Abstract: Differences between animals and humans in liver pathways now necessitate the use of in vitro models of the human liver for several applications such as drug screening. However, isolated primary human hepatocytes (PHHs) are a limited resource for building such models given shortages of donor organs. In contrast, human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) can be propagated nearly indefinitely and differentiated into hepatocyte-like cells in vitro using soluble factors inspired from liver development. Additionally, iPSCs can be generated from patients with specific genetic backgrounds to study genotype-phenotype relationships. While current protocols to differentiate hESCs and iPSCs into human hepatocyte-like cells (hESC-HHs and iPSC-HHs) still need improvement to yield cells functionally similar to the adult liver, proof-of-concept studies have already shown utility of these cells in drug development and modeling liver diseases such as α1-antitrypsin deficiency, hepatitis B/C viral infections, and malaria. Here, we present an overview of hESC-HH and iPSC-HH culture platforms that have been utilized for the aforementioned applications. We also discuss the use of semiconductor-driven microfabrication tools to precisely control the microenvironment around these cells to enable higher and longer-term liver functions in vitro. Finally, we discuss areas for improvement in creating next generation stem cell-derived liver models. In the future, stem cell-derived hepatocyte-like cells could provide a sustainable cell source for high-throughput drug screening, enabling better mechanistic understanding of human liver diseases for the development of more efficacious and safer therapeutics, and personalized cell-based therapies in the clinic. [Discovery Medicine 19(106):349-358, May 2015]

Introduction

As the largest internal organ, the liver has over 500 functions, such as glucose and fatty acid metabolism, and detoxification of endogenous and exogenous (i.e., drugs) substances. Additionally, the liver is the target for several diseases such hepatitis B/C viral infection, malaria, and steatosis (fatty liver). Studies in animals are not always predictive of human outcomes due to the significant species-specific differences in liver pathways (Olson et al., 2000). Thus, several in vitro models of the human liver have been developed to complement animal testing such as microsomes, cancerous hepatic cell lines, isolated primary human hepatocytes (PHHs), and precision cut liver slices (Khetani et al., 2015). Of these, PHHs are widely considered to be the “gold standard” for constructing in vitro models of the human liver due to their intact cellular architecture, ability to function for several weeks in vitro, and precise control of culture conditions. However, both the scarcity of healthy liver tissue and significant interdonor variability in phenotypic functions limit PHH use for high-throughput drug screening. Furthermore, a lack of available PHH donor diversity makes it difficult to explore the genetic basis of several diseases. Finally, PHHs are not suitable for use in personalized drug screening and therapies for live patients.

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) can potentially address the aforementioned limitations of PHHs and serve as...
complementary and sustainable cell sources for several applications. hESCs are isolated from the inner cell mass of a blastocyst, which gives rise to all three germ layers (endoderm, mesoderm, ectoderm) in the embryo (Thomson et al., 1998). These cells maintain their undifferentiated state over many passages (self-renewal) and can be differentiated into all three germ layers both in vitro and in vivo (Agarwal et al., 2008). However, ethical concerns around the destruction of embryos have led to significant limitations in generation of new hESC lines using U.S. federal funding. On the other hand, iPSCs have been generated from adult somatic cells (i.e., skin biopsy) via ectopic expression of select genes and thus offer a nearly unlimited supply of stem cells without the aforementioned ethical concerns of hESCs (Figure 1A) (Takahashi et al., 2007; Yu et al., 2007). iPSCs maintain all aspects of pluripotency and allow for the creation of genetically diverse donor panels to help elucidate inter-individual variations in drug response and disease progression. More recently, iPSCs lacking viral vectors and transgene sequences have been generated with non-integrating episomal vectors for applications in regenerative medicine (Yu et al., 2009). Hepatocyte-like cells have even been generated from somatic cells through transdifferentiation protocols, which bypass the need for a pluripotent stage altogether (Figure 1A) (Huang et al., 2014; Zhu et al., 2014).

Several in vitro protocols have been established to differentiate hESCs and iPSCs towards human hepatocyte-like cells (hESC-HHs and iPSC-HHs, respectively) (Agarwal et al., 2008; Gerbal-Chaloin et al., 2014; Schwartz et al., 2014). Such protocols rely on soluble factors (i.e., growth factors) to mimic sequential differentiation of stem cells down various stages of liver development as in vivo (Figure 1B). Protocols that have eliminated serum, feeder cell layers, and undefined reagents lead to better batch-to-batch consistency and higher purity of hepatocyte-like cells. More recently, semiconductor-driven micropatterning approaches and co-culture with stromal cells have further improved the maturation status of iPSC-HHs (Berger et al., 2014). Such advances in differentiation protocols have allowed hESC-HHs and iPSC-HHs to be used in downstream applications. In this review, we discuss hESC-HH and iPSC-HH generation and applications.

