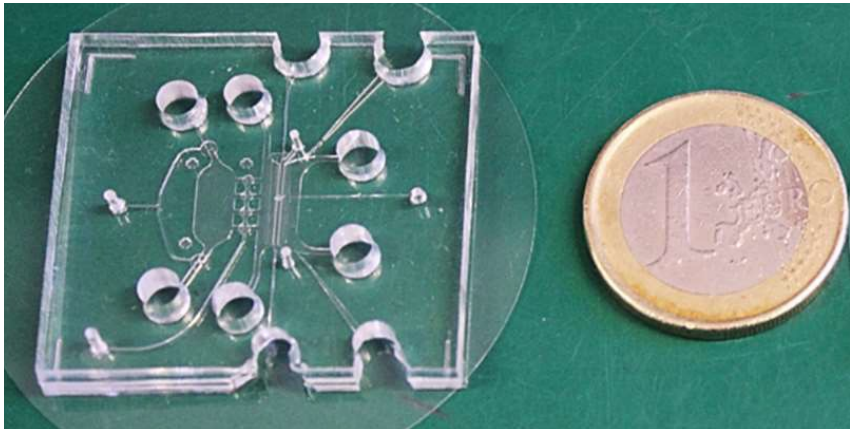


In collaboration with BiomimX THE BEATING ORGANS-ON-CHIP



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

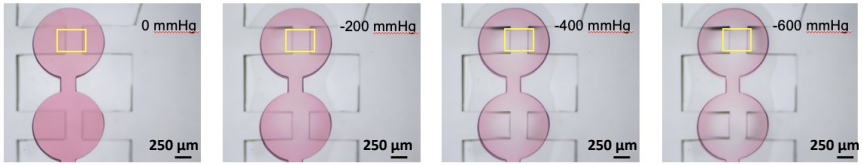
Ferrari E, Visone R, et al. *Adv Mat Tech (2023)*

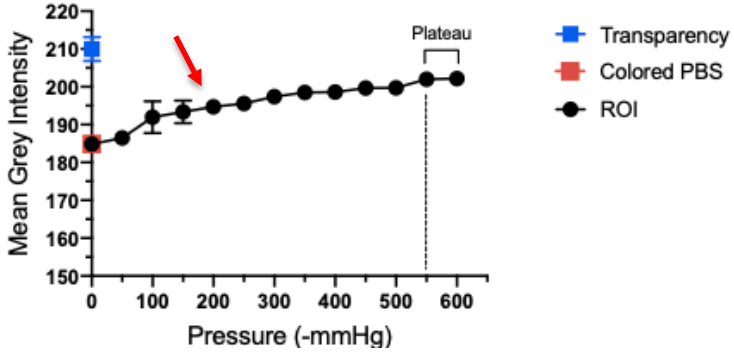
LivHeart platform - technical results

Valve operating pressure

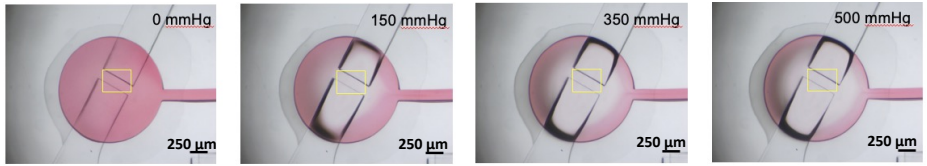
- Colored PBS → communication and reservoirs valves
- Application of decreasing pressure values

