



Encapsulated spheroids applications

Dr. Jérôme Caron, R&D & Operations Manager

CONTEXT: THE CRUCIAL NEED FOR NEW PREDICTIVE CELL MODELS

➔ Towards the end of animal experimentation?

Predictivity issues

A lot of drugs failed in clinical trials



Why animal studies are often poor predictors of human reactions to exposure

Michael B Bracken J R Soc Med 2008; 101: 120-122. DOI 10.1258/jram.2008.08k033

Limitations of Animal Studies for Predicting Toxicity in Clinical Trials

Is it Time to Rethink Our Current Approach?

Gail A. Van Norman, MD

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 4, NO. 7, 2019
NOVEMBER 2019:845-54

nature

Misleading mouse studies waste medical resources

Erika Check Hayden

26 March 2014

Science 10 JAN 2023 • 5:30 PM • BY MEREDITH WADMAN

FDA no longer needs to require animal tests before human drug trials

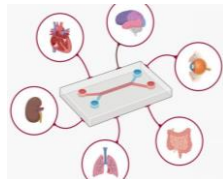
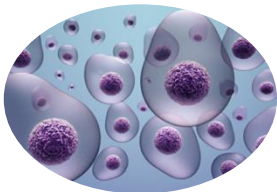
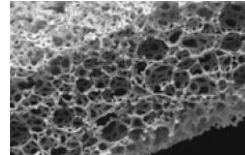
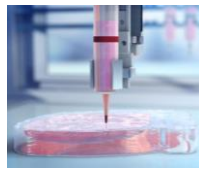
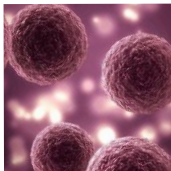
Ethical concerns and 3Rs reglementation

EU already banned animal testing for cosmetics

➔ Going faster and further in development & therapy

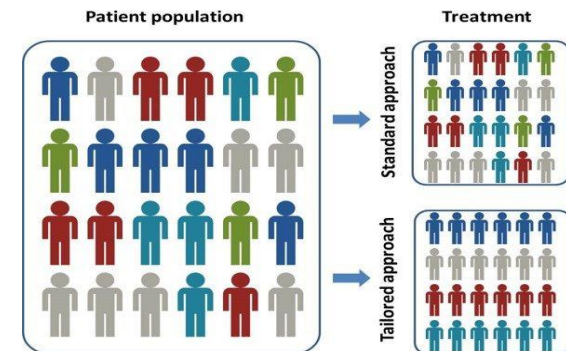
Complex solutions : Cells & technology

Closer and closer to organs



For precision and personalized medicine

Quickly find the right treatment



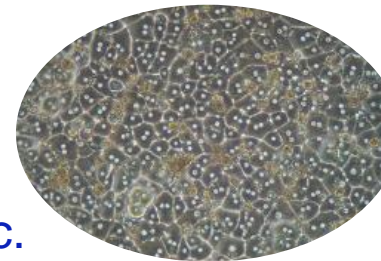
LIMITATIONS OF 2D CELL CULTURE

→ Some **advantages**

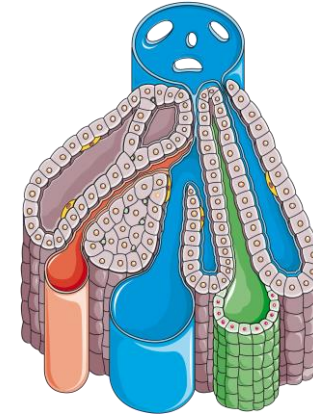
- Cheap: low-cost maintenance
- Widely used, well-known and user friendly

→ But **non-physiological**

- Do not mimic the real organs
- Limited cell-to-cell contact surfaces
- Poor cell organization, polarization, etc.
- Higher drug sensitivity



Hepatocytes monolayer



Liver lobule

→ **Challenging for mass production**

- Huge surfaces cultures: CellStack, large surface flasks, etc.
- Numerous incubators
- Spoil of culture media, time and energy

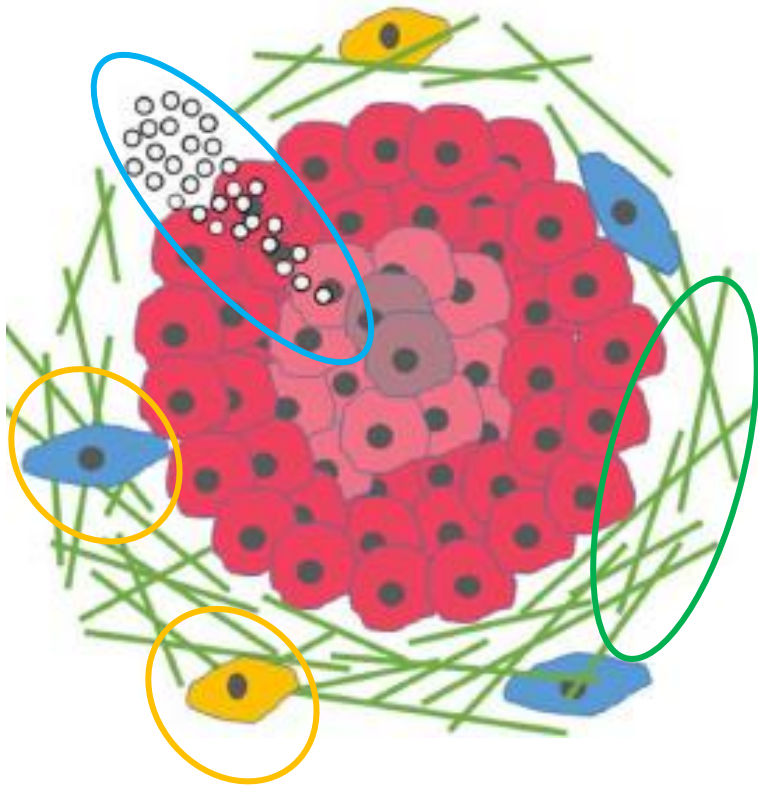


Add a dimension to be **closer to *in vivo***

Kapałczyńska M *et al.*, 2018, *Arch Med Sci*
Jensen C and Teng Y, 2020, *Front. Mol. Biosci*

3D CELL CULTURE

→ Mimic *in vitro* what is happening *in vivo*



- **Multiple cell types**
 - Homo- and heterotypic cell interaction
 - Paracrine signaling
- **Extra-cellular matrix**
 - Composition
 - Stiffness
 - Attachment to cells
- **Diffusion gradients**
 - O₂
 - Nutrient & soluble factors
 - Proliferation

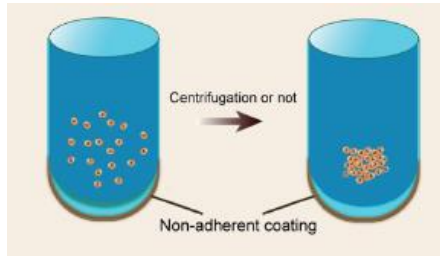
Observe cell phenotype, drug response, etc.

