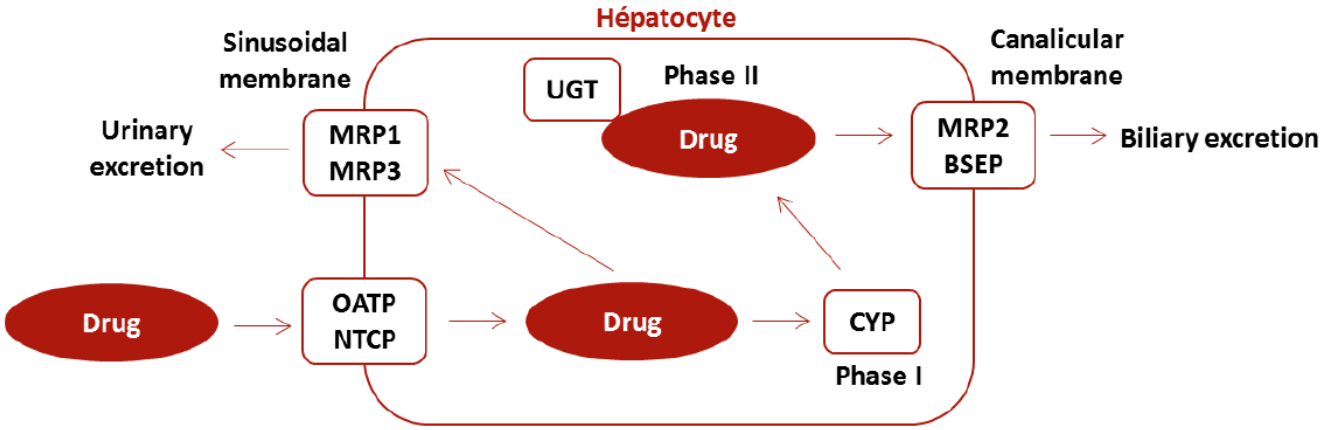
MAINTAIN HEPATOCYTE FUNCTIONS

INTRODUCTION

Maintaining the basal characteristics of primary hepatocytes and HepG2 allows performing metabolic studies.

The functional instability of hepatocytes in 2D culture results in a rapid decrease in specific metabolic pathways (1). Cell culture in BIOMIMESYS®*Liver* shows higher metabolic characteristics of hepatocytes compared to a culture in 2D, such as:

- The albumin secretion, often used as a marker for functionality.
- The presence of active bile ducts (MRP2 and CFDA staining).
- The expression of genes encoding transporters (NTCP, BSEP and MRP2) and phase I enzymes (CYPs) and II (UGT) of drugs metabolism.



(1) 3D cultivation techniques for primary human hepatocytes, Bachmann A. et al. Microarrays. 4:64-83, 2015

Materials required

- Human Albumin Kit Elisa (Abcam)
- DNA quantitation kit, fluorescence assay using bisBenzimide (Sigma Aldrich)
- MRP-2 antibody (Abcam)
- Phalloidin (Lifetechnologies)
- Hoechst (Lifetechnologies)
- CFDA (Sigma)
- Confocal microscopy (LSCM)
- RNA extraction : RNAXS kit (Marcherey-Nagel)
- LightCycler 480 SYBR Green I Mast (S+E-20) (Roche) for qPCR
- BIOMIMESYS®*Liver*
- HepG2 from ATCC & cryopreserved primary human hepatocytes

Hydroscaffold properties
Translucent and porous

Method

- Recovery protocol for secreted proteins (ELISA)
- Staining and lysis of cells by following the manufacturer's instructions

RESULTS

The composition of BIOMIMESYS®*Liver*, similar to the liver extracellular matrix, promotes the functionality of the hepatocytes:

- **Albumin secretion**

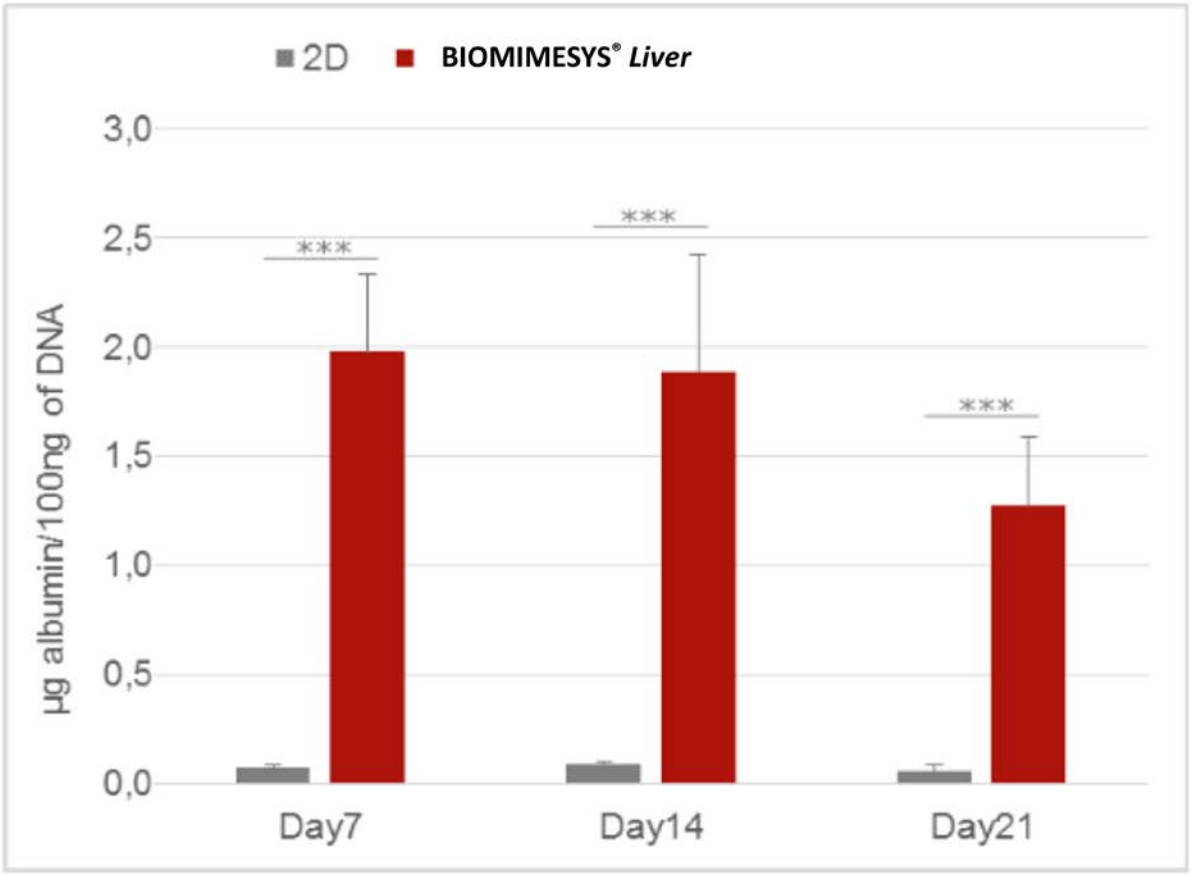


Figure 1: Albumin secreted by HepG2 grown in 2D and in BIOMIMESYS®Hepatocyte (n=4; ***: p<0,001).

The amount of albumin secreted in HepG2 cultured in BIOMIMESYS®*Liver* is 20-30 times higher than that measured in 2D.