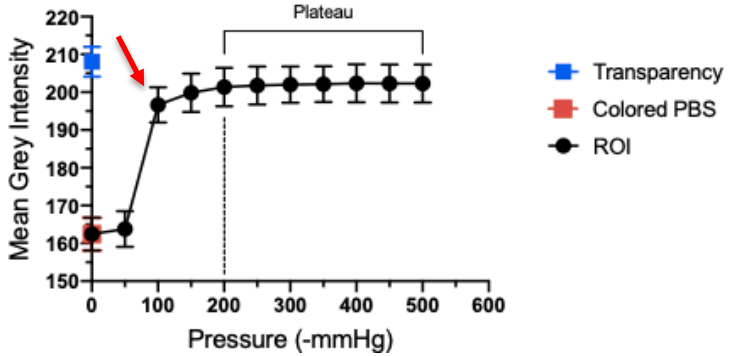
Communication Valves





Reservoirs Valves



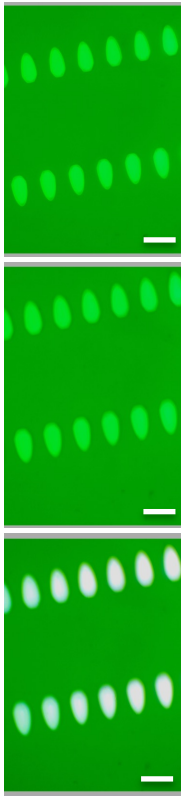


N=4

Ferrari E, Visone R, et al. *Adv Mat Tech (under review)*

LivHeart platform - technical results

Actuation operating pressure - stretching



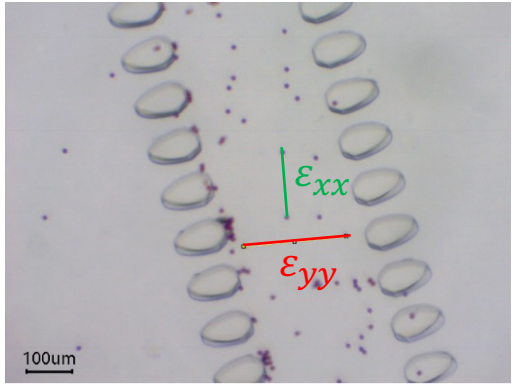
P= 0 mmHg

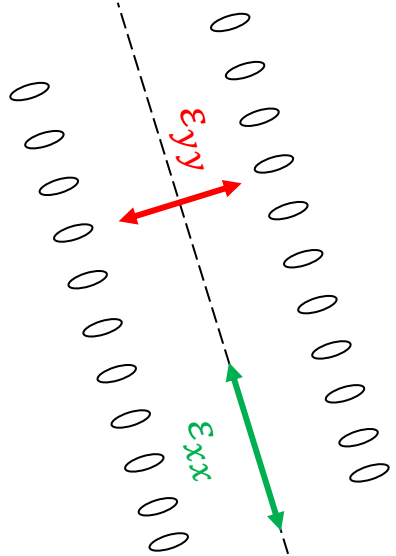
P= 300 mmHg

P= 500 mmHg

Scale bars=100µm

P= 0 mmHg - P= 500 mmHg





$$\varepsilon_{yy} = \frac{\Delta y(P500) - \Delta y(P0)}{\Delta y(P0)} = 0{,}11$$

10-15% physiological uniaxial strain

Marsano et al., 2016

$$\varepsilon_{xx} = \frac{\Delta x(P500) - \Delta x(P0)}{\Delta x(P0)} = 0{,}03$$

