Response to the reviewers' comments

The authors appreciate that the reviewers share the significance of the manuscript and their insightful comments. I believe I have satisfactorily addressed all reviewers' comments.

Specific responses to the comments are as follows:

Reviewer's code: 02551224

Dear Authors, I have a minor observation regarding the chapter "Needs for bioartificial liver systems in clinical practice", line four. It is not clear in the text which liver transplant list it refers to. You need to refer to Reference 4 for that. Anyway, I think your article is clear and well written, and can be published without changes.

The authors appreciate the reviewer's suggestion. Reference 4 was added to the suggested sentence on page 3 line 5.

Reviewer's code: 02860897

Shortage of donated organ is a world-wide health problem in transplantation. In the field of the liver disease, artificial liver support (ALS) is temporary alternative method and it can maintain patient to maintain favorable condition until liver transplantation or regeneration of liver. ALS is divided into non-biological type and biological type. Non-biological type comprises plasma exchange and another blood purification method such as hemodiafiltration. This method has been established and widely used in Japan whose number of transplantation is very small. This method can easily replenish depleted clotting factors and arouse patient with deep coma. On the contrary, the role of biological artificial liver support is not clear. Efficacy in elimination of toxic substance is not clear and clinical effect is questionable. Author's statement is reasonable; however, these are already known matters. High time to publish review article is just after settlement of these clinical problems.

The authors appreciate the reviewer's comments and agreement with our statement regarding the limitation of non-biological type ALS. We added the description about the clinical implementation of non-biological ALS in Japan on Page 3, line 14-16. We would like to clarify that this mini-review paper focuses on the possibility of producing BAL systems with iPSC-derived hepatocytes, which could re-introduce this therapeutic option to the readers of World Journal of Gastroenterology.

Reviewer's code: 03213892

This is an interesting topic and a clinically relevant treatment type. The authors have introduced clinical translation of bioartificial liver support systems with human pluripotent

stem cell-derived hepatic cells. The manuscript described in detail "Needs for bioartificial liver systems in clinical practice", "Sources of hepatocytes for BAL systems" and "Successes and challenges of developing clinical BAL systems". Indeed I find the work compelling and worthy of publication pending minor revisions.

The authors appreciate the reviewer's suggestion. To address the reviewer's comments, we added paragraphs and a figure in the manuscript.

1. I am hampered by a lack of about BAL's introduction, eg. different types of BAL and their respective characteristics? Which type is more suitable for hPSC-derived hepatic cells culture? Which type is more suitable for clinical uses?

The author added a paragraph on page 3-4 as follows;

"There are several types of BAL systems that have been proposed which differ in their cell housing mechanism, including hollow fiber-based, membrane-based, sponge/scaffold -based, and floating cell/spheroid culture-based systems (Table 1). Although most of these housing mechanisms have successfully cultured cells on the small experimental scale, hollow fiber-based BAL systems are widely used in clinical trials."

2. Are there any problems in the proliferation and differentiation of hPSC-derived hepatic cells in the BAL support system? How to solve these problems?

Since the application of hPSC-derived hepatic cells to BAL systems is a relatively new field, there are only a few papers that have been published on the subject. Therefore, it is not clear that there is a problem with culturing the hPSCs in BAL systems. These problems may need to be addressed in the future.

Reviewer's code: 00069130

It will be nice if you can discuss on bioreactors which are essential for iPSC-hepatocyte like cell production in large scale.

The authors appreciate the reviewer's suggestion. To address the reviewer's comments, we added a paragraph in the manuscript on Page 6-7 as follows:

"The most critical factor for large-scale cell culture is oxygen and nutrient supply. The oxygen and nutrients must be uniformly supplied to a large number of cells. It is well known that the anchorage-dependent hepatocytes easily form aggregates, and if the diameter of the aggregates exceeds 100um, central necrosis occurs resulting from lack of oxygen and nutrition. This fact indicates that the organization of the cell culture space in the large-scale BAL system must allow for sufficient oxygen and nutrient penetration of the cell aggregates. A sophisticated controlling system and well-engineered bioreactor will be required to monitor oxygen and nutrient supply. In addition, since hPSCs are sensitive to environmental factors, the shear stress from the culture medium must be minimized. Ideally, the bioreactor should mimic the structure within the liver, which provides appropriate pressure and shear

stress similar to the Space of Disse."