**Figure 1.** Generation of human hepatocyte-like cells from somatic cells. (A) Flow diagram of various methods for generating human hepatocyte-like cells. (Top) A series of growth factors and small molecules convert somatic cells into endoderm progenitor cells, which then enter hepatic differentiation (Zhu et al., 2014). (Middle) Somatic cells are converted to an induced pluripotent stem cell (iPSC) intermediate via non-integrating plasmid vectors (i.e., OCT3/4, SOX2, C-MYC, KLF4, NANOG, LIN28) and differentiated down the hepatic lineage (Schwartz et al., 2014). (Bottom) Somatic cells are converted directly to hepatocyte-like cells via transcription factors (Huang et al., 2014). (B) Typical protocol for the directed differentiation of iPSCs down the hepatic lineage along with representative growth factors used by various groups (Schwartz et al., 2014; Gerbal-Chaloin et al., 2014).
Stem Cell-derived liver Cells for Drug testing and Disease Modeling

Several groups have implemented hESC-HHs in the respective (Basma et al., 2009). Similarly, CYP3A4 and CYP1A2 in hESC-HHs were inducible with phenobarbital and β-naphthoflavone, whereas similar trends were not seen with the cancerous HepG2 cell line (Hay et al., 2008). Similarly, CYP3A4 and CYP1A2 in hESC-HHs were inducible with phenobarbital and β-naphthoflavone, respectively (Basma et al., 2009).

Several groups have implemented hESC-HHs in the preclinical evaluation of drug-mediated hepatotoxicity. Following 72 hours of dosing with two compounds metabolized by either CYP2C9 or CYP2D6, viability of hESC-HH cultures declined significantly (Medine et al., 2013), suggesting activation of the compounds into reactive metabolites by the CYPs. The same investigators also expanded their compound set to include 20 hepatotoxins and found a system sensitivity of 75% (relative to clinical liver liabilities of the compounds) as assessed by measurement of apoptosis via caspase 3/7 enzyme activity and ATP levels (Szkolnicka et al., 2014). However, this compound set was generally restricted to overtly toxic compounds, and known hepatotoxins such as diclofenac were not found to be toxic in hESC-HHs. On the other hand, Yildirimman et al. (2011) used hESC-HHs and principal component analysis to correctly bin 15 compounds into genotoxic carcinogens, non-genotoxic carcinogens, and non-carcinogens.

Stem Cell-derived Liver Cells in Drug Development

hESC-derived human hepatocyte-like cells

Since their first discovery, hESC-HHs have undergone extensive characterization for their drug metabolism enzymes (i.e., cytochrome P450s or CYPs), which can either detoxify drugs and/or metabolize them into reactive metabolites that can cause cell toxicity. When cultured on a feeder layer of mouse embryonic fibroblasts, multiple hESC lines differentiated down the hepatic lineage were shown to have detectable levels of many CYPs, although CYP2C9, CYP2C19, CYP2D6, and CYP3A4 were undetectable and fetal CYP3A7 was highly expressed, suggesting an immature phenotype (Ek et al., 2007). Ulvestad et al. (2013) also found similar trends in CYP gene expression. Duan et al. (2010), on the other hand, showed that most CYP proteins were expressed in several batches of hESC-HHs. Albumin and α1-antitrypsin gene expression levels were 75% and 64% of freshly isolated PHHs, respectively. Moreover, liquid chromatography/mass spectrometry analysis revealed that phase-II conjugation metabolites of bufuralol in hESC-HHs and PHHs were nearly identical. Several glutathione transferases (GSTA1-1, GSTM1-1, GSTP1-1, and GSTA1-1) have also been found to be positive at the protein level in hESC-HHs (Söderdahl et al., 2007). However, another study described hESC-HHs that had significantly low CYP3A4 activity (most abundant CYP in the liver) with a 1,000-fold reduction in CYP3A4 transcripts relative to PHHs (Wobus and Löser, 2011). Altogether, these studies suggest that further maturation of hESC-HHs towards an adult liver phenotype is needed. However, potential differences in the maturation status across different cell lines and differentiation protocols cannot be entirely ruled out to explain the variable findings of the aforementioned studies. Nonetheless, despite having high alpha-fetoprotein (fetal marker) expression, hESC-HHs treated with rifampin showed an induction of CYP3A4 activity, whereas similar trends were not seen with the cancerous HepG2 cell line (Hay et al., 2008). Similarly, CYP3A4 and CYP1A2 in hESC-HHs were inducible with phenobarbital and β-naphthoflavone, respectively (Basma et al., 2009).

