Human Hepatic Organoids – Technology Overview

Human Hepatic Organoids derived from Human Hepatic Stem Cells

Product Overview

AvantiCell Science (ACS) isolates and derives human hepatic organoids from human liver samples obtained with full ethical permission.

ACS product portfolio includes:

- Human hepatic organoids – in vial
- Human hepatic organoids – in plate

Cells are banked using ACS cryopreservation technology and stored at -150°C

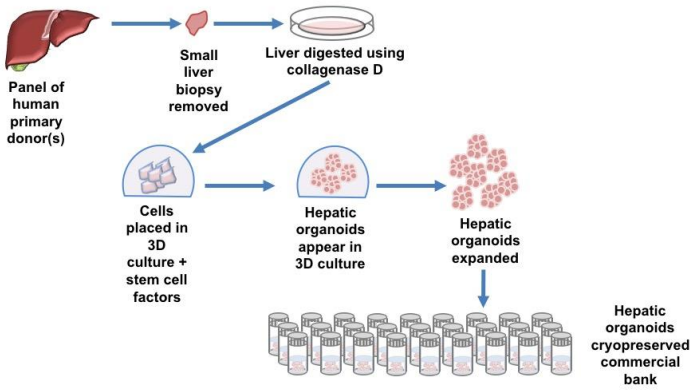
Industrial relevance of human hepatic organoids

Primary hepatocytes are an industrial cell-based standard that has been extensively utilised in preclinical drug discovery to predict toxicity and evaluate biological responses. Despite recent advances in hepatocyte culture techniques both in 2D and 3D (Godoy P *et al*., 2013 and Desai PK *et al*., 2017), the requirement for a more robust and reproducible drug model that is amenable to drug discovery still persists. Coupled to this, the high attrition rate of developed drug molecules in successful clinical trials extensively highlights the need for cell-based models that better reflect the complexity of the target host. Hepatic organoids have emerged from these model developments as a key basis for an *in vitro* model to examine hepatotoxicity and metabolism with organoid 3D culture techniques, allowing the isolation and culture of single stem cells that, when under the correct conditions, develop into structures reflective of the *in vivo* hepatic micro-environment. Hepatic organoids are now positioned as a key element to providing systems that may allow the development of sophisticated medicine

Preparation of human hepatic organoids from liver stem cells

Culturing of hepatic organoids was first presented in the literature by Huch *et al.*, in 2013, and interest in the field since has grown exponentially. ACS LGR5^{+} liver stem cell isolates are produced from human liver tissue obtained as surgical excess, with a growing number of donor adults, representing a distinct and unique panel of hepatic organoids. The hepatic organoids can be differentiated into functional hepatocytes, and industry attention to the potential of these non-engineered, renewable human stem cell cultures is currently driving a significant emerging organoid base market.

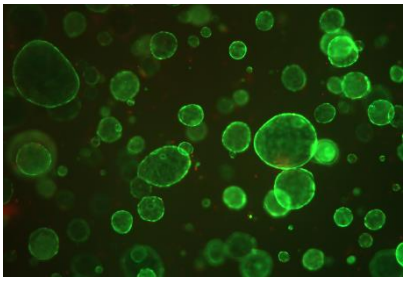
LGR5 is a stem cell marker in several tissues, such as the liver and intestine. This G-protein coupled receptor and its ligands, R-Spondin proteins, regulate Wnt/β- catenin signalling.



Lgr5⁺ tissue-resident stem cells are isolated from human liver samples and expanded as organoid cultures in extracellular matrix and stem cell niche factors-containing media.

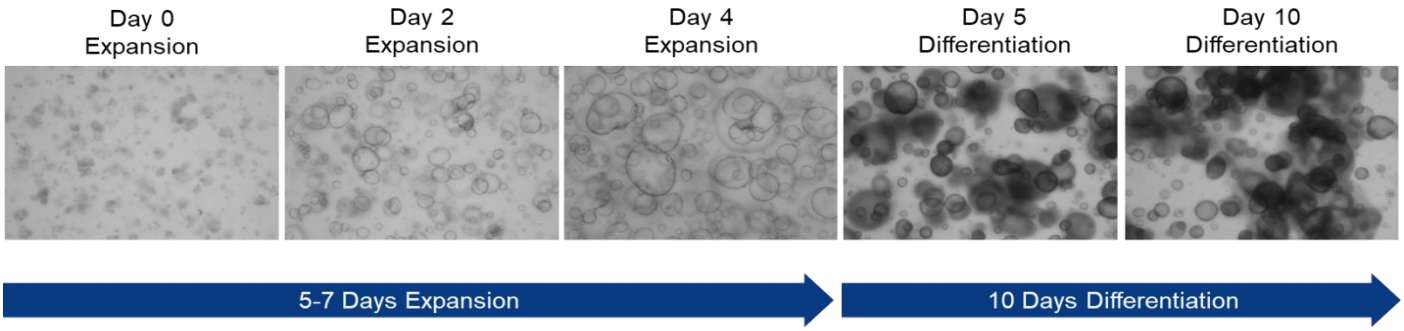
Culturing of human hepatic organoids

The hepatic organoids grow in a unique and fully optimised expansion medium formulation (Broutier *et al*., 2016) in combination with a select extracellular matrix (ECM) which allows organoid cultures to be expanded over several passages without loss of stemness.



