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Dear Dr. Jing Yu,

Thanks for your mail dated on June 20, 2016 (deadline extension request: July 5, 2016) about our manuscript entitled **"Elucidation of the early infection machinery of hepatitis B virus by using bio-nanocapsule"** by Qiu-shi Liu, Masaharu Somiya, and myself. We hereby revised the MS by according to three reviewer's comments (revised sentences are indicated in RED) and sent various files you requested. We would be pleased if you could consider this manuscript for publication as a Minireview in *World Journal of Gastroenterology*.

We look forward to hearing from you at your earliest convenience.

Yours sincerely

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Reviewer 1:

This minireview summarizes recent data using bio-nanocapsule (BNC) as a mimic of HBV virion to investigate functional segments of HBV Large Envelope protein in HBV entry. The authors identified a fusogenic sequence
located in the N terminal of pre-S1 protein, which might mediate step of late
endosomal escape of HBV (uncoating). Overall, the topic is interesting and it provides new information regarding HBV entry process.

However, there are several concerns:

1. A lack of convincing rationale to use BNC as a mimic of HBV virion. The authors argue that HBV particles for infection experiment are difficult to acquire and there is a need to use BNC.
   This statement is not entirely true because several stably HBV DNA transfected cell lines can produce high level of HBV DNA in medium. A probably more convincing rationale is that BNC is easier for manipulations (loaded with color molecules) than HBV virion. This advantage can simplify the experimental procedure, possibly generate reproducible data.

   Our response:
   We agreed with the reviewer's comment.
   We modified the following sentences: lines 53-56; 76-78, 237-242.

2. There is no valid comparison between HBV virion and BNC. This comparison should be clearly stated in the text in addition to Fig.1. Main differences include size, density and ratio of three envelope proteins. BNC is composed of exclusive L protein while the L protein in HBV virion only accounts to very small portion. Any conclusions resulting from BNC should be verified with HBV virion.

   Our response:
   We added the differences between HBV and BNC at the following sentences: lines 173-180.

3. It is not clear whether the authors performed similar experiment with HBV virion. Otherwise Fig.3. can be misleading. Where did HBV results come from or just an assumption?

   Our response:
   The idea described in Figure 3 was already examined by using BNC, myristoylated BNC, and HB patient-derived HBsAg (as a model of HBV virion), and recently published in our paper (Ref. 41). We added the experimental data at lines 258-273. We hope Figure3 is acceptable for the publication in this journal.

Minor issues:

1. there are no line numbers in the manuscript

   Our response:
   We added line numbers.

2. P4. "...HBV as a molecule" incorrect statement

   Our response:
   We deleted it (line 94).

3. P6. there is a lack of clarity in introducing HBV receptors that were mixed with ligand identification contained in HBV.

   Our response:
   By consulting with English-speaking researcher and English editing service, we modified the paragraph “HBV AND ITS RECEPTORS” to improve its readability (lines 113-146).

4. P7. Percentage of HBV vaccination. Exact numbers should be given

   Our response:
   We added new Ref. 21 and percentage (approx. 5%) at line 158.

5. P8. " BNC infects cells", an inaccurate description

Our response:
We modified the word to “BNC target and enter” at line 200.

6. P9. More detailed information should be presented in describing no enhancement of interaction and internalization when NTCP was overexpressed. Credible evidence is needed to challenge NTCP as a major receptor or suggest a different one

   Our response:
   The data (Somiya et al. submitted) has been recently published as Ref. 41. We added the experimental data at lines 258-273.

7. P9. the authors appeared to suggest HBsAg can enter into cells via a same or different receptor? Is this understanding correct? evidence is required if so.

   Our response:
   Yes. We suggested both Myr-BNC and HBsAg enter into cells via same receptor. The evidence has been recently published as Ref. 41.

8. English is understandable, but not idiomatic or professional terms were ot used.

   Our response:
   For revision, our MS was checked by English editing service.

Reviewer 2:

Comments to the Author The authors reviewed bio-nanocapsule (BNC) could be used as a model of HBV for elucidating the HBV early infection machinery. They described the model in which each domain of L protein respectively contributes to the attachment onto human hepatic cells through HSPG, the initiation of endocytosis, the interaction with NTCP in endosomes, and consequently the provocation of membrane fusion followed by the endosomal escape.

Major points:

1. The paragraph “BNC AS NANOCARRIER” just illustrate BNC infects human hepatic cells by using HBV-derived infection machinery. This paragraph should be re-written.

   Our response:
   We added the detailed functions of BNC as nanocarrier at lines 188-234.
   And, the section was separated into three sections for improving the readability.

2. The format of references is not consistent. The format of reference 40 if different from others. There are two reference 28.

   Our response:
   We corrected it.

3. The format of paragraghs is not consistent. There is space in line header sometimes.

   Our response:
   We corrected them.

Reviewer 3:

This is a well written review of the research that has been done to establish the sequence of events that takes place as the HBV virus infects a hepatocyte. The authors synthesized a hollow bio-nanocapsule (BNC) in a fungi to deliver HBV –derived infection, machinery. In this way they could study the mechanism of HBV infection in a more controlled atmosphere. Their experiments are both elegant and simple at the same time. I am not a molecular biologist but I found the paper easy to understand and did not find anything to criticize.

Our response:
Thank you.