In contrast to hESC-HHs, there have been much fewer attempts to characterize the enzymatic profile of iPSC-HHs, partly due to the more recent introduction of this technology to the field. Ulvestad et al. (2013) differentiated iPSCs from human dermal fibroblasts transduced with OCT4, SOX2, KLF4, and C-MYC cultured on a feeder layer of growth-arrested human dermal fibroblasts. These iPSC-HHs were shown to stain positive for CYP1A, CYP2B6, CYP2C9, and CYP3A, and enzymatic activities were verified by measuring metabolites generated from prototypical drug substrates. However, metabolites generated in iPSC-HH cultures were at least an order of magnitude lower than PHHs after 4 hours in culture. Gene expression also validated these findings, showing CYP1A1 upregulated (>100-fold higher) and many other CYPs (1A2, 2B6, 2C9, 3A4) downregulated (most >100-fold lower) compared to PHHs. Several key transporter proteins were expressed in iPSC-HHs, albeit not to the same level as PHHs.

Several investigators have utilized iPSC-HHs for drug toxicity screens. iPSC-HHs were incubated with the same compounds as hESC-HHs in the aforementioned study (Medine et al., 2013). The CYP2D6-metabolized compound caused near-complete loss of iPSC-HH viability after 72 hours of dosing, while the CYP2C9-metabolized compound caused only a nominal decrease in viability compared to vehicle-only controls, suggesting that CYP2C9 levels were very low compared to hESC-HHs. However, such a result could potentially be explained by polymorphisms in the CYP2C9 gene, which would need to be verified using genotyping analysis. In contrast to HepG2, iPSC-HH cultures were...
more sensitive to time- and dose-dependent toxicity of amiodarone, aflatoxin B1, and troglitazone (Holmgren et al., 2014). Additionally, iPSC-HHs treated with staurosporine showed a similar dose response curve for ATP, caspase 3/7, and glutathione as three separate donors of PHHs (Sjogren et al., 2014). iPSC-HHs have also been dosed with a library of 240 compounds and subsequently subjected to high-content (multi-fluorescent) imaging (Sirenko et al., 2014). Calcein-AM (cell viability stain) and MitoTracker-Orange (mitochondrial health stain) were found to be the most sensitive markers. However, comparisons to PHH cultures dosed with the same drug set were lacking. Nonetheless, this study showed the compatibility of iPSC-HHs with high content imaging analysis to evaluate mechanisms of drug toxicity at the organelle level.

One of the great advantages of iPSC technology is the ability to study the impact of genetics on cell responses to drugs. For instance, in a recent study, iPSC-HHs with or without a single nucleotide polymorphism (SNP) in CYP2D6 were co-cultured on a transwell membrane with MCF7 breast cancer cells (Takayama et al., 2014). In comparison to iPSC-HHSs with wild-type CYP2D6 alleles, cells with the CYP2D6*4 SNP (slow metabolizer phenotype) showed a higher resilience to tamoxifen, which is metabolized by CYP2D6 into a toxic metabolite. These same cells showed a higher susceptibility to desipramine, which is detoxified by CYP2D6. While these results showed proof-of-concept in evaluating the effects of CYP polymorphisms on drug toxicity, a panel of iPSC-HHs with different polymorphisms would be useful to get a more complete picture of effects on drug responses. Recently engineered nucleases such as CRISPR/Cas9 and TALENs allow precise gene editing with minimal off-target modifications (Smith et al., 2015) and thus could also be used to generate such a panel of genetically diverse iPSC-HHs.