Organoids derived from Lgr5⁺ adult stem cells in culture visualized with FDA/PI

The LGR5⁺ liver stem cell-containing organoids can be successfully differentiated *in vitro* to express key hepatocyte cell markers. These include; hepatocyte-specific CYP450 enzyme CYP3A4, the ductal marker SOX9, the transcriptional HNF4α and albumin ALB. Differentiation of hepatic organoids is possible over a period of 10 days using an optimised differentiation medium formulation. qPCR analysis of transcript levels in the differentiated organoids related to transcript levels measured in un-differentiated hepatic organoids provides a fold-change due to differentiation.



Brightfield images of hepatic organoids pre- and post-differentiation.

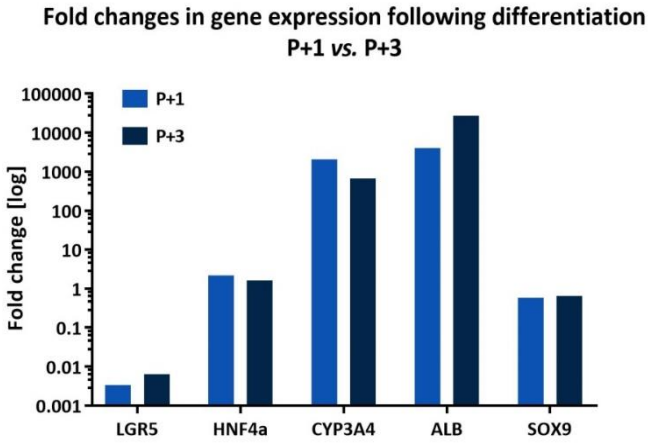
Analysis of differentiated ACS human hepatic organoid samples shows increased expression of CYP3A4 and albumin, expression traits associated with a mature hepatocyte phenotype. Critically, levels of LGR5 are reduced, indicating the differentiation procedure has induced a more mature hepatocyte phenotype in the differentiated hepatic organoids. The differentiation capability of each donor batch is shown within their respective product information.

ACS human hepatic organoid donor panel gene expression post-differentiation

		Fold change upon differentiation		
Gene	Target Description	Donor #18-28043A	Donor #18-12058A	Donor #18-29063A
SOX9	Ductal marker	0.44	0.27	0.19
HNF4α	Hepatocyte marker	1.73	0.76	0.67
ALB	Mature/functional hepatocyte marker	1315.00	173.60	67.93
CYP3A4	Mature/functional hepatocyte marker	578.90	5.15	12.63
LGR5	Stem cell marker	0.02	0.00050	0.001

Stability of human hepatic organoids

To evaluate differentiation potential across hepatic organoid passages, ACS human hepatic organoids were sampled and tested across three passages. Hepatic organoids were cultured in differentiation medium for a period of 10 days before RNA organoid samples were generated post-differentiation, and key hepatic gene markers were analysed at both passage 1 (P+1) and passage 3 (P+3) post-thaw. Fold changes in all hepatic gene markers have not been shown to differ in P+1 compared to P+3 analysed samples. Thus, ACS human hepatic organoids maintain their stemness, and ability to differentiate, after the guaranteed three passages.

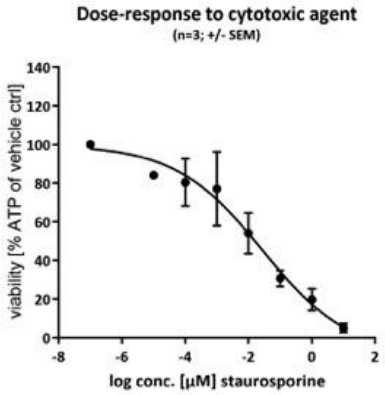


Analysis of key hepatic markers ACS human hepatic organoids post-differentiation at P+1 and P+3 relative to corresponding un-differentiated levels.

ACS has employed over 10 years of expert cell culture knowledge to generate a distinctive panel of hepatic organoids, isolated and cultivated from a range of human donors. This hepatic organoid panel has been cryopreserved and once resurrected, is optimal for a multitude of downstream cell-based applications. Hepatic organoids expanded in this manner represent an ideal 3D model and potent tool for the pharmacokinetic profiling of drug cytotoxicity and metabolic activities.

Differentiated hepatic organoids are biologically-active

Human primary hepatocytes, when exposed to cytotoxic reagents, give a defined biological response. To characterise the equivalent characteristic in ACS human hepatic organoids, the cytotoxic response to a range of concentrations to a known cytotoxic agent, staurosporine, was used to measure the biological sensitivity of ACS human hepatic organoids.