Adapted from Langhans SA, 2018, [Front. Pharmacol.](#)

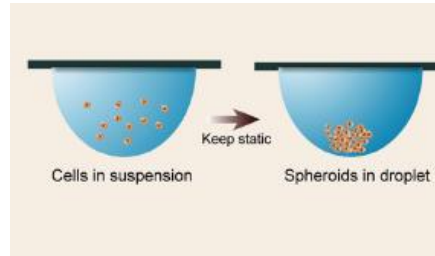
DIFFERENT METHODS OF 3D CELL CULTURE

→ Scaffold-free (or floating) methods

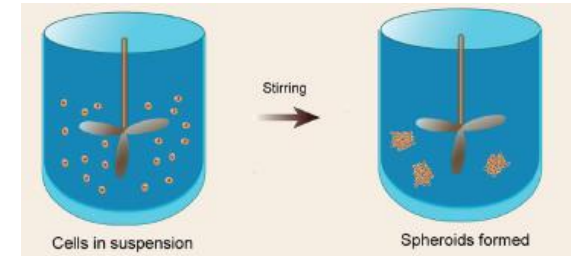
Non-attachment surfaces



Hanging drop



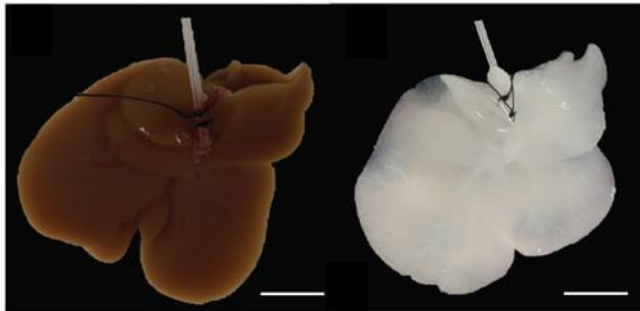
Stirring



He J *et al.*, 2017, [Oncotarget](#)

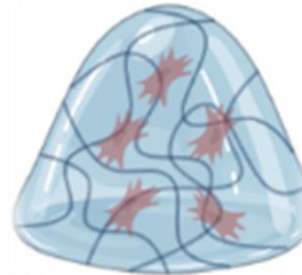
→ Scaffold-based methods

Decellularized organs

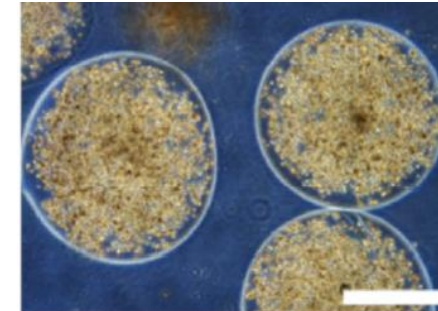


Uygun BE *et al.*, 2010, [Nat. Med](#)

Embedding in a matrix



Encapsulation in hydrogel beads

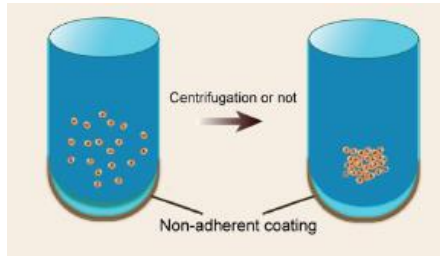


Pasqua M *et al.*, 2020, [Biotechnol. Bioeng.](#)

DIFFERENT METHODS OF 3D CELL CULTURE: LIMITATIONS

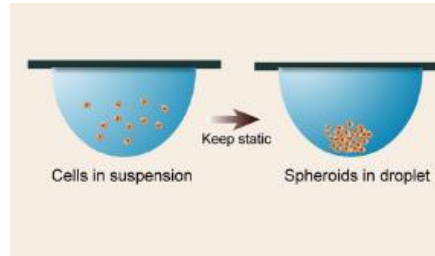
→ Scaffold-free (or floating) methods

Non-attachment surfaces



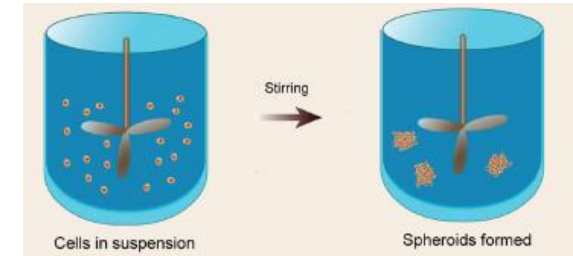
Low throughput

Hanging drop



1 spheroid per well

Stirring

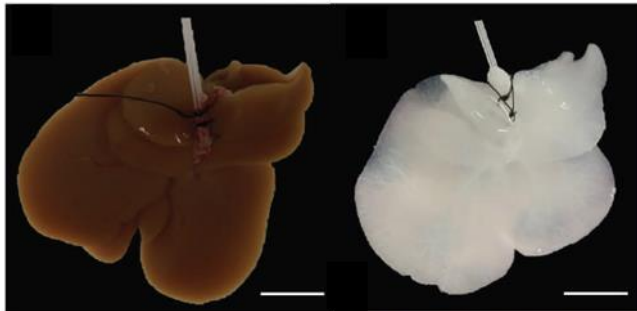


Shear stress

He J *et al.*, 2017, [Oncotarget](#)