**Engineered cultures of stem cell-derived hepatocyte-like cells**

Engineering tools such as micropatterning, microfluidics, and synthetic biomaterials have been used extensively for the culture of primary hepatocytes in order to enable higher and longer-term functions than possible with conventional (declining) confluent monolayers (Khetani et al., 2015). While the application of such tools to stem cell-derived hepatocyte-like cells is still in its infancy, a handful of groups have embarked on such efforts. We have recently engineered a micropatterned co-culture platform (iMPCC) in which commercially available iPSC-HHs are organized onto collagen-coated domains of empirically optimized dimensions and subsequently surrounded by 3T3-J2 murine embryonic fibroblasts (Figure 2A) (Berger et al., 2014). Such control over cell-cell interactions between iPSC-HHs and their stromal neighbors enabled high, stable levels of liver-specific functions and a significant reduction in fetal markers for at least 4 weeks in vitro as compared to a declining phenotype in conventional iPSC-HH monolayers. Activities of major CYPs in iMPCCs ranged from ~5% (CYP2C19) to ~70% (CYP1A2) of PHH activities. CYP3A4 activity in iMPCCs was at 90% of levels observed in conventional PHH cultures after 24 hours in culture and up to 40-55% of levels observed in stable cultures of PHHs from multiple donors in the same micropatterned co-culture format. Furthermore, CYP activities in iMPCCs could be induced by severalfold following treatment with prototypical drugs, which enables utility of iPSC-HHs in assessing potential for drug-drug interactions in the clinic.

**Figure 2.** Engineered cultures of induced pluripotent stem cell-derived human hepatocyte-like cells (iPSC-HHs). (A) Generation of iPSC-HH-based micropatterned co-cultures (iMPCCs) with stromal fibroblasts. Tissue culture polystyrene (TCPS) wells coated with collagen and then subjected to soft lithography-based patterning. iPSC-HHs are seeded to fill the islands, and 3T3-J2 murine embryonic fibroblasts complete the iMPCC model (Berger et al., 2014). Adapted with permission from Wiley. (B) Nanopillar plate used to generate 3D spheroids of iPSC-HHs (Takayama et al., 2013). Adapted with permission from Elsevier.
Even though the iPSC-HHs in iMPCCs are not fully differentiated to the same level as PHHs with respect to several functions, we were still able to utilize this platform effectively for predictive toxicology studies. iMPCCs, created in industry standard 96-well plates, were treated for 6 days with 47 drugs, and functional endpoints (albumin, urea, and ATP) were evaluated in dosed cultures with comparison to control cultures treated with solvent vehicle alone (Figure 3) (Ware et al., 2015). We found that iMPCCs correctly identified 24 of 37 hepatotoxic drugs (65% sensitivity), while all 10 non-toxic drugs tested were classified correctly (100% specificity). Conventional confluent cultures of iPSC-HHs, on the other hand, failed to detect several liver toxins that were picked up in both iMPCCs and PHH cultures. The sensitivity for hepatotoxicity detection in iMPCCs was similar to data obtained in MPCCs created using PHHs (65% versus 70% sensitivity) that were also dosed with the same drug set (Khetani et al., 2013). Finally, iMPCCs detected the relative hepatotoxicity of structural drug analogs and duplicated known bioactivation mechanisms of acetaminophen toxicity in vitro, which suggests that the platform may be useful for prospective drug screening and investigative toxicology efforts.

In contrast to organizing iPSC-HHs in a 2D format as in iMPCCs, Takayama et al. (2013) differentiated iPSC-HHs and hESC-HHs in 3D spheroids on a nanopillar surface (Figure 2B). Several liver functions (i.e., albumin secretion, urea synthesis, CYP activities) in the spheroids were higher than those measured in monolayers, potentially due to the establishment of homotypic cell-cell contacts and presence of key ECM components within and around the aggregates. The investigators also treated iPSC-HH spheroids with multiple doses of 24 drugs and compared results to HepG2 spheroids. Most drugs showed a higher sensitivity in iPSC-HH spheroids as opposed to HepG2 spheroids. However, acetaminophen and troglitazone had a much lower sensitivity in iPSC-HHs than in PHH monolayers.

