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Mirjam B. Zeisel, Marine Turek, Thomas F. Baumert. Getting closer to the patient: upgrade of hepatitis C virus infection in primary human hepatocytes.. *Journal of Hepatology*, 2010, 53 (2), pp.388-9. 10.1016/j.jhep.2010.04.004 . inserm-00517102

**HAL Id: inserm-00517102**

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Submitted on 13 Sep 2010

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**Getting closer to the patient: Upgrade of hepatitis C virus infection in primary human hepatocytes**

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**Word count :** 798

**References :** 10

**Figures :** 1

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**Abbreviations:**

CLDN1: claudin-1; HCV: hepatitis C virus; HCVcc: cell culture-derived HCV; HCVpp: HCV pseudoparticles; JFH1: Japanese fulminant hepatitis 1; MPCC: micro-patterned cocultures; OCLN: occludin; PHH: primary human hepatocytes; SR-BI: scavenger receptor class B type I

Comment on : Ploss A, Khetani SR, Jones CT, Syder AJ, Trehan K, Gaysinskaya VA, et al. Persistent hepatitis C virus infection in microscale primary human hepatocyte cultures. Proc Natl Acad Sci U S A 2010, 107:3141-3145.

### **Abstract**

**Hepatitis C virus (HCV) remains a major public health problem, affecting approximately 130 million people worldwide. HCV infection can lead to cirrhosis, hepatocellular carcinoma, and end-stage liver disease, as well as extrahepatic complications such as cryoglobulinemia and lymphoma. Preventative and therapeutic options are severely limited; there is no HCV vaccine available, and nonspecific, IFN-based treatments are frequently ineffective. Development of targeted antivirals has been hampered by the lack of robust HCV cell culture systems that reliably predict human responses. Here, we show the entire HCV life cycle recapitulated in micropatterned cocultures (MPCCs) of primary human hepatocytes and supportive stroma in a multiwell format. MPCCs form polarized cell layers expressing all known HCV entry factors and sustain viral replication for several weeks. When coupled with highly sensitive fluorescence- and luminescence-based reporter systems, MPCCs have potential as a high-throughput platform for simultaneous assessment of *in vitro* efficacy and toxicity profiles of anti-HCV therapeutics.**

## Comment

With an estimated 170 million infected individuals, hepatitis C virus (HCV) has a major impact on public health. The majority of HCV-infected individuals develop chronic hepatitis that may progress to liver cirrhosis and hepatocellular carcinoma. Although new antivirals are entering clinical trials, current treatment options are limited and a vaccine is not available.

In recent years, tremendous progress has been made in deciphering HCV-host cell interactions. The establishment of novel and robust infectious model systems has allowed a detailed study of the entire HCV lifecycle *in vitro* [1]. However, these model systems rely on unique HCV isolates (i. e. JHF1) and on specific cell lines (i. e. Huh7) for a reproducible and standardized analysis of HCV-host cell interactions. *In vivo*, the hepatocyte is the primary target cell of HCV. The liver architecture is elaborated and hepatocytes are characterized by a complex and highly-polarized morphology [2]. Human hepatoma cells differ from primary human hepatocytes (PHH) with respect to morphology, proliferation, gene expression and innate immune responses but are widely used as surrogate model for human hepatocytes because PHH are difficult to maintain in cell culture and infection with recombinant or serum-derived HCV is not robust. Indeed, PHH do not proliferate and tend to rapidly lose their phenotype and viability upon isolation. Therefore, innovative strategies allowing to circumvent these drawbacks and to improve PHH culture are needed. Furthermore, a PHH-based HCV infectious model system would be of great interest to determine the efficacy and toxicity of novel antivirals. Interestingly, transgenic immunodeficient mice with hepatocyte-lethal phenotype have been successfully transplanted with PHH and are susceptible to HCV infection [3, 4]. In the transplanted mice, PHH may occupy the majority of the liver parenchyma and preserve their phenotype for several months, suggesting that the *in vivo* microenvironment stabilizes the PHH phenotype.