→ Scaffold-based methods

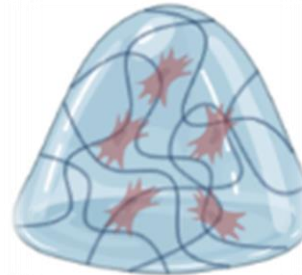
Decellularized organs



Scarcity

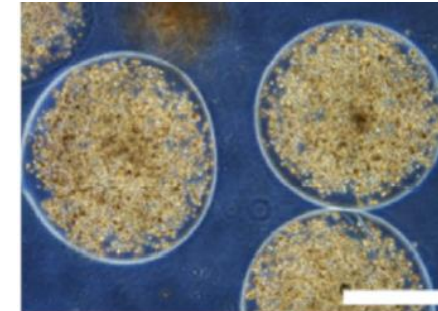
Uygun BE *et al.*, 2010, [Nat. Med](#)

Embedding in a matrix



Diffusion problems

Encapsulation in hydrogel beads



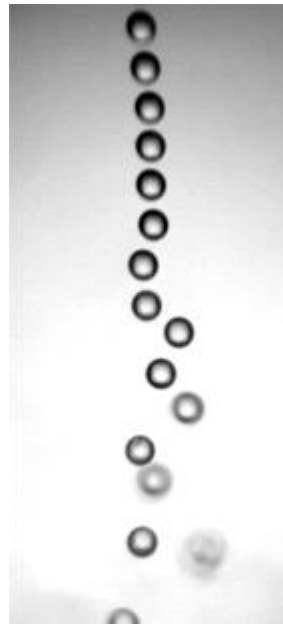
No self-organization

Pasqua M *et al.*, 2020, [Biotechnol. Bioeng.](#)

CYPRIO'S ENCAPSULATION TECHNOLOGY



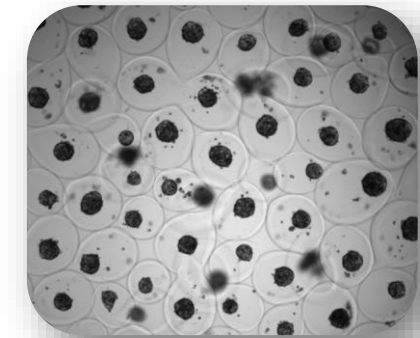
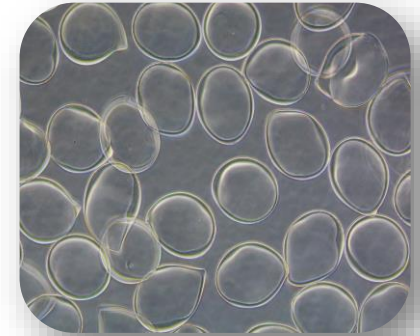
Co-extrusion
of cells and alginate



Generation of
uniform droplets



Reticulation of
alginate hydrogel

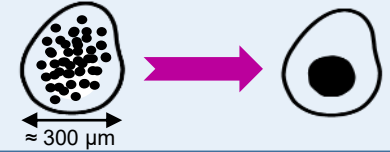


Liquid-core capsules

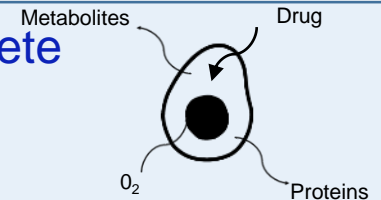
→ **Proprietary** technology protected by a family of 4 patents

ADVANTAGES OF ENCAPSULATING CELLS

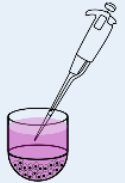
1. Micro-compartmentalization: our technology promotes spheroid formation as this **increases probability of gathering**



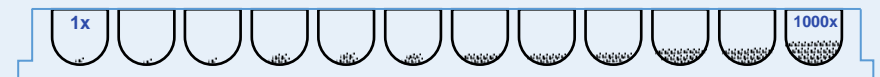
2. Physiological environment: the **capsule porosity** ensures a complete exchange of oxygen, nutriment, growth factors, proteins & small molecules between external and internal media



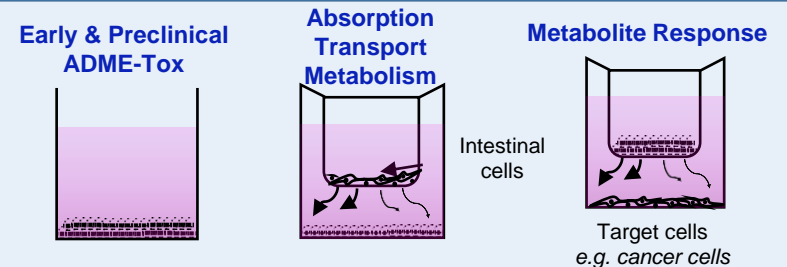
3. **Easy handling & Spheroid Protection**: supernatant media may be aspirated easily while the capsule protect organoids from aspiration and any shear stress



4. **Flexibility**: the alginate barrier allows the sorting of an accurate number of spheroids per well with no risk of fusion and no need for pooling wells

