Large-scale Production of Pluripotent Stem Cell-derived Liver Organoids through Liquid-core Encapsulation

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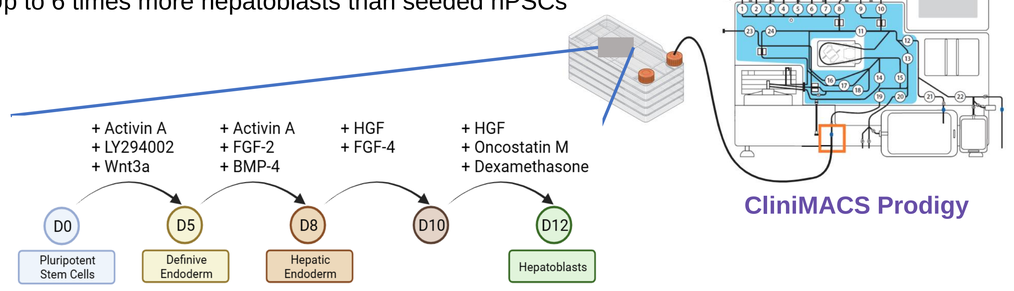
- Hepatocytes cultured as a monolayer are widely used in the pharmaceutical industries for multiple *in vitro* applications, such as drug metabolism and pharmacokinetics (DMPK), hepatotoxicity or drug-induced liver injury (DILI). However, this model is not optimal to predict toxicity and/or efficacy of a new drug due to the rapid loss of functionality and viability of these cells.
- Liver organoids have a promising potential for these industries and the therapeutic field, but challenges such as scale-up production and long-term viability remain. The scarcity and heterogenous quality of primary human hepatocytes hinders the possibility of conducting large-scale and reliable studies.
- Here, we aim to exploit the unique properties of human pluripotent stem cells (hPSCs) to be largely amplified and differentiated into hepatic progenitors (hepatoblasts) using an automatized production system. These cells will then be encapsulated in liquid-core alginate capsules to produce iPearls®, functional liver organoids with a long-term viability that will be used as a model for toxicology studies.

Large-scale Production of Hepatoblast Progenitors from hPSCs

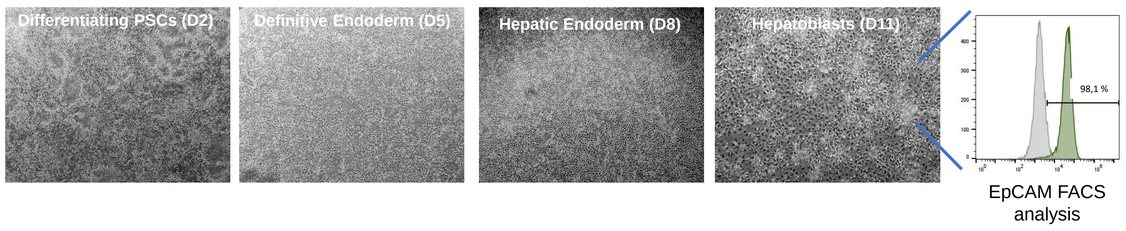
Automatization of the hPSCs differentiation

