Results on functional CYP and transporter expression in hiPS-Hep™ and the next generation of hES-Hep™

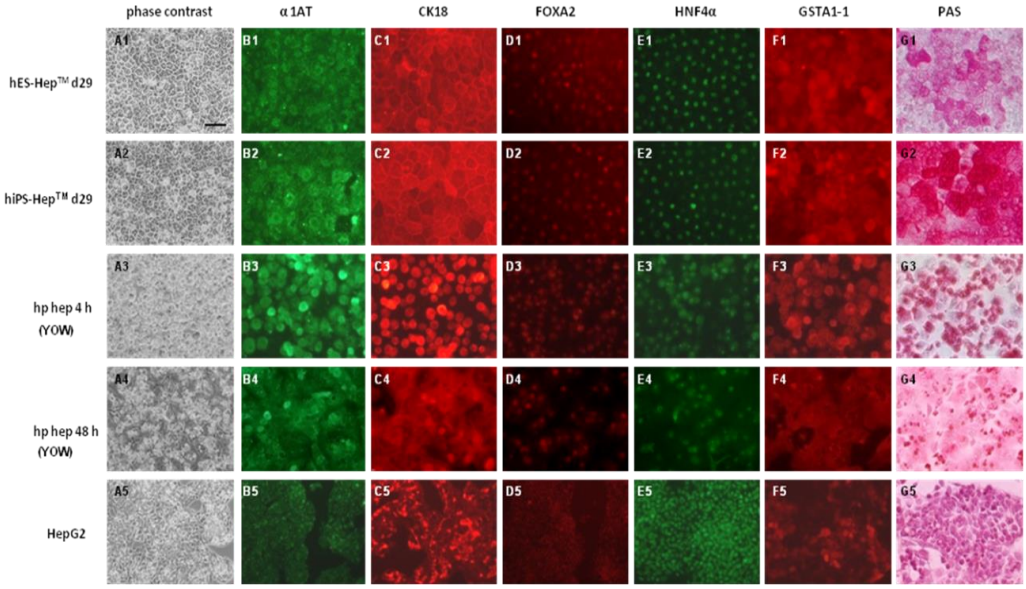


Figure 1. Immunocytochemical analysis of the expression of hepatic markers and glycogen detection in hES-Hep™ and hiPS-Hep™ (both on day 29), hphep (donor YOW) cultured for 4 and 48 h, as well as HepG2 cells. A) phase contrast pictures. B-F) Immunocytochemical stainings for α1-Antitrypsin (B), Cytokeratin 18 (C), FOXA2 (D), HNF4α (E), and GSTA1-1 (F). G) Glycogen storage detected by periodic acid Schiff (PAS) staining. Scale bar: 100µm in A1-5, B5, C5, D5; 50 µm in all other pictures. Abbreviations: α1AT = α1-Antitrypsin; CK18 = Cytokeratin 18; HNF4 α = hepatic nuclear factor 4 alpha; GSTA1-1 = glutathione-S-transferase A1-1; hphep = human primary hepatocytes; PAS = periodic acid Schiff staining.

Figure 1- Comparative study of CEL hepatocytes (hES-hep = hepatocytes derived from a hESC cell line & hIPS-hep = hepatocytes derived from a hIPS cell line) vs human primary hepatocytes 4h and 48h after thawing. The study is represented by immunostaining images.