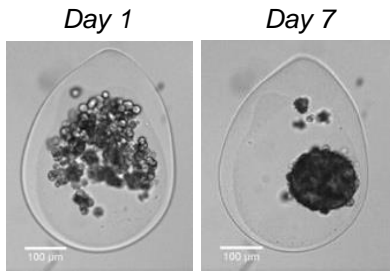


5. Versatility: Integration to diverse experimental plans like **Drug Discovery and Development**

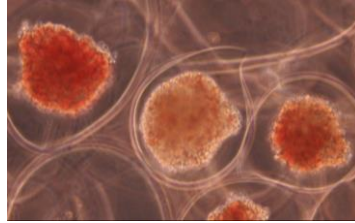


A TECHNOLOGY FOR ALL TYPES OF CELLS & COMPLEX SYSTEMS

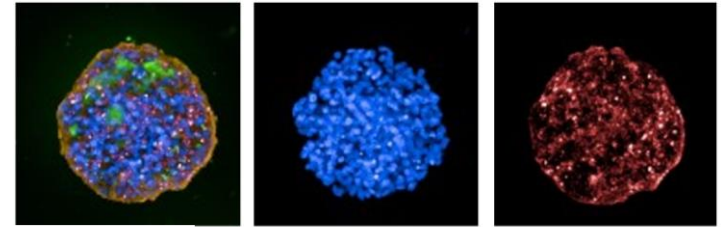
→ HepatoPearls®



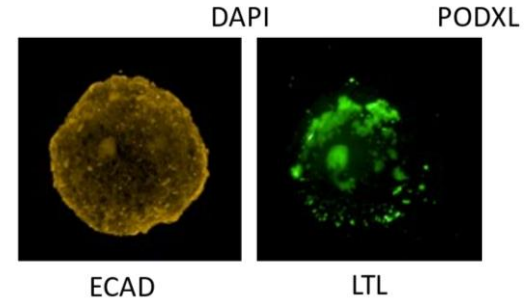
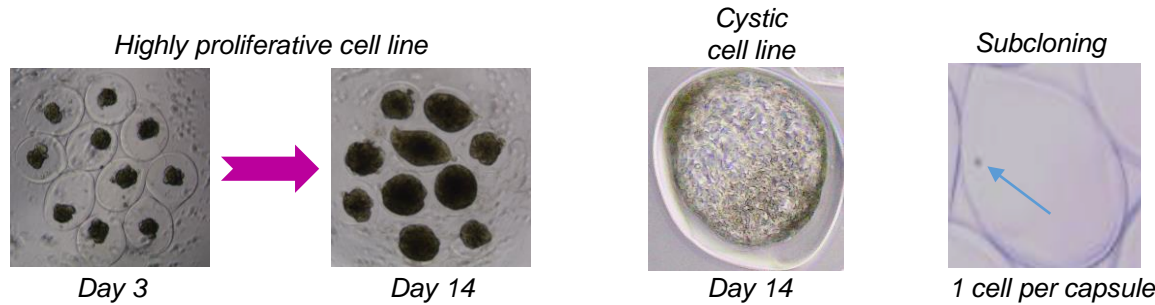
→ Hematopoietic stem cells



→ Kidney-derived iPSCs

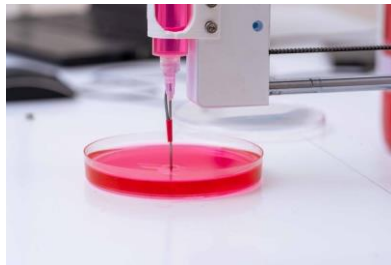


→ Cancerous cell lines

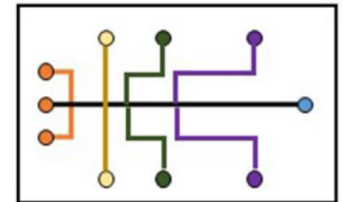


Classical bioassays & integration to specific devices

→ Bioinks



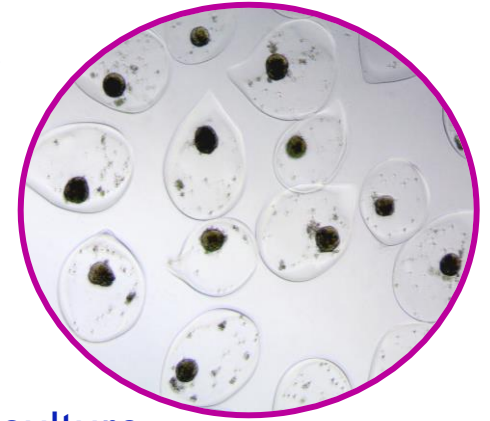
→ Microphysiological systems



HEPATOPEARLS®: ENCAPSULATED LIVER SPHEROIDS

HEPATOPEARLS: A NOVEL LIVER MODEL FOR LONG TERM STUDIES

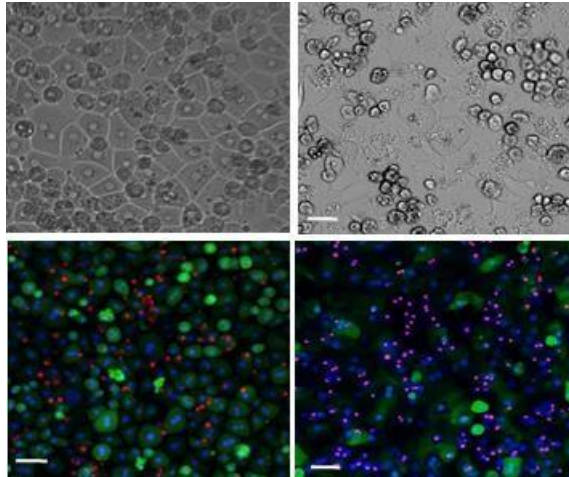
- Primary human hepatocyte spheroids using Cyprio technology
- Spheroids protected with an **alginate shell**
- **Size-controlled** organoids
- **No necrotic core**
- Viability of PHHs **up to 45 days** versus 2 weeks maximum in 2D culture



2D Hepatocytes

Day 1

Day 3



Nucleus – Live cells – Dead cells

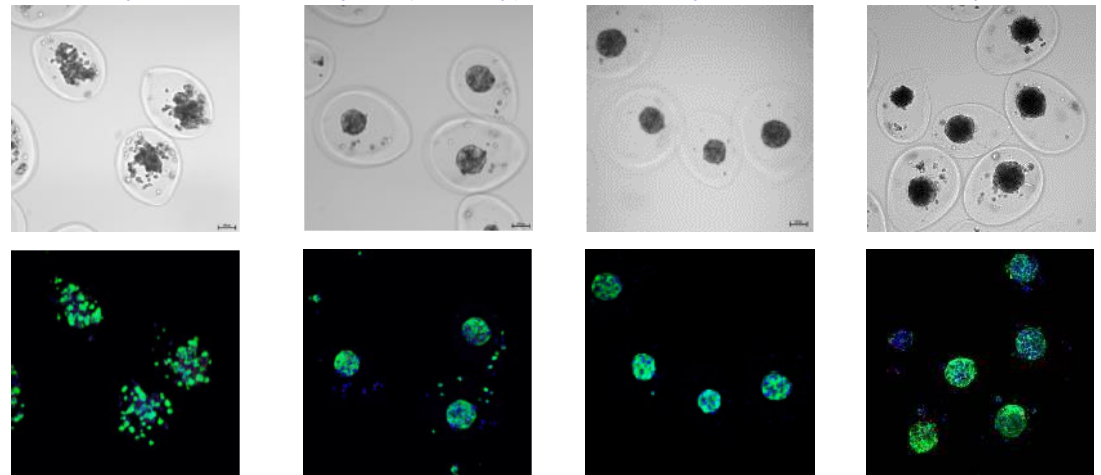
HepatoPearls®

Day 1

Day 10 (delivery)