Ferrari E, Visone R, et al. *Adv Mat Tech (2023)*

LivHeart platform - technical results

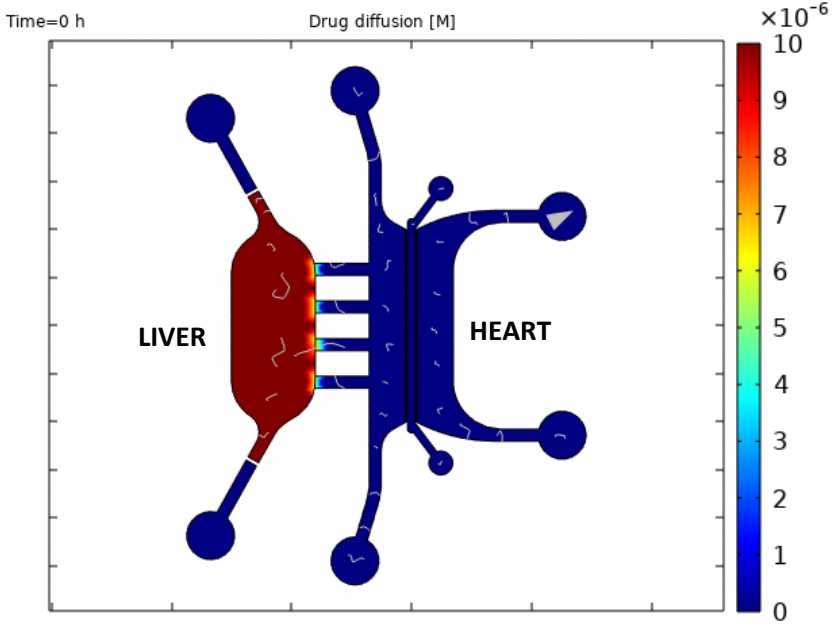
Diffusion characterization - numerical

- 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

  *ŋ DMEM w/10% FBS: 9,4e10-4 Pa*s*

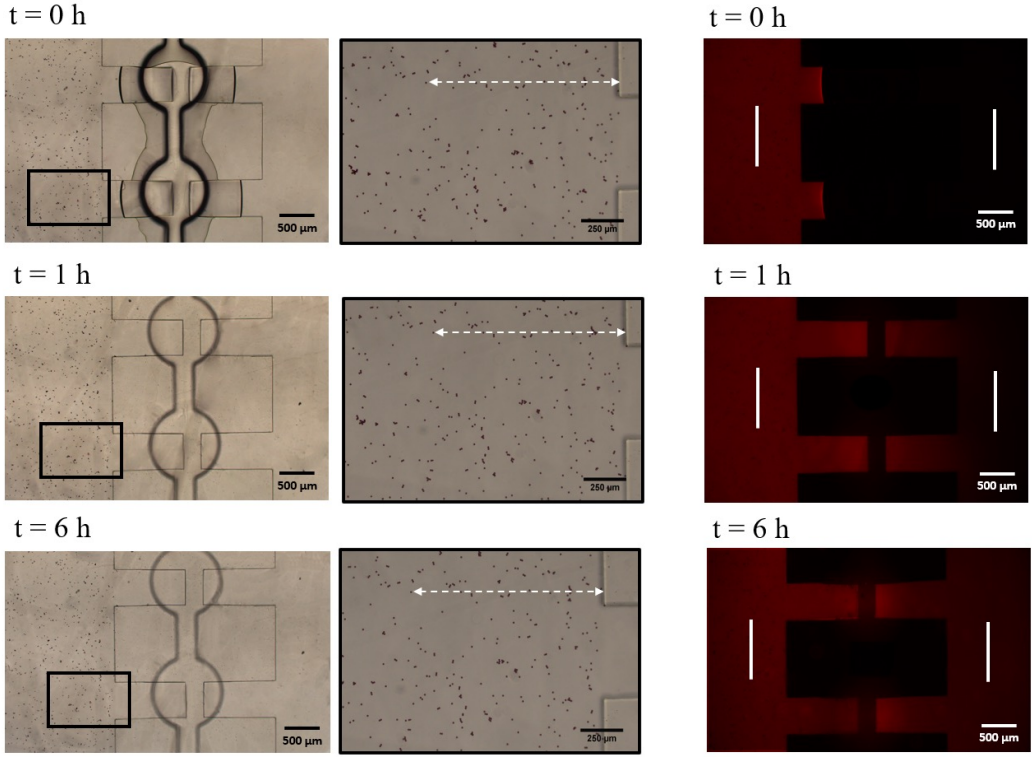
- t = [0, 48] h & time step = 1 h



	0 h	12h	24h	36h	48h
Concentration value liver [μM]	10	5.83	4.48	3.85	3.53
Concentration value cardiac channel [μM]	0	2.41	2.92	3.09	3.15