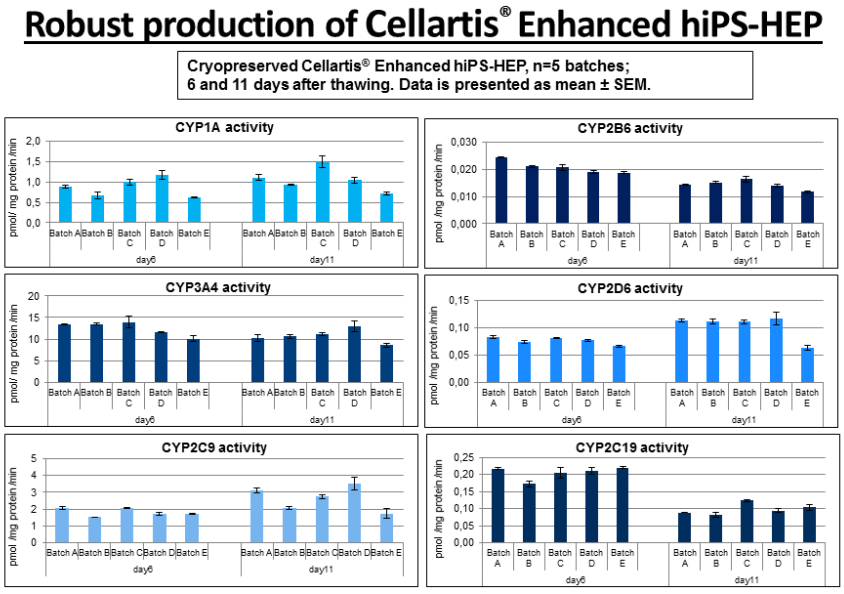
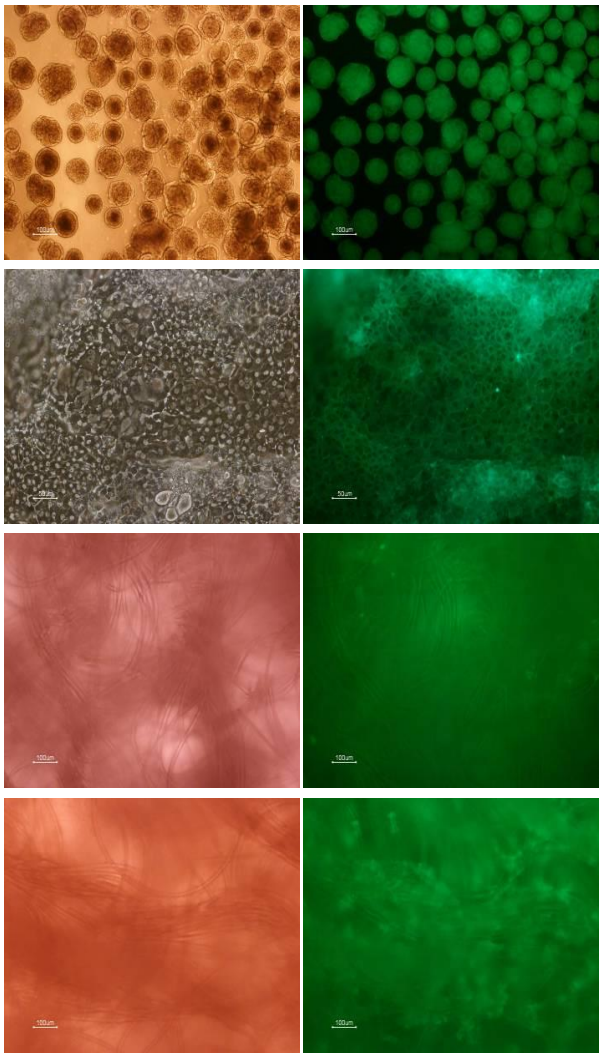


Figure 2- The five batches presented in figure 4 were harvested for qPCR analyses 6 and 11 days post thaw. The small batch-to -batch variations as well as the stability over time for several CYPs were evaluated and is presented in the figure.



A) Differentiated cells as spheroids

-Expressing ActinB-GFP construct regulated from ActinB promoter

B) Differentiated spheroids 7 days after reattachment to collagen

-Cells reattach well and form hepatocyte-like morphology

C) Differentiated spheroids 7 days after Seeding onto collagen coated MESH

-Cells did not reattach well

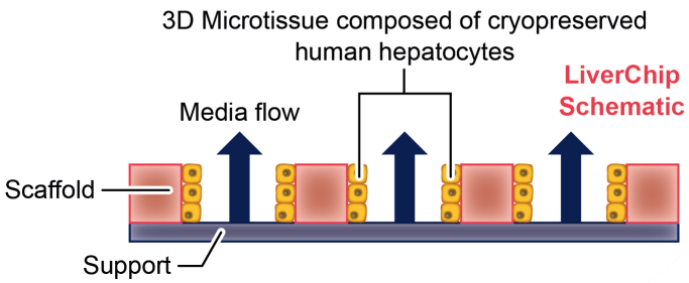
D) Partially differentiated cells reseeded and cultivated for 7 days on MESH