Day 21

Day 45

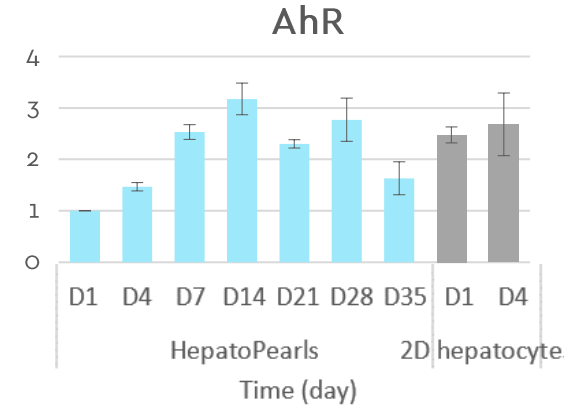
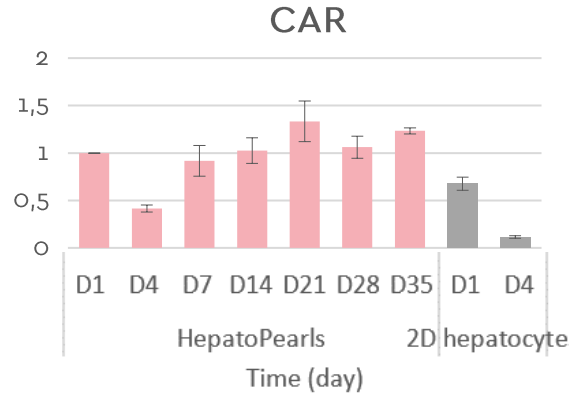
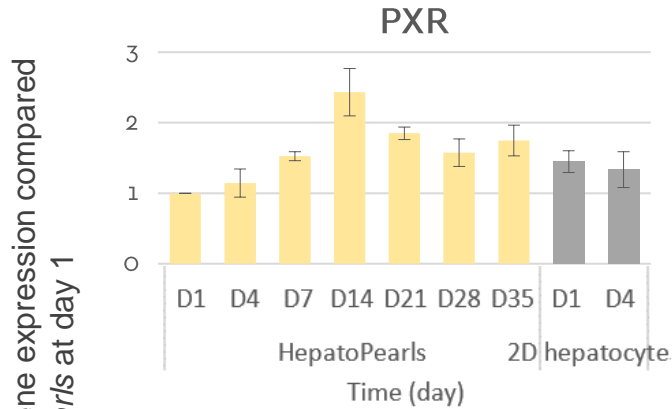


Nucleus – Live cells – Dead cells

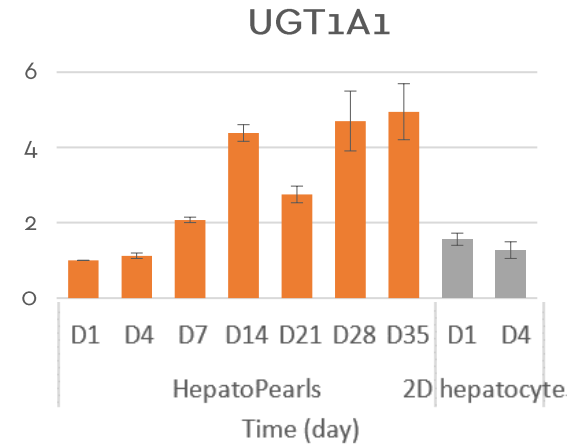
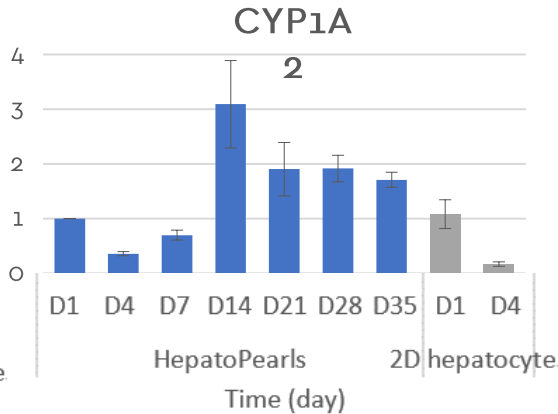
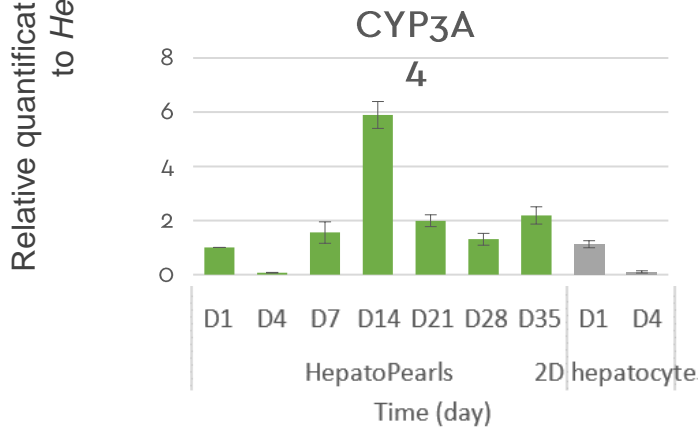
AN *IN VIVO*-LIKE STRUCTURE MIMICKING LIVER ARCHITECTURE AND FUNCTIONS

