Cellular Dynamics international

Application Note

iCell® Hepatocytes:

Using iPSC-derived Hepatocytes as a Functional Model System for Human Hepatitis Research

Hepatitis is defined as a group of viral infections that affect the liver. Two of the most common types of hepatitis are Hepatitis B and Hepatitis C, caused by Hepatitis B virus (HBV) and Hepatitis C virus (HCV), respectively. Both Hepatitis B and Hepatitis C represent major, global healthcare problems; infections can be acute or chronic diseases and can range in severity from a mild illness lasting a few weeks to a serious, lifelong illness. Hepatitis may cause chronic liver disease and lead to liver cirrhosis or liver cancer.

In the US, viral hepatitis is the leading cause of liver cancer and the most common reason for liver transplantation. An estimated 4.4 million Americans are living with chronic hepatitis; most do not even know they are infected.[1] Worldwide, more than 240 million people have chronic liver infections, and about 600,000 people die each year as a result of Hepatitis B infection. In the US alone, there are approximately 38,000 new cases of Hepatitis B each year.[2] About 150 million people across the globe are chronically infected with HCV, and more than 350,000 people die every year from Hepatitis C-related liver diseases. Another 3 - 4 million people (17,000 of them in the US) are infected each year with HCV.[3]

Despite its prevalence, there are no specific therapeutics available for acute Hepatitis B and only limited treatment options (accessible only in wealthy countries) for chronic Hepatitis B. While a combination antiviral therapy with interferon and ribavirin is effective against some genotypes of Hepatitis C, the treatment is costly, poorly tolerated, and not available worldwide – making it unfeasible for most patients.[3] There is a great need for new drugs to treat hepatitis effectively.

One of the major hurdles to the development of new hepatitis virus treatments is the lack of biologically relevant and predictive model systems for studying and understanding hepatitis-induced liver disease. Existing hepatocyte model systems include primary human hepatocytes harvested from cadavers, immortalized cell lines (such as HepG2, HepaRG, and Huh7), and animal models. Unfortunately, each of these model systems suffers limitations in terms of functionality, reproducibility, and/or accessibility. As an example, supplies of primary human hepatocytes are often limited, and those that are available can show great variability in phenotypic responses to stimuli depending on the genotype and environmental background of the donor.[4]

iPSC-derived Hepatocytes. iCell® Hepatocytes were developed to overcome the limitations of existing hepatocyte model systems; they are well-characterized, highly reproducible, functionally stable, and available in limitless quantities. iCell Hepatocytes are a highly pure population of human hepatocytes derived from induced pluripotent stem cells (iPSCs) using proprietary differentiation and purification protocols. These cells exhibit definitive morphological features (including cobblestone patterning, round nuclei with distinct nucleoli, bi-nucleation, and bile canalicular networks) characteristic of hepatocytes and are proven to express alpha-1-antitrypsin (AAT) and albumin at levels indicative of a pure culture. In addition, iCell Hepatocytes display intrinsic metabolism (e.g. glycogen and lipid storage), xenobiotic metabolism, and transporter functions and respond appropriately to known hepatotoxicants. iCell Hepatocytes also maintain better viability and morphology during long-term cultures of >14 days than do primary human hepatocytes.

Studying Viral Infection and Growth Using iPSC-derived Hepatocytes. iCell Hepatocytes have been validated to sustain hepatitis virus infection. Recent data suggest the utility of these cells for supporting HCV host cell attachment and internalization, as well as serving as a suitable environment for studying the viral replication life cycle.

Methods

iCell Hepatocytes (Cellular Dynamics, #HCC-100-010-001-PCC) were infected with the genotype 1a HCV serum at an MOI of 10 at 3 days post-plating. Cells were incubated with virus for 2 - 4 hours at which time the viral inoculum was removed and fresh medium added. Cells were then incubated for 3 days, and immunostaining was performed using an antibody raised against HCV core protein.

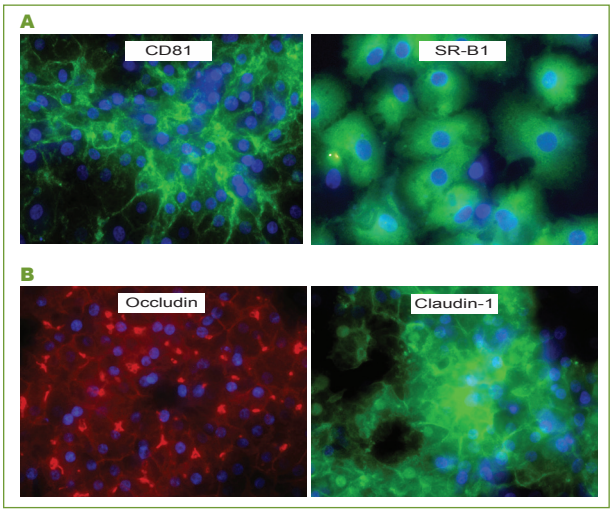
Results & Discussion

Many markers are required to enable HCV attachment to, and uptake by, a human liver cell. Gene expression analysis of four of these markers (CD81, SR-B1, occludin, and claudin-1) across multiple cell types shows that transcriptional levels of iCell Hepatocytes markers are consistent with those of primary hepatocytes. Moreover, iCell Hepatocytes exhibit a high degree of lot-to-lot reproducibility (Table 1).

