POSTER REFERENCE NUMBER: 22a

Development of a cellular platform supporting Hepatitis B virus infection and replication for research, drug design and diagnosis

Rute Lopes[1]; Carla Rios da Cruz[2]; Thaís Oliveira Rodrigues Sanzovo Falcoski[2]; Júlio César de Paula e Silva[2]; Paulo Inácio da Costa[2].

[1] São Paulo State University (UNESP), Institute of Chemistry, Campus Araraquara ; [2] São Paulo State University (UNESP), School of Pharmaceutical Sciences, Campus Araraquara.

Description

Hepatitis B is a disease spread worldwide, with 240-350 million people estimated to be chronically infected, and over 2 billion ever having been infected[1]. *In vitro* replication models of hepatitis B virus (HBV) are very important to understand the molecular mechanisms of viral replication and behavior throughout the infection evolution. The Huh-7.5 cells derived from Huh-7, which is a traditional and differentiated human hepatoma cell line, was already successfully transfected with a sub-genomic replicon of HCV, a positive single-stranded RNA virus, and it has become the gold standard for the *in vitro* research of regulatory mechanisms of virus replication, gene expression, proteins function and the development of various compounds and nanomaterials, with meticulous evaluation of their effects and metabolism[2].

Public Health Impact

Currently in Brazil the molecular control for HBV diagnosis is performed with positive serum for HBV infection from blood donors, a procedure that incurs in manipulation and contamination hazards.

Therefore, the development of an *in vitro* platform of Huh-7.5 cell culture with sustained infection of HBV would allow investigations of the molecular mechanisms associated with viral infection, it would additionally facilitate the advancement of studies focusing on pathways to suppress viral replication, to produce new drugs and vaccines, and it could be applied as a resource for laboratory quality control.

Innovation

So far there are no records of Huh-7.5 cells as a system for HBV infection in the literature. In this regard, experimental models that would precisely reproduce pathogen-host interactions are limited. The goal of our work is to establish a platform of HBV infection in cell culture, with little or no previous chemical treatment, that yields a high amount of infectious particles, in order to contribute to the research of the molecular mechanisms of this infectious disease, and to the development of new and improved drugs and nanomaterials for therapy. Another possible application of this platform would be to provide quality control supplies produced in a more controlled environment for laboratory analyses already in place, and to offer tools for the investigation of cost-effective diagnostic methods. All the results obtained thus far have been promising and an outcome is expected for the ensuing months.

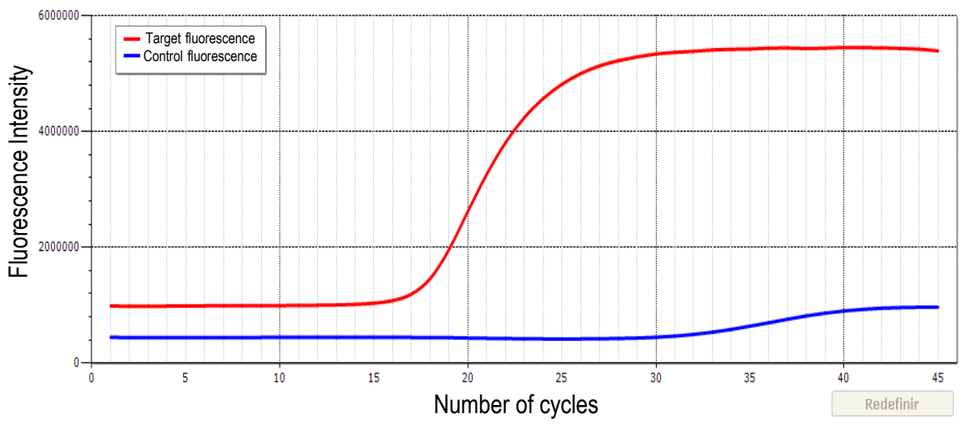


Fig. 1: qPCR internal control (blue) and HBV DNA (red) amplification curves.