➔ Genetic expression of liver-specific genes as high as in 2D culture and maintained over time

➔ Genetic expression of liver-specific nuclear receptors



➔ Genetic expression of phase I & II enzymes



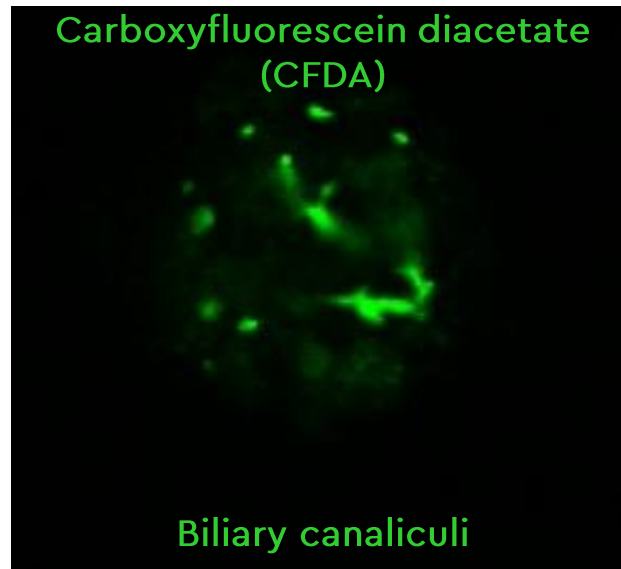
AN *IN VIVO*-LIKE STRUCTURE MIMICKING LIVER ARCHITECTURE AND FUNCTIONS

→ A polarized micro-structure with excretion and synthesis activities

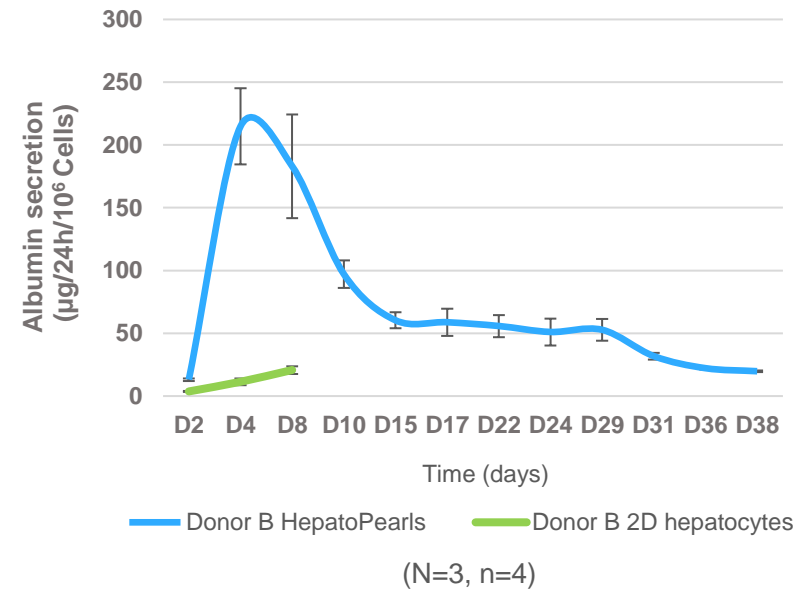
Cell Polarization



Functional Transporters



Albumin secretion

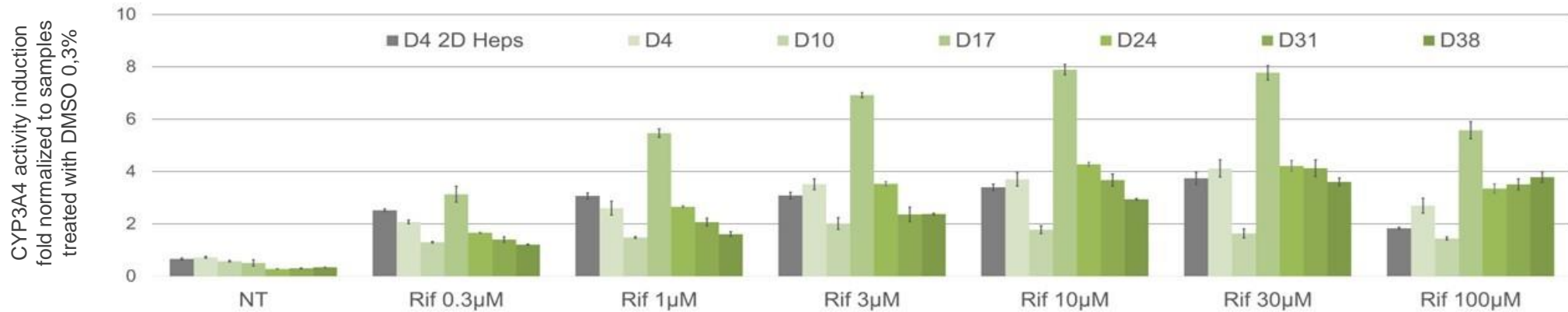


A TOOL TO STUDY DRUG-DRUG INTERACTIONS

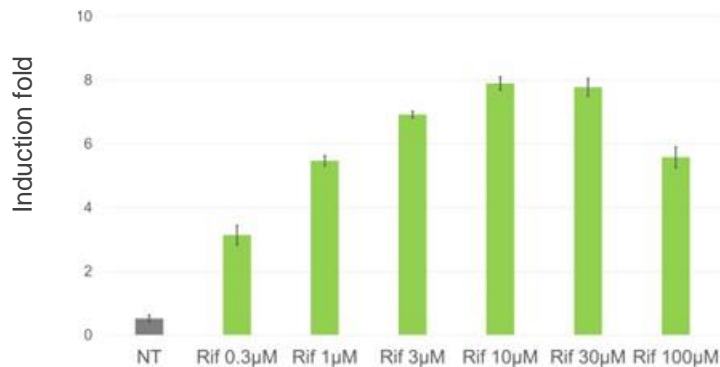
→ *Inducibility of CYP P450 enzymes all along their lifespan*