Figure 3. Utility of induced pluripotent stem cell-derived human hepatocyte-like cells (iPSC-HHs) in preclinical drug toxicity screens. (A) Albumin secretions in micropatterned co-cultures (iMPCCs) containing iPSC-HHs and 3T3-J2 murine embryonic fibroblasts after treatment for 8 days with either diclofenac (hepatotoxic) or aspirin (non-toxic) at various doses. Data was normalized to dimethyl sulfoxide (DMSO)-only controls. C_{max} represents the maximum concentration of a given drug measured in human plasma. (B) Representative images of iMPCCs before and after treatment with drugs. DMSO-treated cultures did not appear morphologically different than aspirin-treated cultures. Scale bars are 400 µm. (C) Comparison of sensitivity (percent of 37 clinical liver toxins correctly identified in various in vitro models) and specificity (percent of 10 non-liver-toxic drugs correctly identified) in extracellular matrix (ECM) sandwich cultures of primary human hepatocytes (SCHH), micropatterned co-cultures containing primary human hepatocytes and 3T3-J2 fibroblasts (PHH-MPCCs), and iMPCCs (Khetani et al., 2013; Ware et al., 2015).
at 48 hours. Thus, it remains unclear whether 3D spheroidal architecture can enable higher sensitivity in iPSC-HHs than what we have observed with 2D iMPCCs as described above. Ultimately, it will be important to derive all of the stromal cell types of the liver (i.e., sinusoidal endothelial cells, stellate cells, Kupffer macrophages) from stem cells and culture them with hepatocyte-like cells (in 2D or 3D) to create a liver model that is more reflective of physiology and can mimic interactions of multiple cell types following drug exposure.

**Stem Cell-derived Liver Cells for Disease Modeling**

*Monogenic disease models*

The first attempts at developing *in vitro* models of liver diseases using iPSC-HHs have focused on monogenic diseases (i.e., single gene mutation), since the effect of the single mutation on disease progression can be studied *in vitro* as opposed to more complex diseases where multiple genes and environmental stimuli have been implicated as triggers (i.e., fatty liver, cirrhosis). Although rare, monogenic liver diseases account for 15% to 20% of the causes for liver transplantation in children (Ordonez and Goldstein, 2012). iPSC-HHs have been generated from patients with known genetic disorders and gene editing tools have been employed to effectively correct the mutation *in vitro*. Such methods allow investigators to obtain deeper mechanistic insights into the progression of disease due to the mutation, since mutated cells can be compared to the same non-mutated genetic background. Below, we present examples of major monogenic liver diseases where iPSC-HHs have been utilized *in vitro*.

Several diseases of the liver involve misfolding and thereby dysfunction of proteins. For instance, α1-antitrypsin (A1AT) deficiency (ATD) is an inherited liver disease where a mutation in the gene coding for A1AT, *SERPINA1*, causes the protein to misfold and polymerize within hepatocytes, effectively lowering blood plasma levels of A1AT (Fairbanks and Tavill, 2008; Fregonese and Stolk, 2008). Rashid *et al.* (2010) were the first to model any liver disease using iPSC technology by using patient-derived iPSC-HHs from ATD and control patients. The ATD iPSC-HHs retained A1AT polymers within their cytoplasm, a hallmark of this disease. However, the amount of polymer accumulation differed between donors, which could help explain the differences in severity of disease progression and hepatoxicity observed across patients in the clinic (Nelson *et al.*, 2012). Indeed, follow-up work by Tafaleng *et al.* (2015) using iPSC-HHs from ATD patients with or without severe liver disease further supported such differences. In particular, greater severity of clinical disease correlated positively with the reduced ability of ATD iPSC-HHs to degrade intracellular A1AT polymers *in vitro*. Yusa *et al.* (2011) successfully corrected the mutation in A1AT at the iPSC stage using zinc-finger nucleases. Corrected cells were then differentiated into iPSC-HHs and showed similar A1AT enzymatic activity as PHHs, suggesting reversal of the disease phenotype. Furthermore, corrected iPSC-HHs transplanted into mice engrafted in the liver and produced liver markers, which identified a potential cell-based therapy for this disease in the future. Choi *et al.* (2013) also used TALENs (transcription activator-like effector nucleases), a cheaper and easier gene editing method than zinc-finger nucleases, to correct the A1AT mutation.

Familial transthyretin amyloidosis (FTA) is another monogenic disease that causes misfolding of transthyretin secreted from the liver. This misfolded protein forms insoluble fibrils which subsequently cause damage to other organs such as the heart and brain (Ando *et al.*, 2005). Leung *et al.* (2013) demonstrated that neurons, cardiomyocytes, and hepatocytes could be generated from iPSCs derived from patients with FTA. Conditioned medium from iPSC-HHs with the FTA mutation caused loss of viability in the iPSC-derived cardiomyocytes and neurons. This was one of the first “multi-organ” interaction studies and highlights the potential of stem cell technologies in identifying unknown disease mechanisms involving multiple tissues.

