# Induced Pluripotent Stem Cells Derived Hepatocyte-like Cells as a Model for Hepatitis B Virus Infection

Pattida Kongsomboonchoke[1] Adisri Charoenpanich[1] Khanit Sa-ngiamsuntorn[2]

[1]Department of Biology, Faculty of Science, Silpakorn University, Nakorn Pathom, Thailand
[2]Department of Biochemistry, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand

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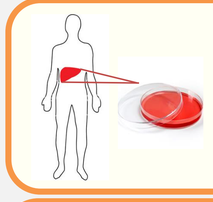
## Abstract

The cell culture models for hepatitis B virus (HBV) infection are still limited. In this study, we developed hepatocyte-like cells derived from human induced pluripotent stem cells (iHeps) as a model to investigate the HBV infection. The expression of hepatocyte's marker genes in iHeps was confirmed in five genes with reverse transcription (RT) and real-time PCR. Next, we tested the susceptibility of iHeps to HBV infection. HBV-positive plasma from patients was used as a source of HBV. The viral infection was evaluated with real-time PCR and gel electrophoresis. Specific PCR products of cccDNA confirmed the success of HBV infection in iHeps. Therefore, iHeps that developed in this study could be a suitable model for HBV infection.

## Introduction

Hepatitis B virus (HBV) is a major medical problem because the most of chronic infection will develop cirrhosis and liver cancer. The study of HBV required suitable hepatocyte culture models but most of them are still limited.

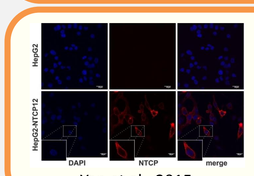
**The timeline of liver cell models for HBV infection studies.**



1988: Primary human hepatocytes
limited by 1) cell availability and phenotypic losing after plating.
2) variation of genetic backgrounds from different donors.

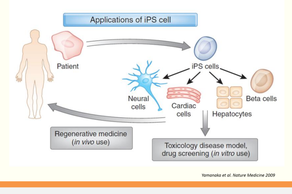
Gripon et al., 2002

2002: HepaRG (a human hepatoma cell line)
susceptible to HBV infection after differentiation into a mixed population of hepatocyte-like and biliary-like cells but it requires significant technical skills to preparation and culturing.




Yan et al., 2015

2015: HepG2-hNTCP
(HepG2 cells that exogenous expression of NTCP that is a key receptor for HBV infection)
limited by very low level of viral releasing and reinfection.

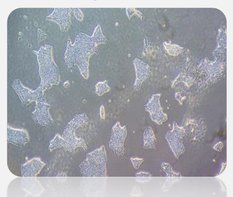



2014 & this study: iHeps
(Induced pluripotent stem cells-derived hepatocytes-like cells)
a novel alternative for HBV infection studies.

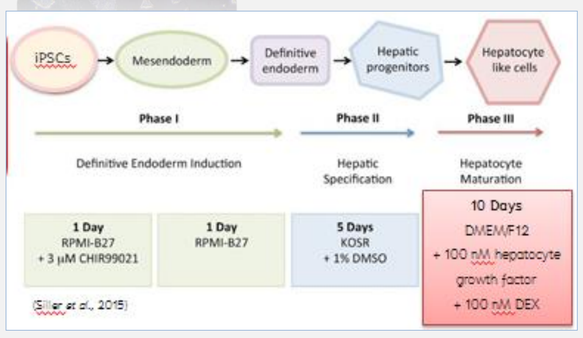
Therefore, our goal is to investigate the HBV infection of induced pluripotent stem cells derived hepatocyte-like cells (iHeps)

## Materials and Methods

### Hepatic Differentiation of iPSCs



iPSCs reprogramed from mesenchymal stem cells provided by Department of Biotechnology, Faculty of Science, Mahidol University.




### Hepatic differentiation was assessed by RT and real-time PCR

**mRNA expression analysis of Five hepatocyte's marker genes**
ALB, AFP, HNF4A, TAT and NTCP

### HBV infection

Patient's plasma provided by Ramathibodi Hospital.

### HBV DNA and cccDNA detection using real-time PCR and gel electrophoresis

**Comparison of HBV infection**
with HLC (normal hepatocyte cell line) & HepaRG

## Results and Discussion

- During hepatic differentiation, iPSCs changed their morphology from one population of pluripotent colonies with non-differentiated cells (Fig 1A) to hepatocyte-like morphology (Fig 1D).
- At the end of differentiation, iHeps expressed the high level of AFP, a hepatoblast's marker gene, compared to the original iPSCs (Fig 2, blue bars). ALB, HNF4α and TAT expression were less different from iPSCs's expression because iHeps were immature hepatocytes. NTCP equally expressed in iHeps and HLC.
- iHeps were infected with patient's plasma and showed the positive results of cccDNA in both infected iHeps and HLC (Fig 3A). However, HBV DNA could not detect (Fig 3B) because of the non specific primers.