→ HepatoPearls® treatment with reference inducer for 3 days

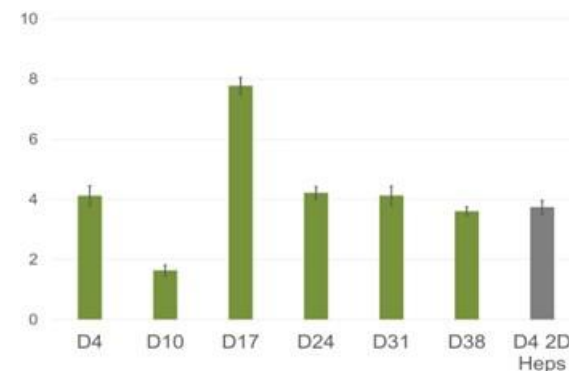
→ Measurement of CYP3A4 enzymatic activity for 6 weeks



• *Dose/response curve at D17*



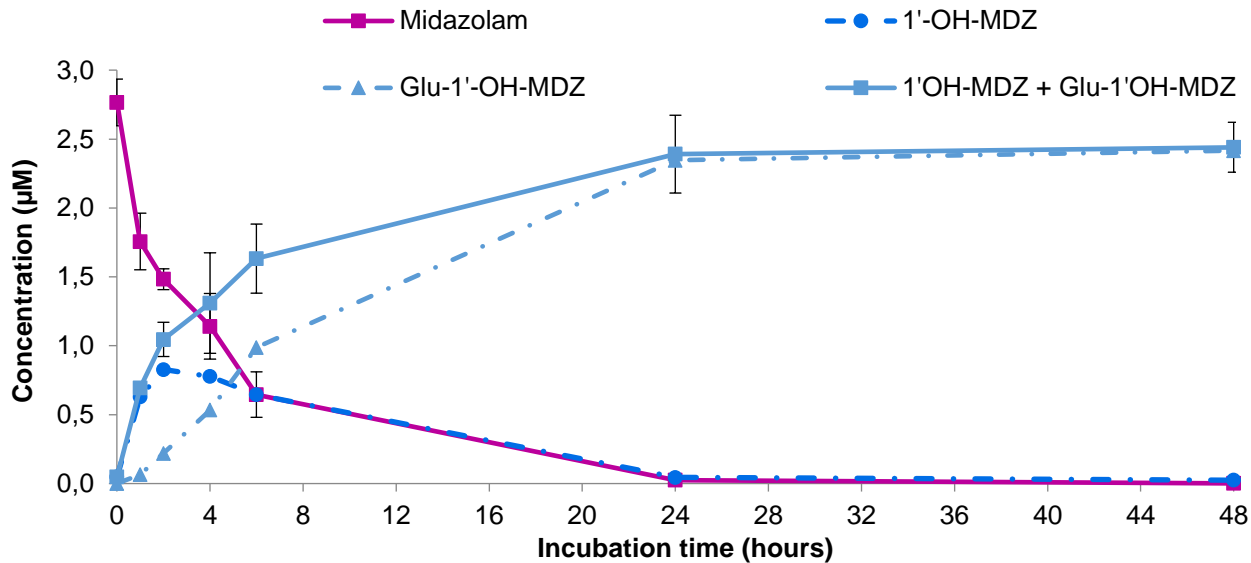
• *Inducibility over time (Rif 30 µM)*



A MINIATURIZED CLEARING SYSTEM FOR DMPK STUDIES

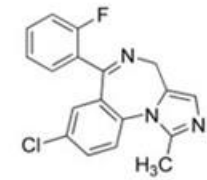
→ Activity of phases I & II enzymes

- \approx 500 HepatoPearls[®] (D8)/well incubated with 3 μ M Midazolam
- Midazolam clearance & metabolites appearance (LC-MS/MS)*



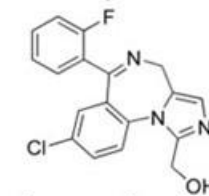
- Metabolism of midazolam was observed with HepatoPearls[®]
- Major metabolites (1'-OH and Glu-1'-OH midazolam) are formed
- Phase I and Phase II enzymes are functional