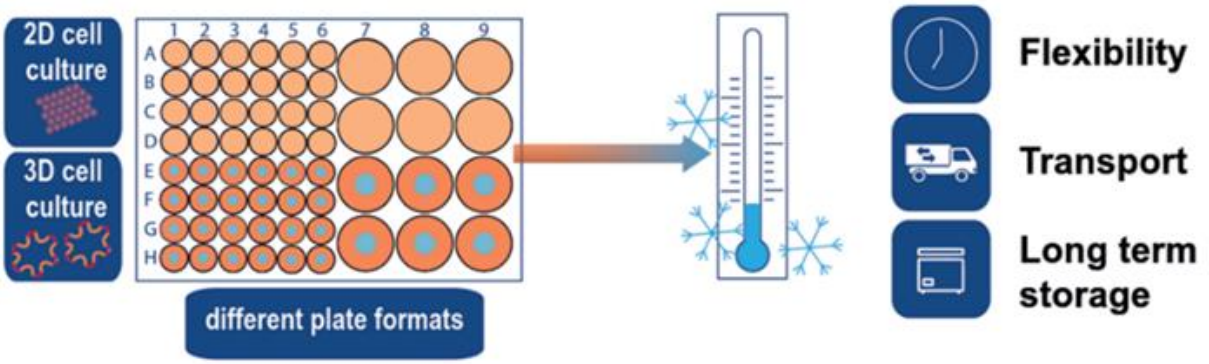


Staurosporine is a potent protein kinase inhibitor and an inducer of apoptosis. It is commonly used as a positive control in *in vitro* cytotoxicity studies.

Dose response of differentiated ACS hepatic organoids in the presence of staurosporine (average of 3 technical replicates from 2 donor isolates)

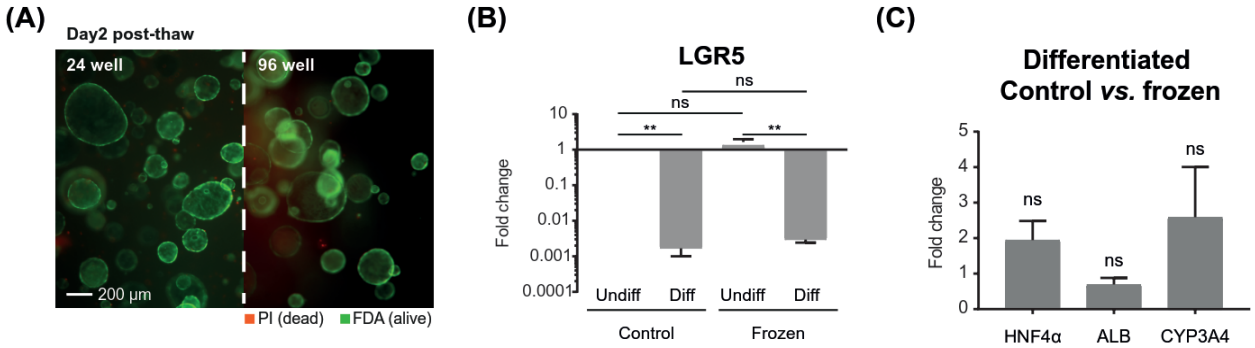
Application of Cryotix™ to human hepatic organoids

To tailor hepatic organoids to specific customer needs and provide the cells in a customer-convenient format, ACS proprietary Cryotix™ cryopreservation technology has been applied to 3D hepatic organoid cultures frozen *in situ.* This provides a plug-and-play format, to be stored under conventional laboratory freezer conditions (-150°C) that, once thawed using an ACS- optimised protocol, can be used in down-stream analysis, without the need for cell propagation before differentiation.



Multi-plate format convenience of ACS Cryotix™ cryopreservation technology.

Application of an optimised cryoprotective agent and ACS Cryotix™ technology, to hepatic organoid cultures in both 24-well and 96-well plates allows the revival of viable hepatic organoids post-thaw. Following storage at -150°C, cultures were revived from frozen and expanded for 4 to 5 days and subsequently differentiated for 10 days. Cultures were analysed using qPCR and the expression of LGR5, HNF4α, ALB and CYP3A4 was measured and compared to unfrozen (control) cultures of hepatic organoids.



Application of ACS Cryotix™ technology allows the provision of viable organoids (A) with no significant effect to their gene expression post-thaw (B and C).

There are non-significant changes to the genes expressed in frozen organoid cultures versus control cultures, showing that *in situ* cryopreservation allows for the provision of hepatic organoids in customer-convenient formats, ready for down-stream analysis, removing the need for stem cell isolation, organoid propagation and manipulation.

References

Godoy P, *et al.*, Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. Arch Toxicology. 2013 Aug;87(8):1315-530.

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Broutier L, Andersson-Rolf A, Hindley CJ, Boj SF, Clevers H, Koo BK, Huch M. Culture and establishment of self-renewing human and mouse adult liver and pancreas 3D organoids and their genetic manipulation. Nature Protocols. 2016 Sep;11(9):1724-43.

Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, Sato T, Hamer K, Sasaki N, Finegold MJ, Haft A, Vries RG, Grompe M, Clevers H. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature. 2013 Feb 14;494(7436):247-50.