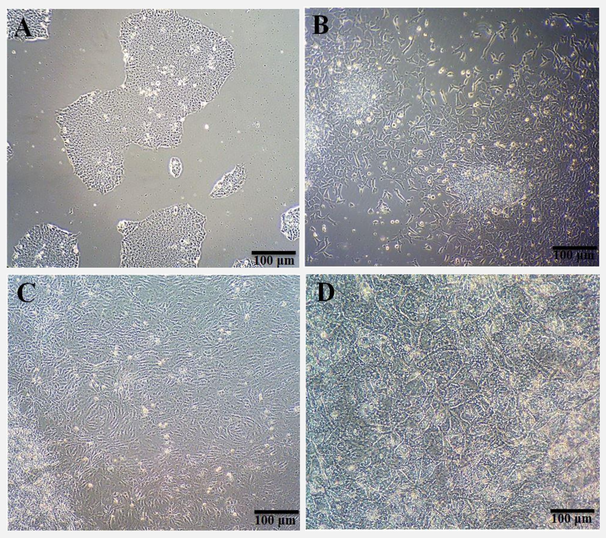



**Figure 1. The morphology of iPSCs at the key stages of hepatic differentiation.**
(A) iPSCs at day 0.
(B) At day 2, differentiated cells migrated away from the pluripotent colonies and formed fibroblast-like structure.
(C) At day 7, cells changed their morphology from fibroblast-like structure to small epithelial-like structure.
(D) At day 17, iHeps displayed hepatocyte-like morphology with a dark cytoplasm, bright junction and polygonal-shaped cells.

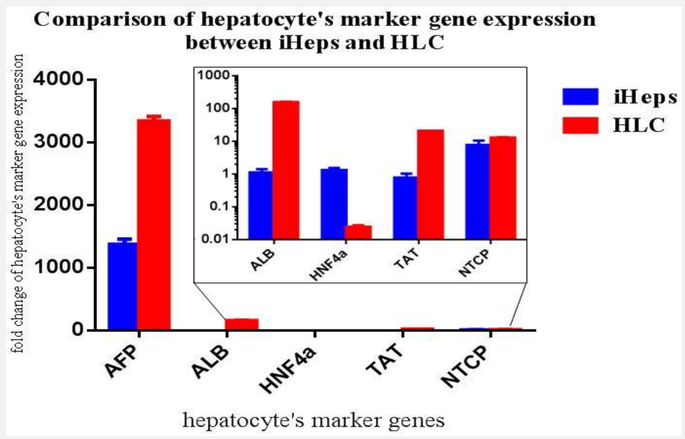



**Figure 2. Relative Hepatocyte's marker gene expression of iHeps.**
iHeps (blue) and HLC (orange), compared to iPSCs, normalized to GAPDH. Data were presented as the mean of triplicates; error bars represent SD.

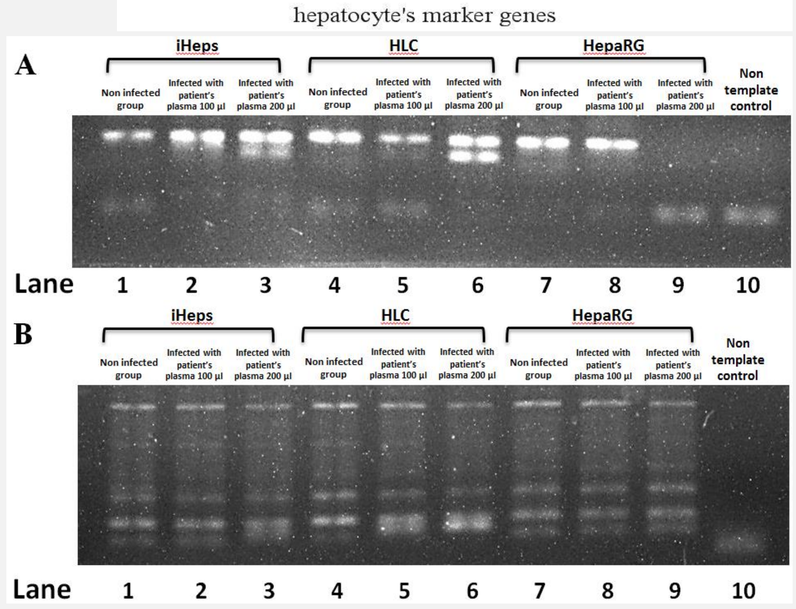



**Figure 3. HBV infection analysis.**
Agarose gel separation of amplified cccDNA (A) and HBV DNA products (B).

## Conclusion

iPSCs differentiated to hepatocytes as iHeps and were able to infect with HBV. However, improvement of HBV infectious system and detection are still required. Further studies of iHeps are about HBV life cycle and viral carcinogenesis.

### Acknowledgements

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### References

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