* This experiment was performed in collaboration with SANOFI



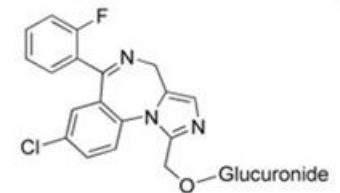
Midazolam

CYP3A4



1'-OH Midazolam

UGT

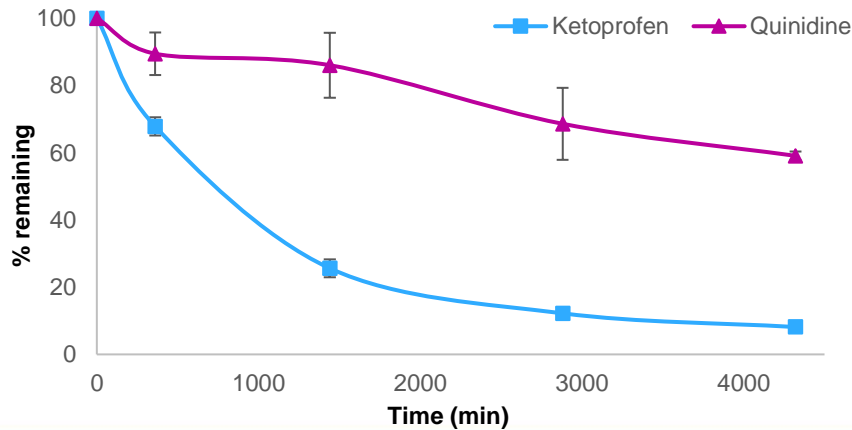
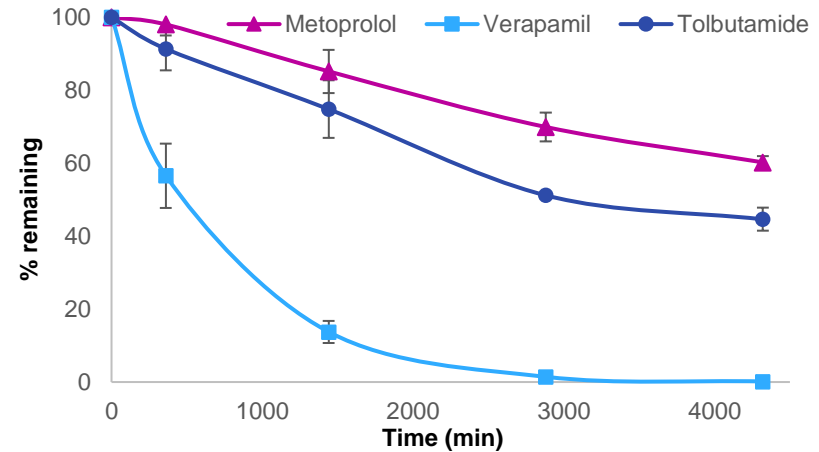
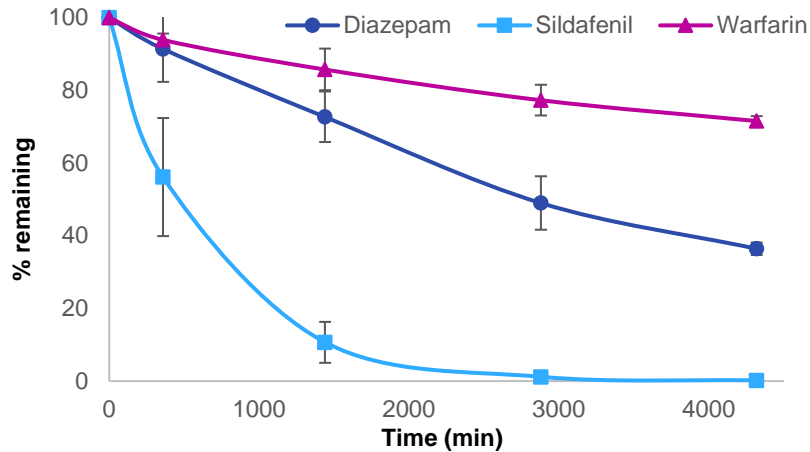


Glucuronide 1'-OH Midazolam

A MINIATURIZED CLEARING SYSTEM FOR DMPK STUDIES

→ Clearance of 8 compounds (LC-MS)

- Low to high-clearance compounds
- 72h analysis without medium changing



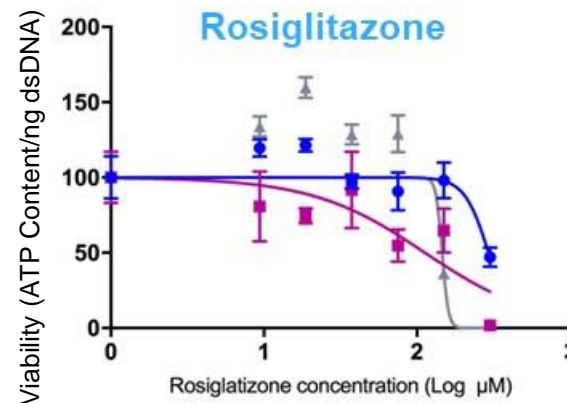
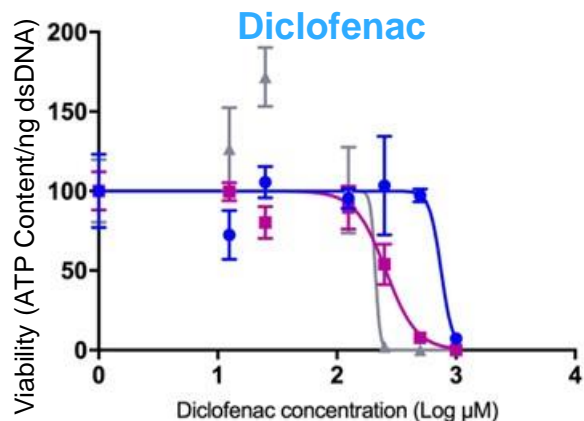
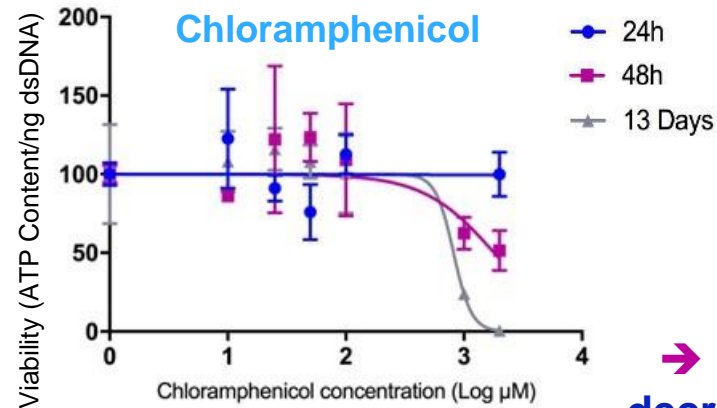
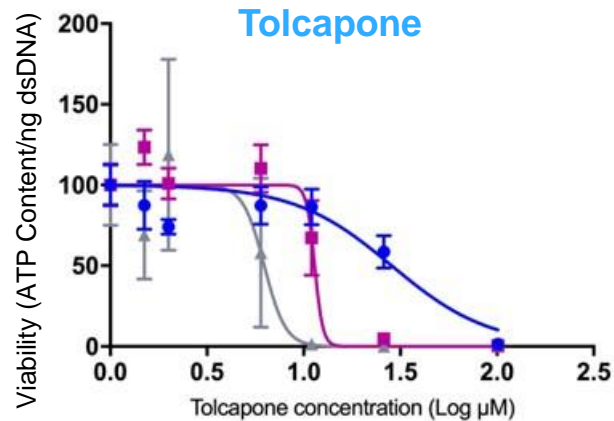
→ Metabolism of all the 8 compounds

A NOVEL MODEL FOR HEPATOTOXICITY ASSAYS

→ *Chronic exposures of potential hepatotoxic compounds*

→ **Pool of HepatoPearls®** from 3 different donors

→ Treatment with different drugs for **acute (24 & 48h)** and **chronic (13 days)** injuries



→ IC50 values were decreased by prolonging exposure duration

THANK YOU FOR LISTENING

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