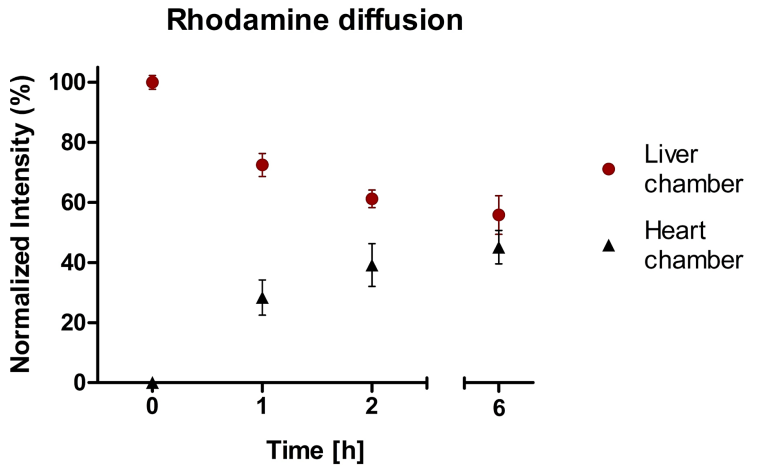
LivHeart platform - technical results

Diffusion characterization - experimental



N=4

Rhodamine MW=479g/mol
Terfenadine MW=472g/mol
Fexofenadine MW=502g/mol



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

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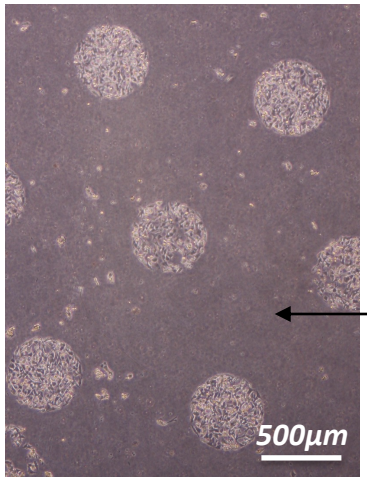
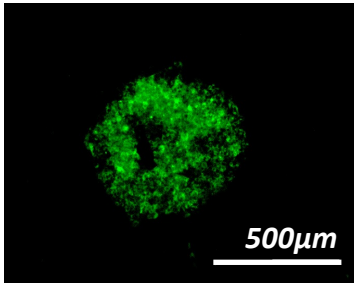
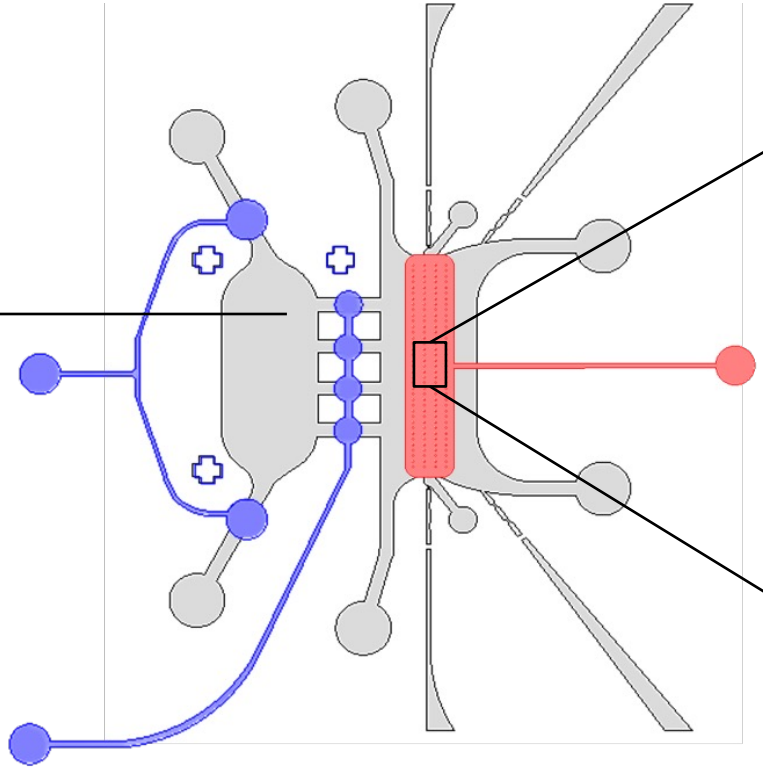
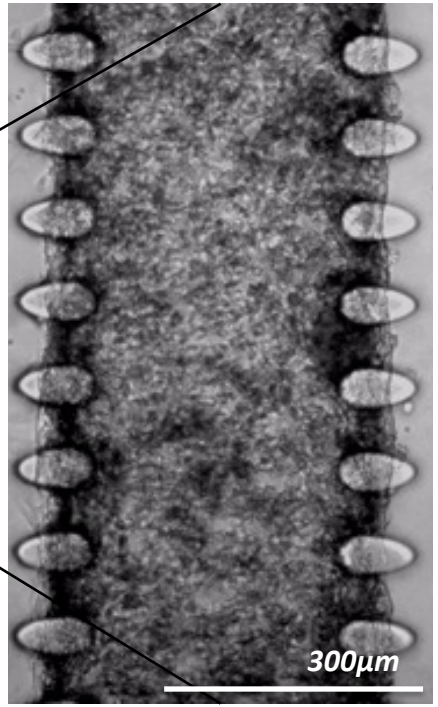


