circumvent this, by growth arrest of cells by adding 1-2% dimethyl sulfoxide (DMSO, a polar aprotic solvent) to the cell culture medium (Sainz et al. (2006) J Virol 80: 10253-10257), resulting in the induction of expression of hepatocyte-specific genes.

[004] Freshly isolated primary human hepatocytes are logically a more representative in vitro model to study HCV infectivity. However, the amount of virus produced in these cells is low (typcially less than 10^3 RNA copies/ml), and for long term experiments (more than a few days) these cells have to be co-cultured with other cell types (Banaudha et al (2010) Hepatology, 51: 1922–1932; Ploss et al. Proc. Natl. Acad. Sciences 2010 vol. 107 no. 7 3141-3145). Primary hepatocytes are thus not suitable for large scale virus production.

[005] HCV viral titers have been achieved in HuH-7 or HuH-7-derived cells of approximately 10^6 to 10^7 RNA copies per ml (about 1 virus per cell). However, these viral titers are generally too low to provide for commercial scale production of viral particles. Additionally, infection in HuH7.5 cells has only been possible with an atypical HCV variant, JFH-1.

[006] There is a need in the field for a culture system that can serve as an in vitro model of primary human hepatocytes.

SUMMARY

[006a] In a first aspect of the present disclosure, there is provided a method of producing a cell culture comprising cells having a primary human hepatocyte phenotype, the method comprising:

culturing a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for more than 11 days

wherein said culturing induces differentiation of the hHCC cell line into a cell having a primary human hepatocyte phenotype.

[006b] In a second aspect of the present disclosure, there is provided a cell culture comprising cells produced by the method of the first aspect.

[006c] In a third aspect of the present disclosure, there is provided a cell culture comprising:

cells having a phenotype of a human primary hepatocyte, wherein the cells are the differentiated progeny of an hHCC cell line; and

a culture medium comprising human serum.

[0006d] In a fourth aspect of the present disclosure, there is provided a method for assessing an effect of a candidate agent on a cell having a phenotype of a human primary hepatocyte, the method comprising:

contacting the cell culture of the third aspect with a candidate agent; and

assaying for the presence of absence of an effect of the candidate agent on a phenotype of the cell having a phenotype of a human primary hepatocyte.

[0006e] In a fifth aspect of the present disclosure, there is provided a method for assessing metabolism of an agent by a cell having a phenotype of a human primary hepatocyte, the method comprising:

contacting the cell culture of the third aspect with an agent; and

assaying for the presence of absence of a metabolite of the agent and/or the agent.

[0006f] In a sixth aspect of the present disclosure, there is provided a method for assessing toxicity of an agent on a cell having a phenotype of a human primary hepatocyte, the method comprising:

contacting the cell culture of the third aspect with an agent; and

assaying for the presence of absence of a change in a phenotype of the cell which is indicative of toxicity of the agent for the cell.

[0006g] In a seventh aspect of the present disclosure, there is provided a method of producing viral particles, the method comprising:

incubating a cell culture comprising a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for more than 11 days,

wherein said incubating induces differentiation of the hHCC cell line into a cell having a primary human hepatocyte phenotype;

introducing a genome of a hepatotrophic virus into at least one of the hHCC cell line or the cell having primary human hepatocyte phenotype; and

maintaining the cell culture under conditions suitable for production of viral particles.

[0006h] In an eighth aspect of the present disclosure, there is provided a virally-infected cell culture comprising cells produced by a method comprising:

incubating a cell culture comprising a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for more than 11 days, wherein said culturing

induces differentiation of the hHCC cell line into a cell having a primary human hepatocyte phenotype; and

introducing a genome of a hepatotrophic virus into at least one of the hHCC cell line or the cell having primary human hepatocyte phenotype.

[006i] In a ninth aspect of the present disclosure, there is provided a method for screening a candidate agent for antiviral activity, the method comprising:

incubating a cell culture comprising a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for more than 11 days, wherein said incubating induces differentiation of the hHCC cell line into a cell having a primary human hepatocyte phenotype;

introducing a genome of a hepatotrophic virus into at least one of the hHCC cell line or the cell having primary human hepatocyte phenotype;

contacting the cell culture with a candidate antiviral agent;

maintaining the cell culture under conditions suitable for viral replication; and

detecting the presence or absence of an effect of the candidate agent upon viral replication;

wherein a decrease in viral particle production in the presence of the candidate agent as compared to the absence of the candidate agent indicates the candidate agent have antiviral activity.

[006j] In a tenth aspect of the present disclosure, there is provided a method for screening a sample suspected of containing an antibody for antiviral activity, the method comprising:

incubating a cell culture comprising a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for more than 11 days, wherein said incubating induces differentiation of the hHCC cell line into a cell having a primary human hepatocyte phenotype;

introducing a genome of a hepatotrophic virus into at least one of the hHCC cell line or the cell having primary human hepatocyte phenotype;

contacting the cell culture with a sample of suspected of containing an antibody;

maintaining the cell culture under conditions suitable for viral replication; and

detecting the presence or absence of an effect of the sample upon viral replication;

wherein a decrease in viral particle production in the presence of the sample as compared to the absence of the sample indicates the sample contains an antibody having antiviral activity.

[006k] In an eleventh aspect of the present disclosure, there is provided a method for screening for a candidate agent for the treatment of a lipoprotein mediated disease, the method comprising:

incubating a cell culture comprising a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for at least 3 days, wherein said incubating induces differentiation of the hHCC cell line into a cell having a primary human hepatocyte phenotype;

contacting the cell culture with the candidate agent; and

assaying the cell culture for the presence or absence of an effect of the candidate agent on the levels of lipoprotein secreted by the differentiated hHCC cell line as compared to a control sample;

wherein an effect of the candidate agent on the levels of lipoprotein secreted by the differentiated hHCC cell line indicates that the candidate agent can be used for the treatment of the lipoprotein mediated disease.

[006l] In a twelfth aspect of the present disclosure, there is provided a method of infecting a cultured hepatocytic cell with a hepatotrophic microorganism, the method comprising:

contacting a cultured hepatocyte cell with an infectious hepatotrophic microorganism in a culture medium comprising serum depleted of LDL-receptor binding lipoproteins;

wherein said contacting is for a time sufficient to provide for infection of the cultured hepatocyte cell with the hepatotrophic microorganism.

[007] The present disclosure provides methods and compositions relating to in vitro cultures of human hepatocyte cell lines which exhibit a primary human hepatocyte phenotype. Such cell lines are susceptible to infection by a hepatotrophic virus, such as HCVor HBV, and support both viral replication and high levels of viral particle production. Such in vitro cultures find use in production and study of hepatotrophic virus, as well as methods of screening (e.g., for antiviral drugs, assessing drug metabolism), and study of primary human hepatocytes.

[008] The present disclosure provides methods of producing a cell culture comprising cells having a primary human hepatocyte phenotype, which method comprises culturing a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for more than 11 days, wherein said culturing induces differentiation of the hHCC cell line into a

cell having a primary human hepatocyte phenotype. In some embodiments, the culture medium comprises from about 1% to 20% human serum, optionally from about 2% to 10% human serum. In some embodiments, the hHCC cell line is a HuH-7 or HuH-7-derived cell line. In some embodiments, the culturing is conducting without subculturing after 10 days of culturing in the