Some diseases of the liver lead to abnormal accumulation of otherwise physiological molecules. For instance, glycogen storage disease type 1a (GSD1a) is caused by a mutation in the glucose-6-phosphatase gene, which inhibits hepatocytes from releasing glucose into the blood and leads to the irregular buildup of glycogen and lipids in the cytoplasm (Rake *et al.*, 2002). iPSC-HHs derived from patients with GSD1a developed an increased amount of glycogen and lipids when cultured *in vitro* (Rashid *et al.*, 2010). GSD1a iPSC-HHs also responded to the pancreatic hormone glucagon and mimicked the increased lactic acid production seen in patients. This is an important first step towards developing therapies for GSD1a, which currently does not have a cure.

Besides GSD1a, Wilson’s disease (WD) is characterized by accumulation of copper in the liver, which leads to hepatocyte damage and liver failure. This disease is caused by a mutation in the gene ATP7B, which codes for a protein that allows copper to be transported out of hepatocytes (Roberts *et al.*, 2008). iPSC-HHs derived
from patients with WD recapitulated the abnormal copper transport and ATP7B cytoplasmic localization in vitro (Zhang et al., 2011; Yi et al., 2012). The phenotype of mutated WD cells could be reversed by overexpression of the wild type gene ATP7B or treatment with curcumin, suggesting a potential therapy for WD.

Niemann-Pick disease type C (NPC) is characterized by abnormal cholesterol metabolism in the brain and liver, which can be fatal (Carstea et al., 1997). iPSC-HHs derived from patients with NPC were used to identify new compounds that alleviated the toxicity caused by cholesterol accumulation (Maetzel et al., 2014; Soga et al., 2015). Importantly, Maetzel et al. (2014) successfully corrected the mutation in NPC iPSC-HHs using TALENs, which rescued the cells from toxicity.

Diseases due to drugs and alcohol

Valproic acid (VPA) is a versatile drug used to treat a variety of issues ranging from seizures to bipolar disorder, but it is associated with idiosyncratic liver injury (Nanau and Neuman, 2013). Alpers syndrome, a monogenic disease caused by a mutated mitochondrial polymerase, places patients at an increased risk of acute liver failure when treated with VPA (Schwabe et al., 1997). Li et al. (2015) recently developed iPSC-HHs from patients with Alpers syndrome and showed increased sensitivity to VPA-induced apoptosis via a mitochondrial-dependent mechanism. Chemical modification of mitochondrial pathways was then used to rescue Alpers syndrome iPSC-HHs from VPA-induced toxicity, which may open up new avenues for multidrug therapy to reduce incidences of drug toxicity in Alpers syndrome patients treated with VPA.

Excessive and chronic alcohol consumption leads to alcoholic liver disease (ALD), which can display several phases ranging from steatosis (fat accumulation) to steatohepatitis (inflammation with fat accumulation), cirrhosis (severe scarring of liver with excessive extracellular matrix deposition), and hepatocellular carcinoma (Tilg and Day, 2007). Excessive alcohol use during pregnancy can also lead to fetal alcohol spectrum disorders (FASD), which cause similar pathology in fetal livers as adult ALD (Hofer and Burd, 2009). Tian et al. (2014) exposed iPSC-HHs to ethanol at different stages of differentiation and found that hepatic progenitor cells were especially sensitive to apoptosis during their transition from endoderm to hepatic progenitor cells, which may shed insights into susceptibility of the fetus to ethanol at different stages of embryonic development in vivo. Furthermore, mature iPSC-HHs exposed to ethanol recapitulated the pathology of ALD such as increased lipid accumulation.

Hepatotropic infectious diseases

The use of iPSC-HHs for hepatitis C viral (HCV) infection has been extensively reviewed elsewhere (Cheng et al., 2015; Sampaziotis et al., 2014), and thus here we will focus on the more recent developments for hepatitis B viral (HBV) infection. HBV is currently incurable and affects approximately 400 million people worldwide. Due to the chronic nature of this disease, it poses considerable risks for developing cirrhosis and hepatocellular carcinoma. Very few model systems accurately depict virus-host interactions, which limits mechanistic studies of HBV and thus the development of novel therapies. Recently, Shlomai et al. showed for the first time that iPSC-HHs could be infected by HBV-positive human serum, but that such infection was dependent on the differentiation status of the iPSC-HHs (Shlomai et al., 2014). Chemically inhibiting the innate immune response increased viral permissiveness. However, iPSC-HHs did not show the same level of infection as PHHs, which could potentially be further improved by differentiating iPSC-HHs closer to the adult PHH phenotype.