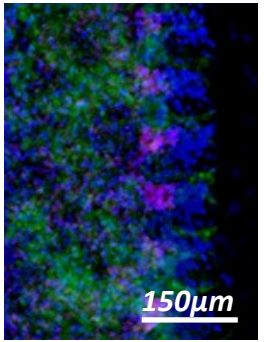


Cytoplasmic Albumin





Troponin I
Cx43 DAPI



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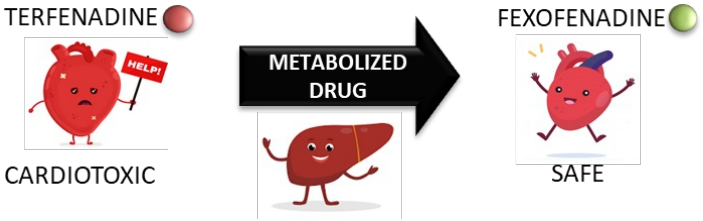
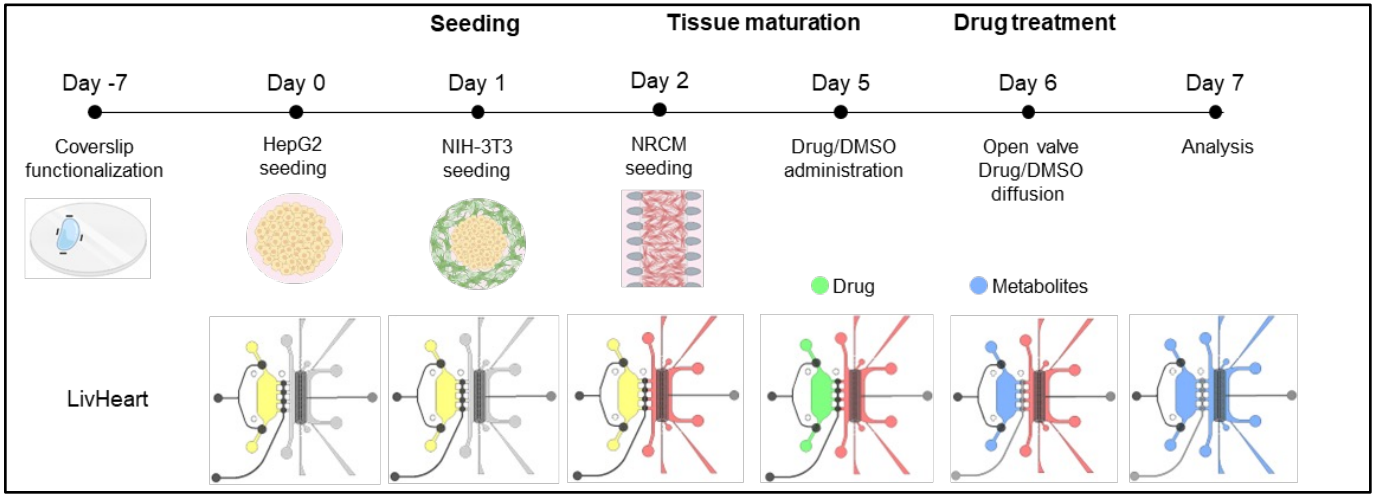
Model validation with Terfenadine



" 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"