-High concentration of single cells allowed rebinding

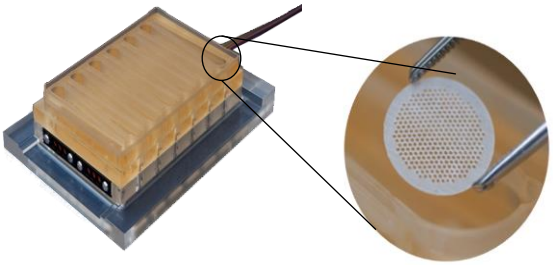
-Hard to regulate cell density

Figure 3- In attempt to study the rebinding potential of either spheroids differentiated to hepatocytes (A-C) or hepatoblasts (D) to a MESH filter (PGA 20mmx30mmx1.1mm pre-coated with collagen 10ug/ml solution, MESH provided from partner TransTissue), a monoclonally derived cell line constitutively expressing Actin-GFP was used. Cells were grown as spheroids and differentiated to hepatocytes (A-C), or differentiated in a 2D format to hepatoblasts (D) and re-plated to the MESH filter. Seven days post re-seeding, the filters (C, D) were studied in a fluorescence microscope. Quite unexpectedly the spheroids had fallen through the MESH, while a high concentration of single cells seeded at hepatoblast stage had successfully attached to the MESH filter. B) Represents a successful rebinding of spheroids to collagen surface when reseeded to a 2D format.

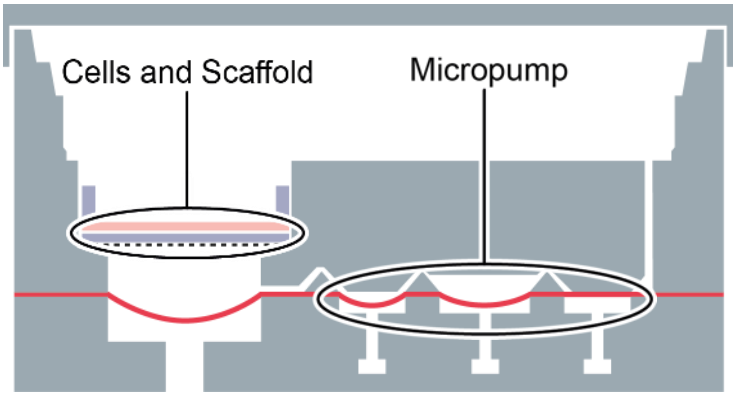
a)



b)



c)



d)

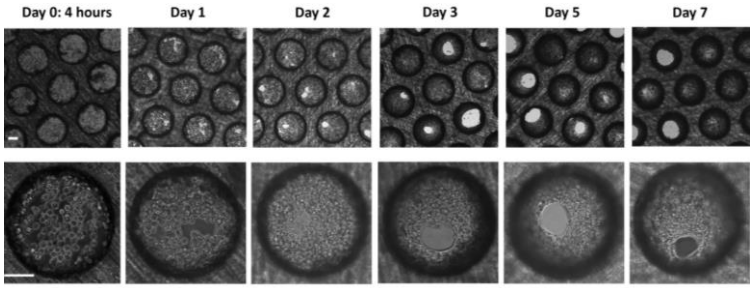


Figure 4- a) Schematic of the LiverChip® scaffold, b) LiverChip® 12 well system, c) Cross Sectional view of a single LiverChip® bioreactor, d) Microtissues formed within the scaffold in LiverChip®