- Up to 6 times more hepatoblasts than seeded hPSCs



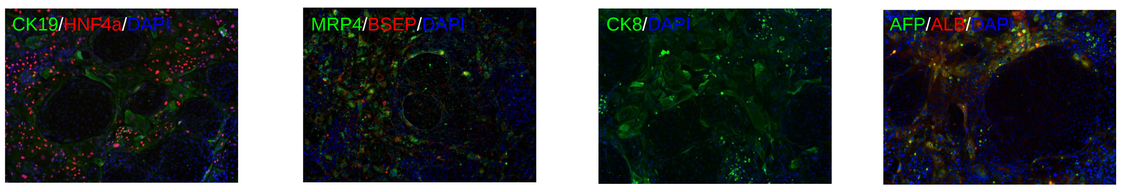
CliniMACS Prodigy

Homogeneous cell populations



EpCAM FACS analysis

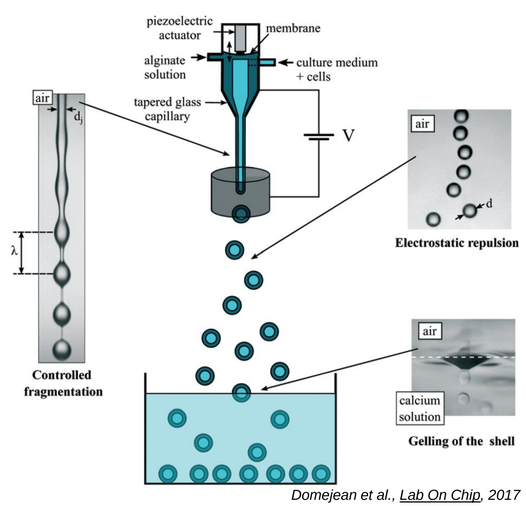
Hepatoblasts differentiate into hepato-biliary cells in 2D cell culture



- Cells differentiated from hepatoblasts express either hepatocyte (HNF4, BSEP) or cholangiocyte markers (CK19) but some cells still express fetal markers (AFP) → **3D cell culture**

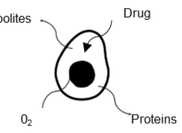
High-throughput Encapsulation

Liquid-core encapsulation

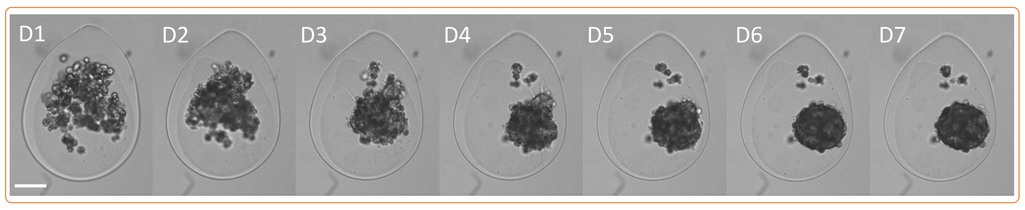


Domejean et al., Lab On Chip, 2017

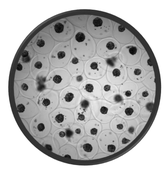
- Co-extrusion of an alginate solution and cells + medium
- Homogeneous droplet generation in the air
- Reticulation in a core-shell structure
- More than 1,000 capsules per second
- Flexible in number of cells per capsule
- Alginate porosity allows diffusion of small molecules and nutrients



Self-assembling of the cells into a single 3D structure



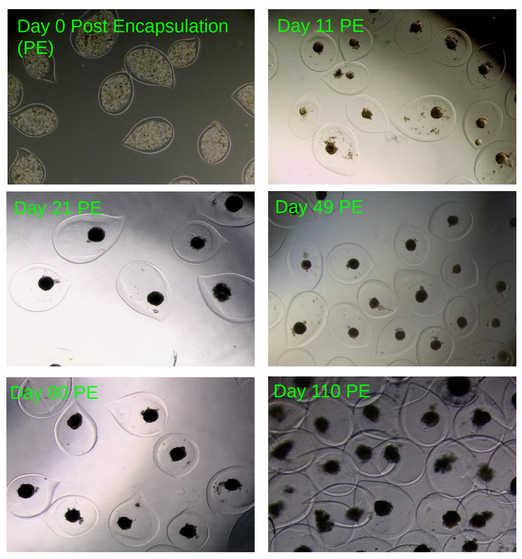
Time-lapse of organoid formation (scale bar : 100 µm)



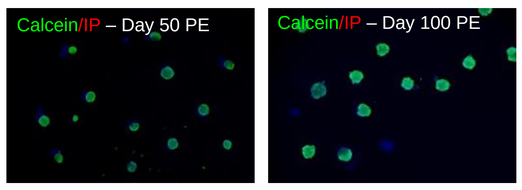
- One organoid per capsule (no necrotic core)
- Up to 200 organoids per well thanks to the alginate barrier: No risks of fusion