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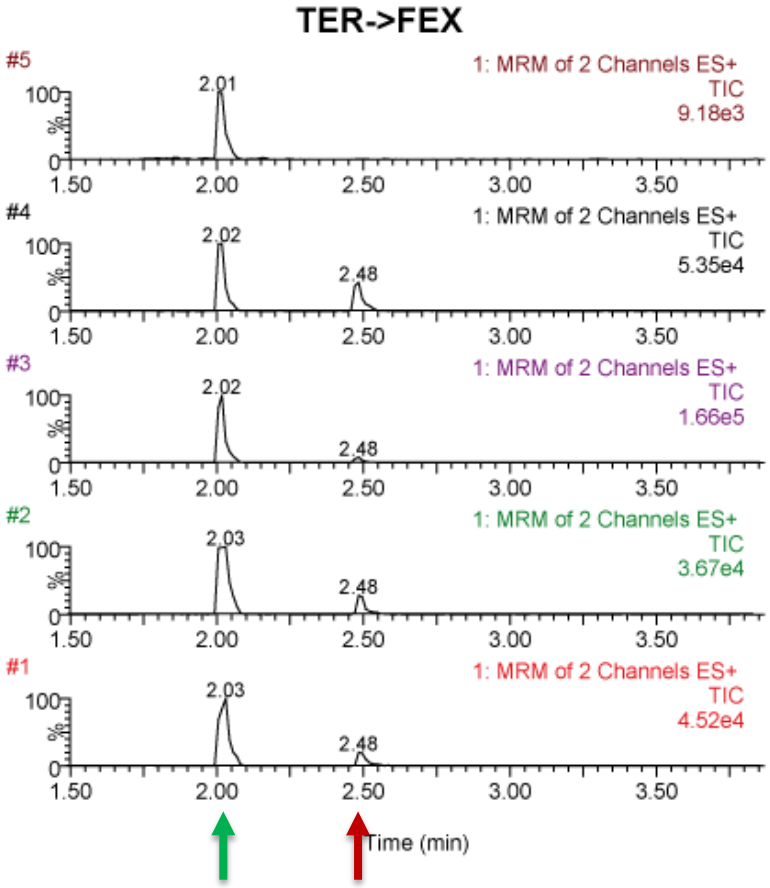
Mass Spectrometry



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

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

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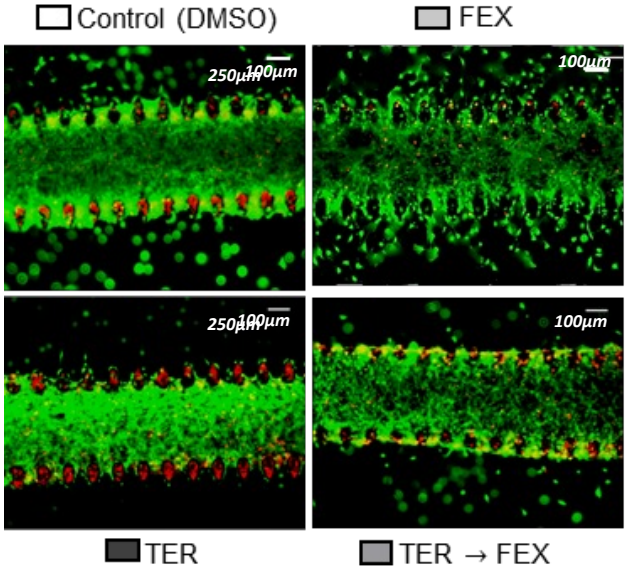
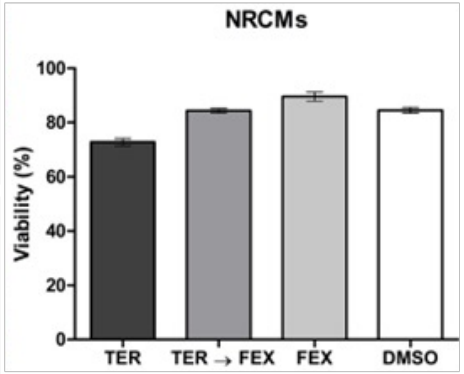
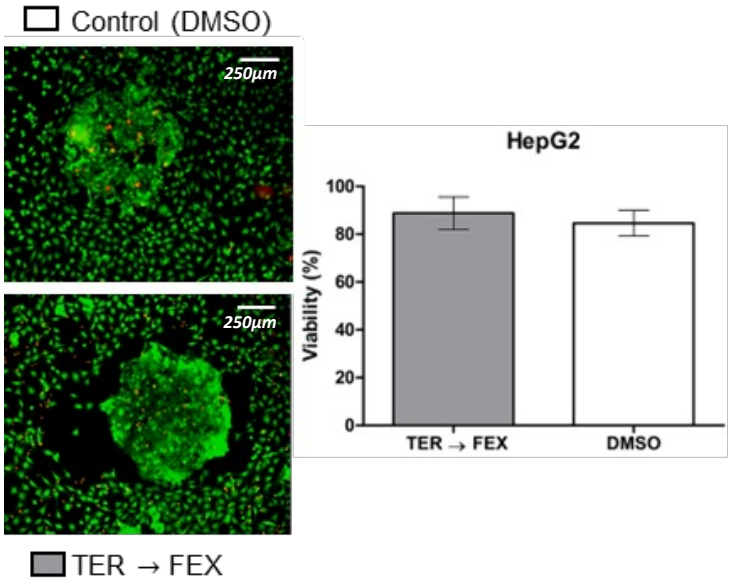
Live/**Dead**