❖ **Bile canaliculi formation**

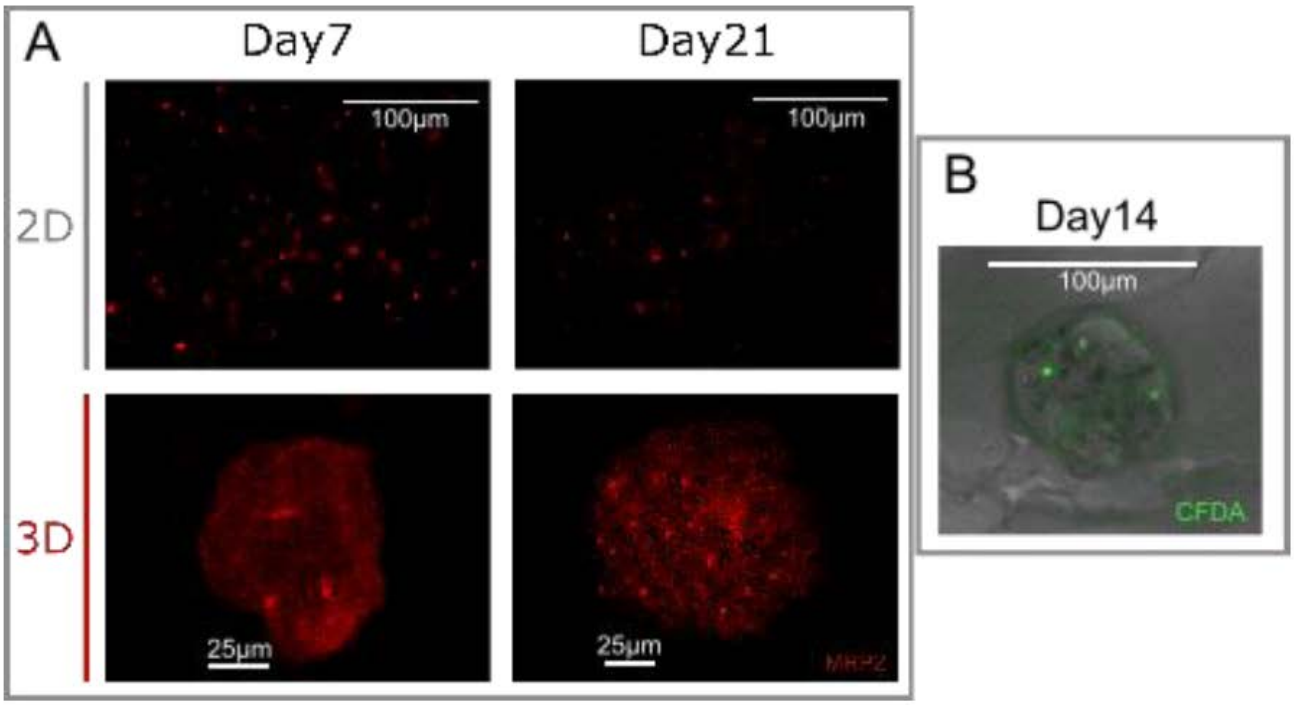


Figure 2: A. Detection of the bile canaliculi by MRP2 protein staining (red) during the culture of HepG2 in BIOMIMESYS®*Liver and 2D culture (top panel), after 7 and 21 days. B. Active bile ducts highlighted by CFDA (green) staining in HepG2 culture in* BIOMIMESYS®*Liver.*

Cells grown in BIOMIMESYS®*Liver* show constant MRP-2 staining during 3 weeks compared to cells grown in 2D, where the expression of MRP2 drops after 1 week of culture.
Observation of CFDA fluorescence in HepG2 grown in BIOMIMESYS®*Liver* shows active bile ducts. Indeed, CFDA is internalized by hepatocytes, cleaved by intracellular esterases and excreted into bile canaliculi as fluorescent CDF by active MRP2.