iPearls® Characterization

Long-term integrity and viability



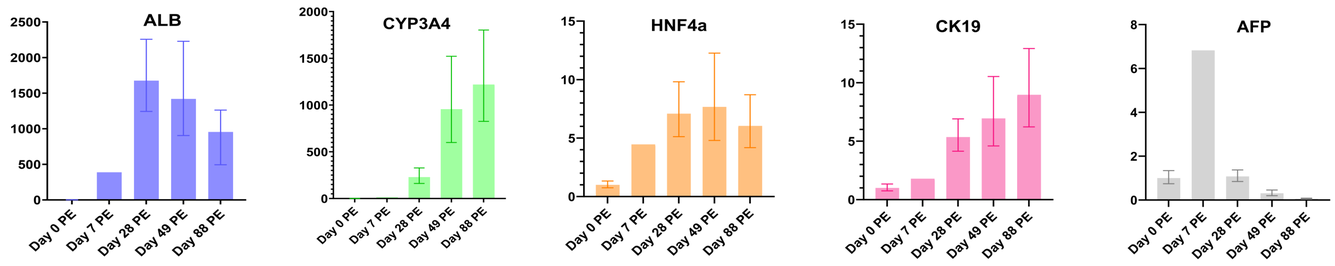
- Organoids maintain their integrity…



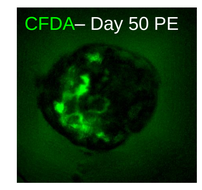
… and their viability after 4 months of culture

Encapsulated hepatoblasts differentiate into hepato-biliary organoids

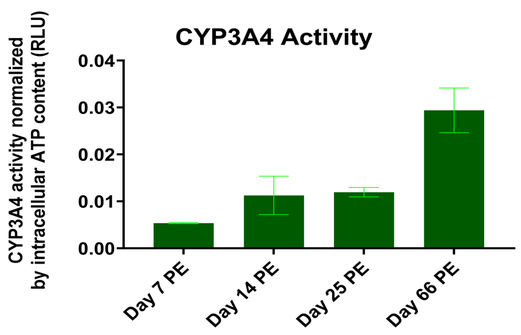
Relative mRNA quantification compared to Day 0 post encapsulation (PE)



- Over time, organoids express higher levels of hepatocyte and cholangiocyte markers whereas fetal markers expression decrease as demonstrated by RT-qPCR

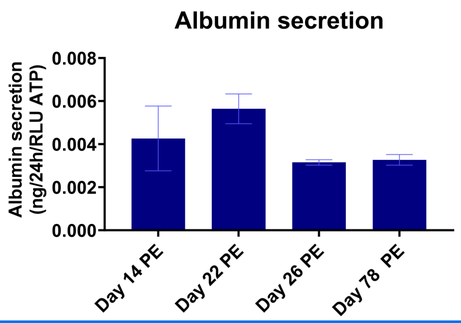


- Appearance of bile canaliculi

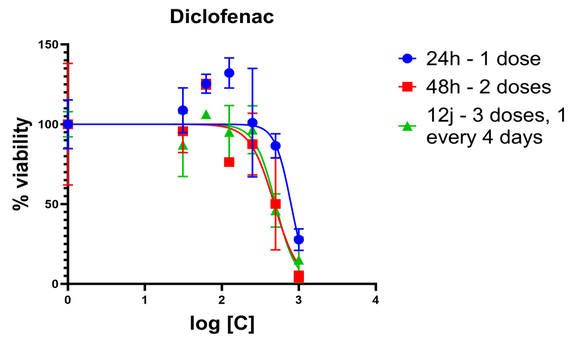


- Stable secretory functions during the lifespan of the organoids

- Increasing enzymatic activity of detoxification by the cytochrome CYP3A4



A model for hepatotoxicity studies



- Decrease of IC_{50} with repeated exposures of the toxic compound

CONCLUSIONS AND PERSPECTIVES

- ***iPearls®*, encapsulated hPSC-derived hepatoblasts, rapidly differentiate in 3D culture into functional liver organoids, viable for more than 4 months and expressing hallmarks of hepatocytes and cholangiocytes. They are the basis for long-term studies, such as chronic hepatotoxicity, and therapeutic applications for the encapsulation technology, as a huge number of capsules is needed.**
- ***Ongoing : Toxicity of other compounds (acetaminophen, chloramphenicol). Comparison with encapsulated human primary hepatocytes (HepatoPearls®)***