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TITLE: Microfluidic liver cultures as preclinical tool for the study of hepatitis B and C virus as well as malaria

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ABSTRACT BODY: Abstract Body (Late-Breaking Submission): Liver-tropic pathogens including hepatitis B virus (HBV), hepatitis C virus (HCV) and malaria are a major health concern with more than 620 million people infected worldwide. The rapid de-differentiation of primary human hepatocytes in 2D cell culture poses significant limitations to study host-pathogen interactions and to develop novel therapies against these infectious diseases in physiological settings. Here, we describe a novel 3D microfluidic primary hepatocyte culture system permissive to HCV, HBV and malaria, which, in contrast to all other available model systems, maintains the hepatic phenotype for at least 40 days without alteration of hepatic metabolism, cell viability or degree of differentiation. Cells form functional microtissues including bile canaliculi and tight junctions. We demonstrate, for the first time, that HBV patient-derived viral isolates can successfully launch infection and maintain robust levels of replication, resulting in the production of HBV cccDNA as well as infectious progeny virus. Additionally, 3D hepatocyte cultures become susceptible to non-JFH1-derived HCV including genotype 1a patient samples. We demonstrate proof of concept data for the evaluation of novel direct-acting antivirals against HCV, including Ledipasvir and Sofosbuvir for genotypes 1 and 3 as well as Tenofovir alafenamide for HBeAg-positive and –negative HBV isolates. HBV infection induces a pro-angiogenic signature in infected hepatocytes, which is suppressed when co-culturing primary hepatocytes and Kupffer cells. Interestingly, we identify a cellular factor induced by HBV infection, which may be responsible for inactivation of Kupffer cells and the resulting lack of pro-inflammatory responses. Finally, using hepatocyte and erythrocyte co-cultures we show that malaria sporozoites can successfully invade hepatocytes, differentiate to merozoites and transition from liver to blood stage. This platform offers the unique opportunity to evaluate novel drug candidates targeting HBV cccDNA maintenance as well as the malaria liver stage, dissect host/pathogen interactions in multicellular immune networks as well as serve as a personalised medicine platform for the prediction of treatment outcomes for HCV and HBV.
Primary human hepatocytes in 3D cultures form liver-like microtissues that are susceptible to HCV and HBV patient-derived viral isolates. (a, b) Phase contrast and confocal microscopy of actin (red) and DAPI (blue) of hepatic microtissues following seeding and culture of hepatocytes. (c, d) Electron micrographs of hepatocytes in 3D cultures 20 days post seeding demonstrating hepatic microvilli (open arrow) on the basolateral growth area and within bile canaliculi (closed arrow), the formation of tight junctions (asterisk) as well as lipid droplet formation (L). (e) Longitudinal secretion of HCV RNA following infection with patient-derived HCV genotype 1a in the presence or the absence of 1mM Ledipasvir. (f) Infection of 3D hepatocyte cultures with patient-derived HBeAg-negative and HBeAg-positive HBV and the longitudinal secretion of HBV DNA.
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