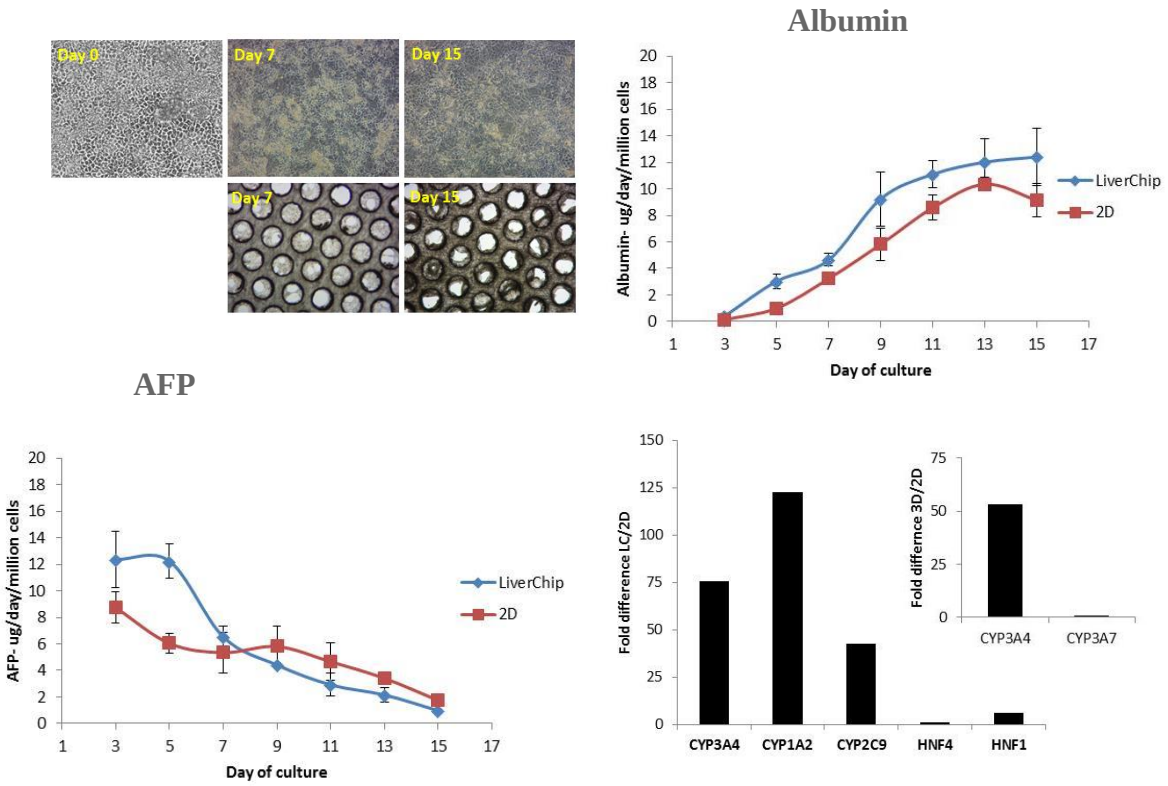


Figure 5- Functional comparison of iPSC-derived hepatocyte like cells in 2D and in ZXLs LiverChip® platform. a) Cells could be reattached to collagen coated plates or scaffolds. b) Excretion of maturity marker proteins albumin and c) AFP were equivalent in LiverChip® and 2D culture but gene expression analysis demonstrated greater expression of mature hepatocyte marker genes. Data is mean +/- SD of 3 wells for albumin and AFP secretion. Gene expression compared using the ΔΔCt method using GAPDH as a stable housekeeping gene