In the past years, tissue engineering has greatly improved PHH culture conditions in order to preserve and modulate PHH phenotype. Micropatterned coculture (MPCC) of PHH with mouse fibroblasts (3T3-J2 cells) (Figure 1) allows stabilizing the PHH phenotype (e. g. synthesis of albumin, urea and cytochrome P450 activity) for several weeks and has been proposed as a model to study drug safety [5]. This model appears robust as stabilization of liver functions was observed with both fresh and cryopreserved PHH from different donors. Of note, like in monoculture, PHH do not seem to proliferate under MPCC culture conditions. On the other hand, virologists have been continuously trying to improve the HCV models and most recently two highly sensitive new reporter models were described [6]: an HCVcc reporter virus expressing secreted Gaussia luciferase (Jc1FLAG2(p7-nsGluc2A) and a fluorescent protein (EGFP or RFP) reporter containing an HCV protease cleavage site (IPS-1). In contrast to current model systems, these new reporter systems offer the advantage of determining HCV infection in living cells without the need for cell lysis (Figure 1).

Combining these two advancements the Rice laboratory established a new model system to study persistent HCV infection in PHH [7]. Interestingly, MPCC PHH appear to polarize [7] and HCV host entry factor distribution seems to be comparable to liver hepatocytes [8]. Thus, MPCC PHH appear to reflect hepatocyte morphology in the liver more closely than PHH monoculture or hepatoma cells. Noteworthy, MPCC PHH were able to replicate HCV for two weeks as shown by luciferase secretion that was reduced in the presence of HCV polymerase and protease inhibitors [7]. In addition, expression of the fluorescent reporter RFP-NLS-IPS allowed assessment of replication of cell culture and serum-derived HCV at a single cell level. Importantly, in line with previous results obtained in hepatoma cells, antibodies directed against CD81 and HCV E2 inhibited HCV infection. Of note, MPCC PHH are able to support the entire HCV lifecycle as supernatants from infected MPCC PHH were infectious in hepatoma cells [7]. Although HCV infection and viral spread occurred only in a small fraction of cells, MPCC PHH appear to be a valuable and robust model system to study HCV infection in primary cells.

The possibility to use fluorescent reporter proteins in MPCC PHH broadens the scope of this model system as it will allow to study HCV infection of a large panel of genotypes and isolates without engineering reporter viruses. Moreover, as HCV associates with lipoproteins *in vivo* and this impacts viral entry and neutralization [9, 10], it will be of interest to compare infectious virions released from MPCC and hepatoma cells with respect to biochemical properties, host factor usage, infectivity and neutralization. Furthermore, this model also offers a unique opportunity to assess the efficacy of novel antivirals but also to determine drug interactions and toxicity in HCV-infected cells [7].

In conclusion, this original model system based on the natural host cell of HCV infection clearly provides an important step forward for the understanding of virus-host interactions in the HCV-infected liver.

### **Acknowledgements**

The authors acknowledge financial support of their work by the European Union (ERC-2008-AdG-233130-HEPCENT and INTERREG-IV-2009-FEDER-Hepato-Regio-Net), ANRS (2007/306 and 2008/354), the Région Alsace (2007/09), the Else Kröner-Fresenius Foundation (EKFS P17//07//A83/06), the Ligue Contre le Cancer (CA 06/12), Inserm, University of Strasbourg, and the Strasbourg University Hospitals, France.

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## Figure legend

**Figure 1: Microscale primary human hepatocyte cocultures to study hepatitis C virus (HCV) infection.** Schematic of micropatterned cocultures (MPCC) of primary human hepatocytes (PHH) for the study of HCV infection. A. A polydimethylsiloxane (PDMS) stencil consisting of membranes with through-holes is applied at the bottom of cell culture wells. B. A solution of collagen is then added to the wells to allow collagen to adsorb to the substrate via the through-holes. C. Micropatterned collagen is left on the substrate upon stencil removal. D. PHH are seeded in the wells and selectively adhere to collagen-coated domains. E. Subsequently, mouse embryonic fibroblasts (3T3-J2) are seeded into the remaining bare areas as feeder cells. F. This generates MPCC PHH co-cultures. G. PHH may be transduced with a fluorescent RFP-NLS-IPS reporter to visualize serum-derived HCV or recombinant cell culture-derived HCV (HCVcc) infection of PHH. HCV infection is indicated by nuclear translocation of the reporter. H. Alternatively, Gaussia luciferase reporter viruses may be used to study HCVcc infection of PHH. Infection is detected by quantitation of luciferase secretion into cell culture supernatants.



Figure 1

