OPEN PEER REVIEW

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Peer Review Report for "Two New Purification Methods of Hepatitis C Virus Particles from Serum-Free Culture System"

Author(s): Shaomei Zhu, Bochao Liu, Yuxia Xu, Ling Zhang, Zhengyuan Xia, Jihong Liu, Xiao Zhou, Yongshui Fu, Jean-Pierre Allain, Chengyao Li , Tingting Li

Review Timeline:

Submit Date: 3 May 2021
Revised Date: 10 Aug 2021
Accepted Date: 19 Aug 2021

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | Revision (0)

7 Jun 2021

Dear associate editor,

The authors of this manuscript aimed to develop two alternative simple and effective purification methods, heparin-affinity chromatography and magnetic separation for hepatitis C virus (HCV) purification from cell culture.

Based on the reported results, these simple methods have been introduced as alternatives to ultracentrifugation. In my opinion, in order to have a better understanding regarding the efficacy of the proposed methods in comparison with ultracentrifugation, both methods should be run in parallel and the final products can be analyzed quantitatively (for example by quantitative molecular tests and/or TCID50). This may help to make a better comparison between two methods.

On the other hand, the authors have mentioned that these methods are superior to ultracentrifugation because the latter needs special instruments. To the best of my knowledge, HCV culture needs biosafety level 3 (BSL3) laboratory which is not easily available everywhere.

I also recommend language editing for the manuscript by a native speaker.

Best regards,

REFEREE: Reviewer | **Revision** (0) 10 Jul 2021

Dear AE,

This manuscript discusses evaluation of two different HCV particle purification methods in a serum free culture media of Huh-7 cells transfected with pJFH-1. The study outcome provides further HCV particle purification methods which can be utilized in HCV drug discovery/drug resistance studies.

The general idea of utilizing heparin affinity-based purification of viral particles is not novel per se. The method has been assessed for purification of baculovirus vectors, HCV and HBV virions from infected patient plasma. This study assessed heparin affinity-based purification of invitro synthesized HCV particles. The results are adequate and reasonable. The paper is well written and prepared properly to follow the topic easily. It provides a nice overview of the methods and corresponding results. However, some revisions are required and questions needed to be addressed prior publishing consideration.

The comments and questions to be addressed are provided below:
Manuscript preparation:
• Minor grammatical and typing revisions are required in manuscript.
• In several instances, “free media” has been used instead of “serum-free media”.

Material/Methods and Results:
• Which genotype of HCV sera from patient was used in magnetic separation method?
• Infectivity of isolated HCV particles can also be evaluated by using neutralizing CD81-specific Ab or by serum from infected patients. Is there any data available for further Infectivity assessment by neutralization/infectivity inhibition assays?
• What are the post-purification recovery percentage of infectious HCV particles in each of the described methods? Recovery percentage is an indicator of purification method efficiency.
• What is the authors’ perspective on described methods scale up process? Drug discovery/resistance studies require large number of infectious particles for high throughput anti-viral library assessments.

With regards,

REFEREE: Associate Editor | **Revision** (0) 10 Jul 2021

Dear EIC,

Manuscript # 115727 entitled, " Two new purification methods of Hepatitis C virus particles from serum-free culture system" was evaluated by two experts. Referees found the manuscript interesting. However, some comments were raised that should be addressed in a "minor revision" of the manuscript.

Thanks

Seyed Moayed Alavian: EIC | **Revision** (0) 11 Jul 2021

Dear Authors,

Thank you for your submission. Please follow the peer-review comments and submit a revision.

Regards,
EIC

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Editor,

Re: 115727 (Two new purification methods of Hepatitis C virus particles from serum-free culture system)

We would like to submit the revised version of our manuscript to Hepatitis Monthly as the journal required. According to reviewers' and your comments and questions, we carefully revised the initial manuscript and the changes are highlighted in yellow. The explanations or answers have been addressed as indicated below for each question point by point.

If you have any more question regarding to this manuscript, please let me know.

Yours sincerely,

Reviewer comments:

Reviewer 1:

This manuscript discusses evaluation of two different HCV particle purification methods in a serum free culture media of Huh-7 cells transfected with pJFH-1. The study outcome provides further HCV particle purification methods which can be utilized in HCV drug discovery/drug resistance studies.

The general idea of utilizing heparin affinity-based purification of viral particles is not novel per se. The method has been assessed for purification of baculovirus vectors, HCV and HBV virions from infected patient plasma. This study assessed heparin affinity-based purification of invitro synthesized HCV particles. The results are adequate and reasonable. The paper is well written and prepared properly to follow the topic easily. It provides a nice overview of the methods and corresponding results.

However, some revisions are required and questions needed to be addressed prior publishing consideration.

The comments and questions to be addressed are provided below:

Manuscript preparation:

• Minor grammatical and typing revisions are required in manuscript.