Malaria is a prevalent pathogen in the developing world with ~250 million cases globally and ~3,000 deaths daily. Liver infection arises shortly after Plasmodium sporozoites from a mosquito bite migrate, via the blood, to hepatocytes, where they undergo maturation and proliferate to form schizonts. Eventually hepatocytes lyse and release pathogenic merozoites into the blood, which can infect red blood cells. PHHs have been successfully used to model the liver stage of infection at a high enough rate to subsequently infect red blood cells, the next phase in the Plasmodium life cycle (March et al., 2013). This same group recently identified the stage in differentiation where iPSC-HHs were susceptible to malaria infection, and thus could be used to model the Plasmodium liver stage of infection and study host-pathogen interactions across several genetic backgrounds (Figure 4) (Ng et al., 2015). Furthermore, small molecules were used to enhance CYP activities in iPSC-HHs in order to allow sufficient metabolism of anti-malaria prodrugs into their efficacious metabolites. The number of iPSC-HHs infected were 10–60% of the number of PHHs infected with Plasmodium. However, infection of red blood cells from merozoites released from the iPSC-HH cultures has not yet been demonstrated.

Summary and Future Outlook

While PHHs are widely used for constructing in vitro models of the human liver, they are ultimately a limited resource with limited genetic diversity and cannot be
used for applications in personalized drug screening and medicine. Over the last two decades, stem cell-derived hepatocyte-like cells have emerged, which can potentially mitigate such limitations inherent with use of PHHs. Hepatocyte-like cells have been generated using both hESCs and, more recently, iPSCs. The use of these cells for evaluating drug metabolism and toxicity as well as modeling several types of liver diseases has increased over the last few years. Engineering tools that can be used to precisely control the microenvironment around liver cells in order to enable more physiologic and long-term liver functions are now being applied to stem cell-derived hepatocyte-like cell culture. However, considerable work needs to be done to further mature stem cell-derived hepatocyte-like cells towards a more adult PHH phenotype with measurements of standardized phenotypic markers such that maturation data across different laboratories can be effectively compared. Furthermore, stem cells need to be differentiated into the various stromal cell types of the liver, and co-cultured with hepatocyte-like cells to enable complex physiologic functions and responses to drugs and diseases. The commercial availability of reproducibly made cells from different genetic and disease backgrounds also needs to improve since many investigators have neither the expertise nor the resources to differentiate stem cells into liver cells, but still want to use the cells for downstream applications. Even with the aforementioned challenges that need to be overcome, the proof-of-concept studies to date in drug screening and disease modeling using stem cell-derived hepatocyte-like cells have been highly promising as we discussed above. We anticipate that, in the future, stem cell-derived liver cells will not only provide a near-infinite source of cells for several applications, but also fulfill the promise of personalized drug screening and

![Figure 4](Image)

**Figure 4.** Drug screening platform development and infection of induced pluripotent stem cell-derived human hepatocyte-like cells (iPSC-HHs) with malaria parasite. (A) Flow diagram of infectious disease-specific drug screening platform development. (B) Characterization of iPSC-HH morphology and host entry factors for malaria sporozoite infection. (C) Representative fluorescent images of iPSC-HHs (large blue nuclei) infected with sporozoites from a human-specific malaria strain (red), *Plasmodium falciparum*, at day 3 (left image) and day 6 post infection (middle image). Right image shows immunofluorescence of the *Plasmodium* maturation marker, MSP1 (malaria merozoite surface protein 1), in iPSC-HHs at day 6 post infection. The right panel shows the size distributions of *P. falciparum* exoerythrocytic forms (EEFs), or colonies, in iPSC-HHs at day 4 and day 6 post infection. Scale bars are 5 µm. (D) iPSC-HHs were treated with small molecule maturation factor, FPH1 (functional proliferation hit 1), or solvent vehicle, DMSO (dimethyl sulfoxide), to enable iPSC-HHs metabolism of primaquine (PQ), a prototypical anti-malaria drug. Small molecule treatment potentially enabled PQ metabolism and subsequent inhibition of *P. falciparum* propagation in iPSC-HHs. Adapted from (Ng et al., 2015) under the Creative Commons Attribution 4.0 license.
therapies.

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Disclosure

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