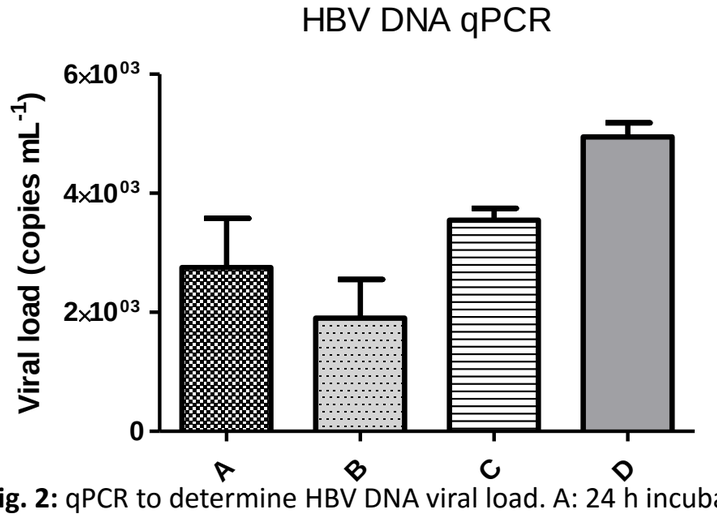
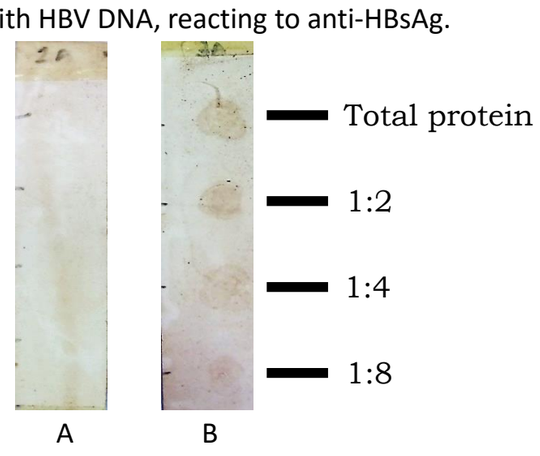


Fig. 2: qPCR to determine HBV DNA viral load. A: 24 h incubation pellet. B: 48 h pellet. C: 24 h supernatant. D: 48 h supernatant.

Fig. 3: Immunoblot strips. A: negative control. B: Serial dilutions of total protein on lysed Huh-7.5 cells, after treatment with HBV DNA, reacting to anti-HBsAg.



Huh-7.5 cells were cultured in complete Dulbecco's modified Eagle's medium (DMEM-C), supplemented with fetal bovine serum, penicillin, streptomycin and incubated at 37°C in a humidified incubator with 5% CO_2. The cells were seeded on a 96 wells plate and incubated for 24 h. Afterwards, HBV DNA diluted in DMEM-C at a known concentration was added, and the plate was incubated for another 24 h. Then, medium was removed and the samples were washed 3 x with PBS, and reconstituted with DMEM-C. 24 and 48 h post-treatment, the supernatants were collected. Also, the adhered cells were removed by trypsinization, washed with PBS and the pellets collected. Abbott Real*Time* HBV viral load assay (qPCR) was performed to quantify HBV DNA in all samples. **Figure 1** shows an amplification curve example. **Figure 2** represents the results obtained on both supernatant and pellet of final viral load. Immunoblotting assay was executed employing the pellet. For that, total protein content was obtained by lysing cells, and then quantified using the Bradford protein quantification method. The blot assay was performed by pipetting serial dilutions of the protein onto nitrocellulose membranes, blocking unspecific site reactions, incubating the strips in an anti-HBsAg solution or a negative control, then in a solution containing antibodies anti-human IgG-biotin and avidin-peroxidase, and finally using 3,3'-diaminobenzidine and H_2O_2 as substrate. **Figure 3** shows a negative control strip and a reactive strip.

Conclusions

The proposed platform for the maintenance of HBV infection in Huh-7.5 cells produced a significant amount of viral DNA after treatment, and the presence of HBsAg was observed by immunoblot assay. Although the results are preliminary, they are still very promising. *In vitro* viral infection would allow the advancement of investigations of the molecular mechanisms related to the pathogenesis of the disease, as well as the development of new strategies for the control of viral replication and it could also be applied in many other areas, such as the design of quality control for laboratory molecular exams, initial studies on the effects of drugs and vaccines, and so forth. Therefore, this research aims to improve and ultimately certify this new platform for *in vitro* infection with HBV.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. MacLachlan JH, Cowie BC. Hepatitis B Virus Epidemiology. **Cold Spring Harb Perspect Med.** 2015;5(5):a021410.

2. Sainz B, Barretto N, Uprichard SL. Hepatitis C virus infection in phenotypically distinct Huh7 cell lines. **PLoS One.** 2009;4(8).

UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

World Hepatitis Summit 2017
SÃO PAULO, BRAZIL 1-3 NOVEMBER