Answer:

Thank you for your comment and suggestion. The English writing was edited carefully and thoroughly in the text by the authors and co-authors.

• In several instances, “free media” has been used instead of “serum-free media”.

Answer:

Thank you for your suggestion. The descriptions “free media”have been changed to“serum-free media” in revised manuscript (page 1, line 16; page 5, line 78; page 6, line 109; page 9, lines 173-174;page 10, line 186;page 11, line 225).

Material/Methods and Results:

• Which genotype of HCV sera from patient was used in magnetic separation method?

Answer:

It was identified as genotype 1b. This information has been added in the revised manuscript (page 7, line 132).

• Infectivity of isolated HCV particles can also be evaluated by using neutralizing CD81-specific Ab or by serum from infected patients. Is there any data available for further Infectivity assessment by neutralization/infectivity inhibition assays?

Answer:

Thank you very much for this suggestion. We have not evaluated the infectivity of

isolated HCV particles by the neutralizing assay. This weakness is now stated in the discussion section of revised manuscript (page 13, lines 259-261).

• What are the post-purification recovery percentage of infectious HCV particles in each of the described methods? Recovery percentage is an indicator of purification method efficiency.

Answer:

The recovery percentage of Heparin-affinity chromatography and Magnetic separation technique was about 30-40% according to RT-QPCR and Westernblot, which was reported that heparin-elution contained less than 50% of the input viral genomes (Ref.18). We have added this in the discussion section of the revised manuscript (page14, lines 256-259).

• What is the authors'perspective on described methods scale up process? Drug discovery/resistance studies require large number of infectious particles for high throughput anti-viral library assessments.

Answer:

This is an enlightening question.The application of direct acting antiviral agents (DDAs) makes the treatment of HCV more effective while the sustained antiviral response (SVR) rate of treatment is more than 90% (Ref.19). However, the therapeutic effects of different virus genotypes are different with high variation frequency of virus drug resistance and high price(Ref.20-23). Drug discovery and resistance studies of HCV becomes more and more important in the Era of DAAs. HCV replicon systems have become indispensable in the development of effective DAAs and the research of drug resistance (Ref.24), which require large number of infectious particles for high throughput anti-viral library assessments. These two new purification methods of Hepatitis C virus particles have shown potential application in this field. (page13-14, lines 264-273) .

Reviewer 2:

The authors of this manuscript aimed to develop two alternative simple and effective

purification methods, heparin-affinity chromatography and magnetic separation for hepatitis C virus (HCV) purification from cell culture.

Based on the reported results, these simple methods have been introduced as alternatives to ultracentrifugation. In my opinion, in order to have a better understanding regarding the efficacy of the proposed methods in comparison with ultracentrifugation, both methods should be run in parallel and the final products can be analyzed quantitatively (for example by quantitative molecular tests and/or TCID50). This may help to make a better comparison between two methods.

Answer:

Thank you for the instructive comments. We agree to the reviewer's comments. Unfortunately, we did not systematically compare the efficacy between ultracentrifugation and the proposed method, which was stated in the discussion section of the revised manuscript (page 13, lines 261-263).

On the other hand, the authors have mentioned that these methods are superior to ultracentrifugation because the latter needs special instruments. To the best of my knowledge, HCV culture needs biosafety level 3 (BSL3) laboratory which is not easily available everywhere.

Answer:

Thank you for your concern of HCV biosafety. As the requirement of facility for HCV culture, the biosafety level 2 (BSL-2) can be used, which is available mostly for virology or microbiology laboratories.

I also recommend language editing for the manuscript by a native speaker.

Answer:

As suggested, the manuscript was edited carefully by an English speaker at University of Cambridge, who had a lot experience for writing English papers.

Associate Editor's Comments:

Associate Editor 1:

Manuscript # 115727 entitled, " Two new purification methods of Hepatitis C virus particles from serum-free culture system" was evaluated by two experts. Referees found the manuscript interesting. However, some comments were raised that should be addressed in a "minor revision" of the manuscript.

Answer:

Thank very much for your requirement. We have made revisions of previous manuscript as reviewers' comments or questions at point by point, which are highlighted in yellow in the revised version of manuscript.

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (1) 19 Aug 2021

Dear Authors,

Thank you for the revised version of the manuscript.
All my comments have been addressed and revised appropriately.
In my opinion, the manuscript is now suitable for publication.

Best regards,

REFEREE: Associate Editor | **Revision** (1) 19 Aug 2021

Dear EIC,

Authors have carefully addressed the reviewer comments in their revised version of the manuscript.

Yours

Seyed Moayed Alavian: EIC | **Revision** (1) 19 Aug 2021

Dear Authors,

I am delighted to inform that your manuscript is accepted for publication in Hepatitis Monthly.

Regards,
EIC