Gene Transcript	Primary Human Hepatocytes (relative expression)	iCell Hepatocytes		
		Lot 1	Lot 2	Lot 3
CD81	12.5	11.1	11.6	10.9
SR-B1	10.9	10.5	11.6	11.0
Occludin	7.6	7.4	7.3	7.7
Claudin-1	9.1	8.9	10.0	9.4

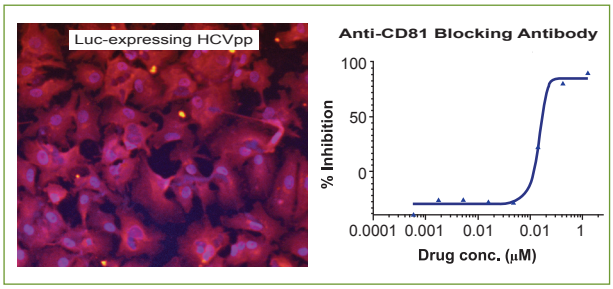
▲ **Table 1: iCell Hepatocytes Are Comparable to Primary Liver Cells and Provide Lot-to-Lot Reproducibility**
iCell Hepatocytes consistently and reproducibly express key markers required for HCV attachment and internalization at levels equivalent to primary human hepatocytes by gene transcriptional analysis.

Immunohistochemical staining of these same four cell surface markers in 2D cultures of iCell Hepatocytes shows strong and uniform expression levels (Figure 1).



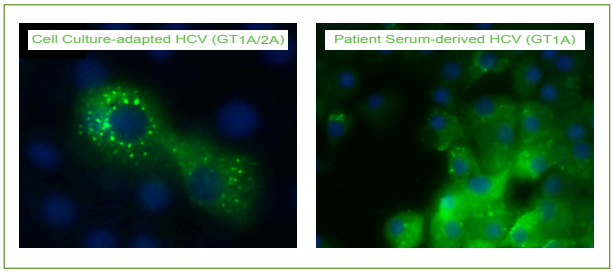
▲ **Figure 1: iCell Hepatocytes Exhibit Uniform Expression of Important HCV Attachment and Uptake Markers**
*Immunohistochemical staining shows the expression of protein markers required for (A) HCV attachment, CD81 and SR-B1, and (B) HCV uptake, occludin and claudin-1.**

Follow-on studies using viral particles strongly confirm that iCell Hepatocytes exhibit markers required for HCV uptake and demonstrate responsiveness to HCV. Fluorescent image analysis of iCell Hepatocytes exposed to a luciferase-tagged viral particle demonstrate successful uptake of that pseudoparticle. Uptake of the engineered HCV pseudoparticle can be inhibited in a dose-dependant fashion by the addition of an anti-CD81 blocking antibody (Figure 2).



▲ **Figure 2: iCell Hepatocytes Exhibit Markers Required for HCV Uptake**
*Sensitivity to HCV is demonstrated in iCell Hepatocytes via the uptake of Luc-expressing HCV pseudoparticles (HCVpp). HCVpp uptake is inhibited by an anti-CD81 blocking antibody, highlighting the importance of CD81 in viral attachment.**

Importantly, data also demonstrate the ability of iCell Hepatocytes to internalize live HCV procured from a cell culture-passaged virus of genotype 1a/2a and a patient serum-derived virus of genotype 1a (Figure 3). The patient-derived HCV genotype 1a is most clinically relevant and difficult to handle in many tissue culture models due to poor infectivity.



▲ **Figure 3: iCell Hepatocytes Internalize Live HCV**
*Cell culture-adapted HCV genotype 1a/2a is internalized by iCell Hepatocytes, showing the sensitivity of iCell Hepatocytes to fully functional HCV. More relevant to the clinical scenario, iCell Hepatocytes are sensitive to patient serum-derived HCV (genotype 1a).**

The data validate that iCell Hepatocytes have the ability to support HCV binding and uptake. While the extracellular receptor responsible for HBV attachment to human liver cells has been discovered only recently to be NTCP[5], it is likely that iCell Hepatocytes, whose functionalities and morphology so closely mimic those of primary human hepatocytes, will similarly sustain HBV binding and uptake.

The hepatitis viral replication cycle begins with virus binding to the host cell and crossing the plasma membrane to gain access to the inner contents of the cell. With the power to enable the first critical step in the replication process, iCell Hepatocytes promise to be the ideal setting for hepatitis researchers to investigate all remaining viral life cycle stages.

Identifying Novel Therapeutics Using iPSC-derived Hepatocytes. As data presented earlier (Figures 2 and 3) demonstrate that iCell Hepatocytes take up both engineered HCV pseudoparticles and intact live virus, iCell Hepatocytes can serve as a highly effective model system for identifying inhibitors of HCV, and potentially also HBV, in the preclinical drug discovery realm. Because iCell Hepatocytes can be produced in limitless quantities, they also easily meet the large volume requirements of high-throughput screening assays typically used to identify new therapeutic hits and evolve leads. For these reasons, iCell Hepatocytes are expected to play a significant role in the development of innovative, novel hepatitis treatments.

Conclusion

iCell Hepatocytes are a predictive in vitro model system for the study of HCV infection in human liver cells. They also hold great potential to serve as a similar model for HBV research. These iPSC-derived cells have demonstrated the ability to support hepatitis virus host cell attachment and internalization and show promise for studying the viral replication life cycle. iCell Hepatocytes are able to overcome the limitations of conventional hepatocyte models and deliver a reliable source of high quality, well-characterized, highly reproducible, and readily available human hepatocytes for hepatitis virus research and preclinical drug development efforts.

References

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2. World Health Organization (2013) Hepatitis B Fact Sheet N204.
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4. Mann D, Einhorn S, et al. (2013) Human iPSC-derived Hepatocytes: Functional Model Tissue for In Vitro Predictive Metabolism, Toxicity, and Disease Modeling. GEN **33**(9):32-33.
5. Yan H, Zhong G, et al. (2012) Sodium Taurocholate Cotransporting Polypeptide Is a Functional Receptor for Human Hepatitis B and D Virus. eLife **1**:e00049.

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