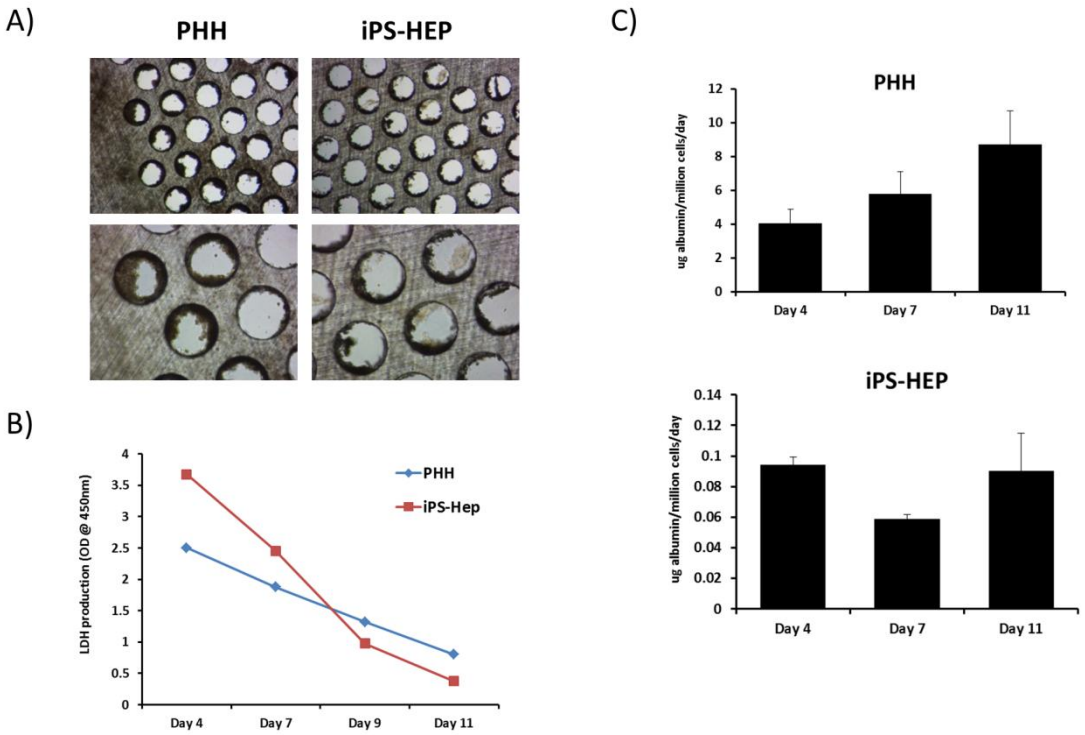


Figure 6- Culture of iPSC-derived hepatocytes and PHH in MICs bioreactor platform A) Light micrographs of collagen coated scaffolds after 11 days of culture. B) LDH production during culture. C) Excretion of albumin during culture measured by ELISA. Data is mean +/- SD of 3 wells.

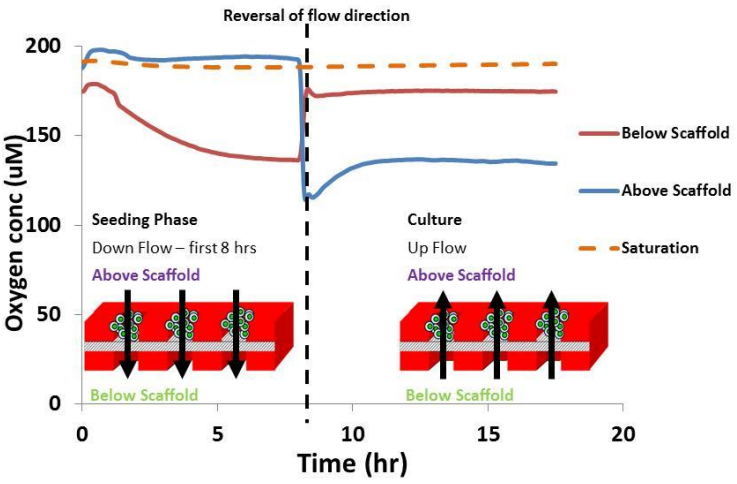


Figure 7- Schematic of the on-line, non-invasive oxygen sensors (Lucid Scientific Inc., U.S.) used to monitor the oxygen concentration in the culture medium before and after the 3D microtissues. The red line indicated oxygen tension below the scaffold. The blue line represents oxygen tension above the scaffold after passing over the cells.

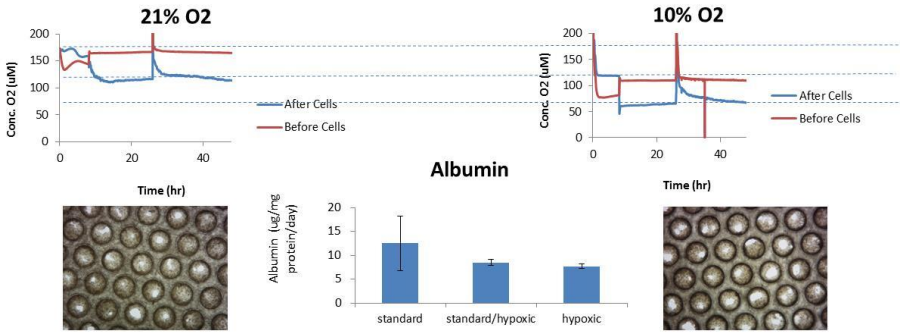


Figure 8- Comparison of oxygen tensions, tissue formation and function of hepatocyte microtissues in conventional and hypoxic incubators. Albumin was quantified on day 3 of culture in a minimum of 3 wells per condition (data is mean +/- Standard deviation). Standard = standard incubator throughout. Standard/hypoxic = seeded in standard incubator and removed to hypoxic after 24h. Hypoxic = seeded and maintained in hypoxic incubator.