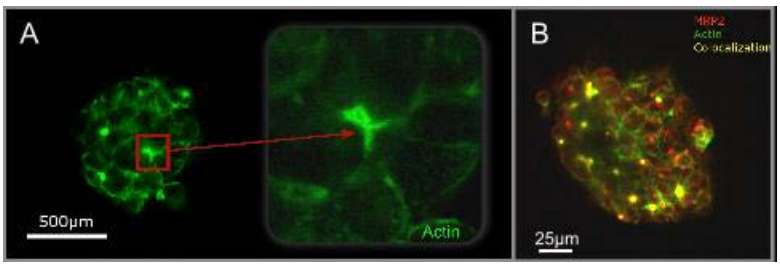


Figure 3: A. Fluorescence microscopy observation of actin (green) in HepG2 grown in BIOMIMESYS®Liver. B. Colocalization (yellow) of actin and MRP-2 (red) in HepG2 (z slice of 20µm depth inside spheroid).

A canaliculi network with an Actin / MRP2 colocalization is observed in HepG2 spheroids.

❖ **Key metabolic gene expression and CYP activities**

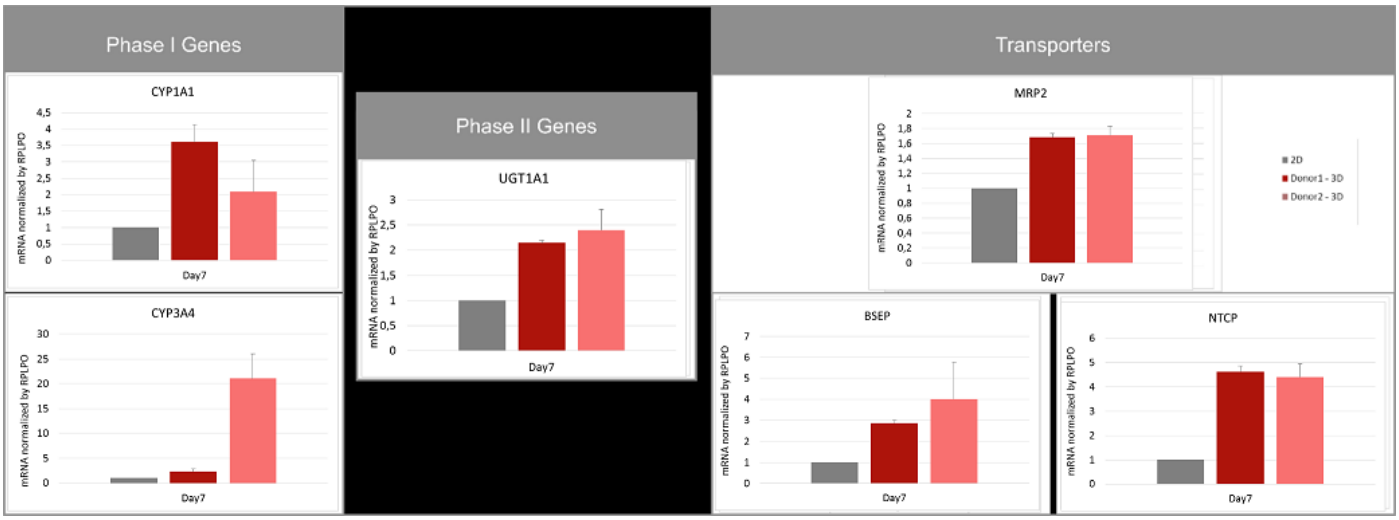


Figure 4: Analysis of gene expression in cryopreserved human hepatocytes by q-PCR. 2D data have been normalized to 1 for 2D and 3D comparison.

In BIOMIMESYS®*Liver*, the analysis of gene expression encoding proteins involved in ADMET functions, after 7 days of culture, shows an increase compared to the level of gene expression of cells cultured in 2D. The expression level is maintained even after 14 days of culture (BSEP, NTCP).

CONCLUSION

Maintenance of gene expression in cryopreserved human hepatocytes allows studying the drug-induced gene regulation at every stage of its management by hepatocytes: transportation (NTCP), the oxido-reductive reactions (Phase I: CYP1A1, CYP1A2, CYP3A4), conjugation (phase II: UGT1A1) and excretion by bile ducts (BSEP and MRP2).

Contact Information

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