VIABILITY RESULTS





Liver metabolized Terfenadine (**TER→FEX**) condition follows the **trend of the control** compared to when Terfenadine (TER) is directly administered on the heart

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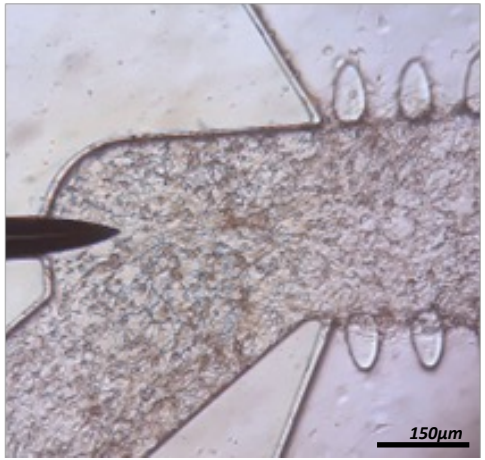
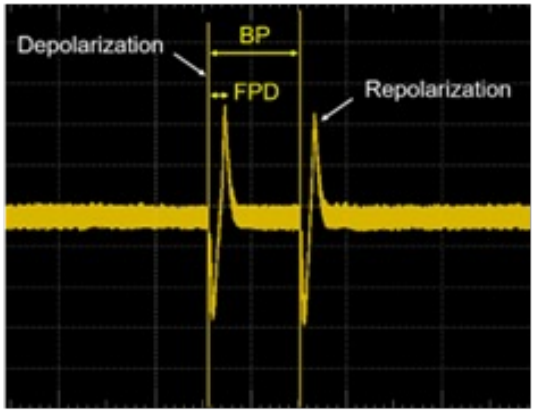
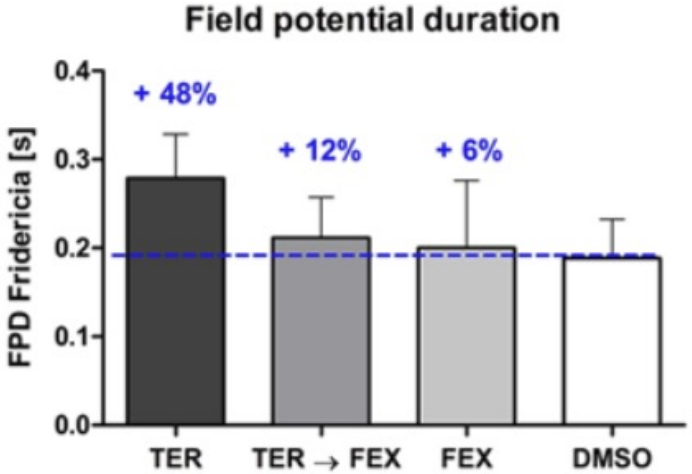
FUNCTIONALITY RESULTS







The LivHeart allowed the testing of a non-cardiotoxic metabolite generated from a cardiotoxic drug

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LivHeart

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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ESTIV

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Thank You!

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Questions?