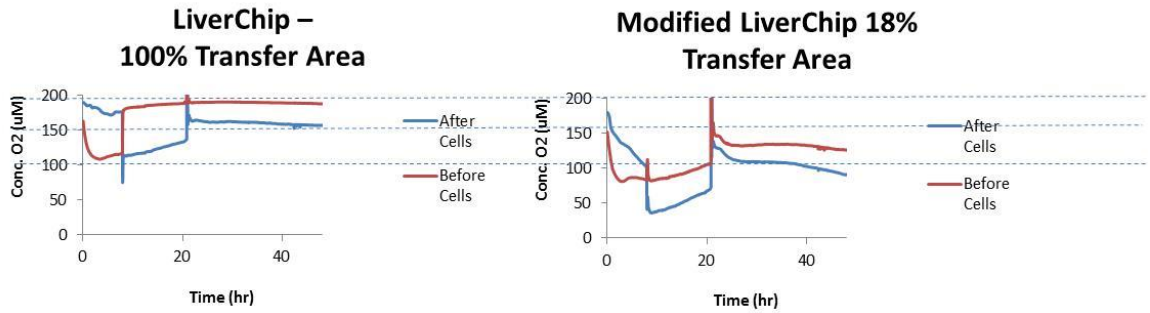


Figure 9- Oxygen concentrations measured in single wells of a regular LiverChip® or a modified plate with a surface channel having only 18% of the oxygen transfer area.

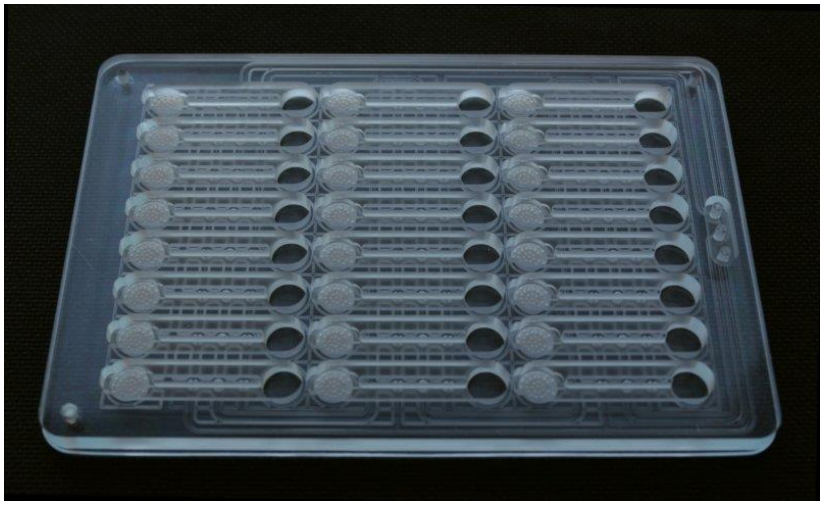


Figure 10- Photograph of a thermally bonded 24-well bioreactor. The size of the device is 128 x 85 x 7 mm^3.

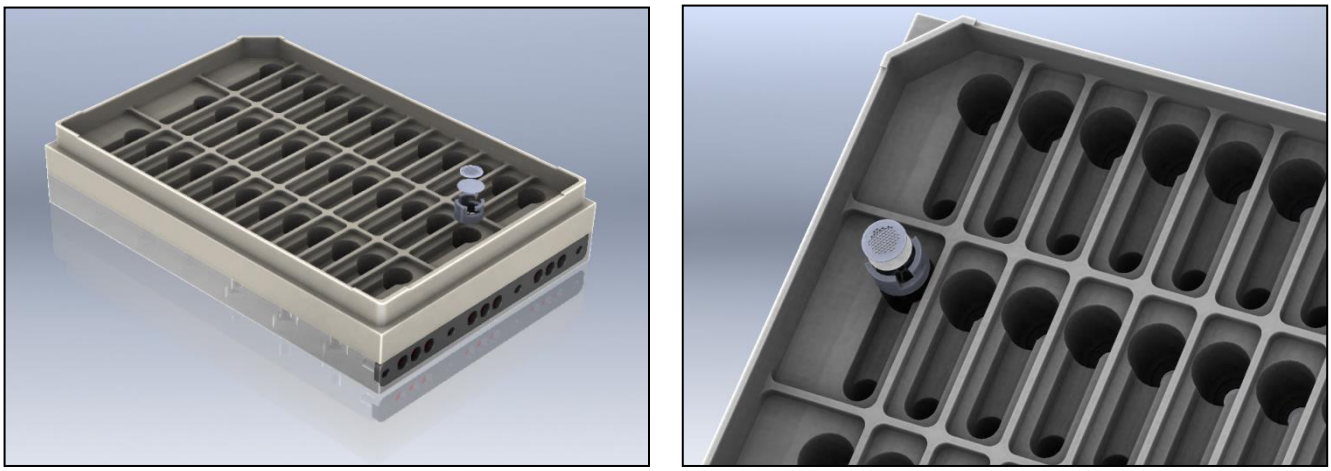


Figure 11- Computer Aided Design (CAD) drawings of the LC36. (a) A Polysulfone top plate which contains 36 individual channels and an acrylic bottom plate which provides the system with pressurised air. (b) Components that fit directly into the culture wells. A filter, scaffold and retaining ring are placed inside an insert accordingly. The assembled component is then pushed down into a culture well

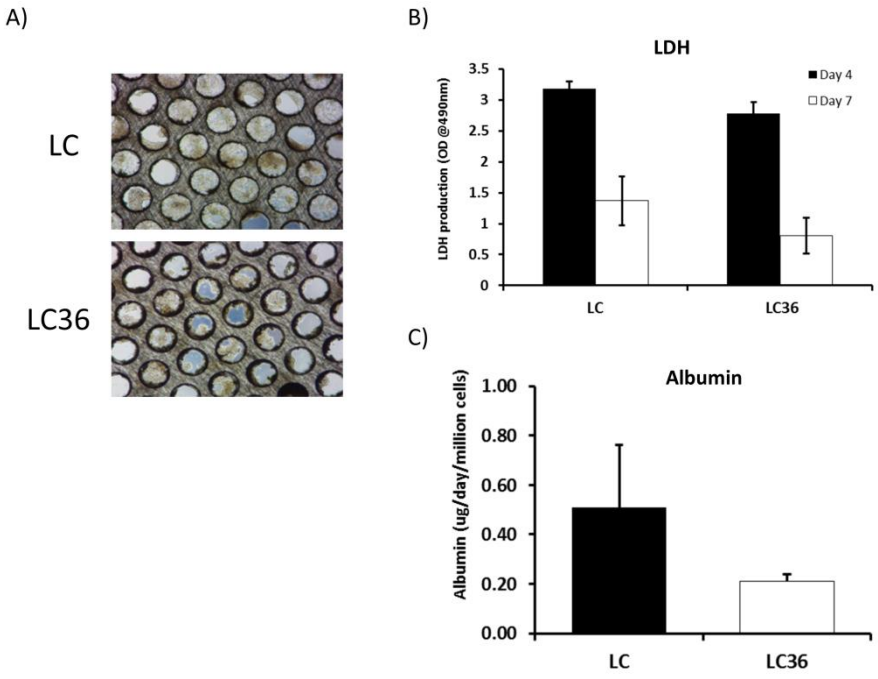


Figure 12- Functional comparison of iPS-derived hepatocytes cultured in LiverChip® and ZXLs LC36 bioreactor platform. A) Light micrographs of collagen coated scaffolds after 7 days of culture. B) LDH production during culture. C) Excretion of albumin during culture measured by ELISA. Data is mean +/- SD of 3 wells.

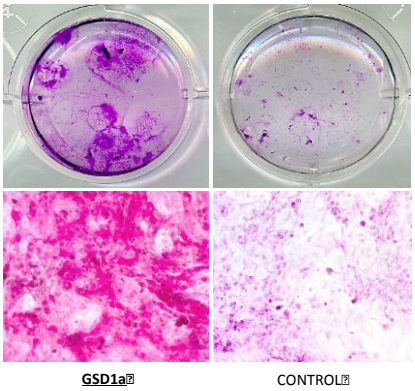


Figure 13- Hepatocytes like cells generated from GSD hIPSCs lines accumulate glycogen when compared to cells generated from control hIPSC lines.

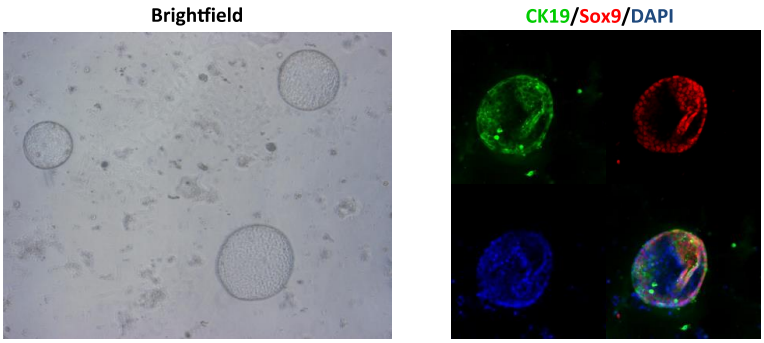


Figure 14- AGLS-hIPSCs can differentiate into cholangiocytes spheroid expressing biliary markers such as CK19 and Sox9.

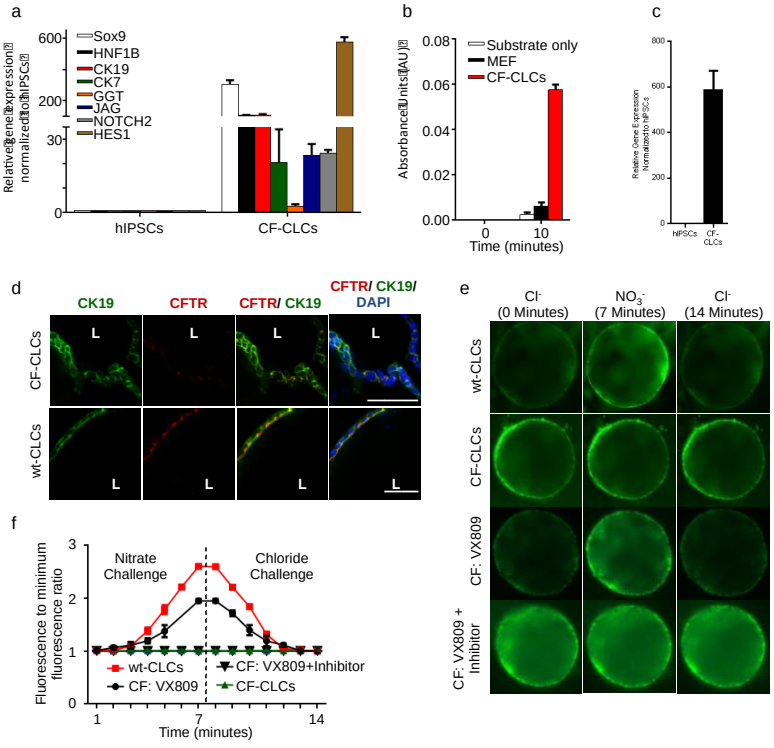


Figure 15- Modelling CF using hIPSCs derived CLCs grown in 3D based matrigel culture system. (a) Q-PCR analyses showing that CF-hIPSCs can be differentiated into CLCs. (b) ELISA showing that CF-CLCs can secrete

gamma GT. (c) Expression of CFTR in CF-CLCs. (d) Immunostaining analyses showing the absence of CFTR in CF-CLCs. (e) MQAE assay showing that CF-CLCs can only transport chloride when grown in the presence of VX809