Dear Lian-Sheng Ma, Science Editor, Company Editor-in-Chief, Editorial Office
Baishideng Publishing Group Inc

Thank you very much for your e-mail of 28, March 2021, with regard to our manuscript (Manuscript NO.: 64598, Basic Study), entitled "**Syngeneic implantation of mouse hepatic progenitor cell–derived three-dimensional liver tissue with dense collagen fibrils**", together with a reviewer.

Here, on behalf of all the authors, I am sending herewith our revised manuscript. In the attached document, we described the changes made in response to the reviewers' comments point-by-point.

This manuscript has not been published and is not under consideration for publication elsewhere. All the authors have read the manuscript and have approved this submission. I believe the manuscript has been improved satisfactorily and hope it will be accepted for publication in *World Journal of Gastroenterology*.

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EDITORIAL OFFICE'S COMMENTS:

(1) ***Science editor:*** I have changed the manuscript type "Frontier" to "basic study". Please provide the Institutional Animal Care and Use Committee Approval Form or Document, Institutional Review Board Approval Form, The ARRIVE Guidelines, and Biostatistics Review Certificate (see attachment).

Thank you for your adequate suggestion. We sincerely agree with your decision. Our manuscript was revised according to the instructions of 'basic study'.

(3) ***Company editor-in-chief:*** I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Before its final acceptance, please upload the primary version (PDF) of the Institutional Review Board's official approval in official language of the authors' country to the system; for example, authors from China should upload the Chinese version of the document, authors from Italy should upload the Italian version of the document, authors from Germany should upload the Deutsch version of the document, and authors from the United States and the United Kingdom should upload the English version of the document, etc.

Those documents were sent as attachment files in Japanese.

Response to Reviewer #1:

Comment 1: As the author addressed in the manuscript, this extracellular matrices containing organoids consist of both hepatocyte and non-parenchymal cell features, however, the result shows most of gene expression are related to the feature of hepatocytes, the marker genes of non-parenchymal cells are not shown. In addition, the author considered CK19 as a marker for hepatic progenitor cell, it is also a marker of biliary epithelial cells, but its gene expression decreased in the 3-D model. Does this 3-D model also bears the feature of biliary system?

Answer 1: We appreciate your adequate indication. In this study, fibroblasts exist in the matrix. We examined confirming fibroblast-specific gene expression, but we did not find a suitable marker.

Since fibroblasts are used to create a collagen matrix like liver, it is considered that the collagen matrix is formed and that it exists from the tissue section. The gene expression of CK19 is 4 times higher in the 3D model than in the fetal liver and 0.73 times higher than that in the adult liver. It shows 73% of CK19 expression compared to adult liver, slightly lower than mature liver but higher than fetal liver.

Comment 2: The author showed the 3-D model exhibits multiple liver-specific function, these functions (production of urea and albumin, P450, etc.) are mostly related to hepatocyte. Does this 3-D model contain the functions of immune cells and other type of cells in the liver?

Answer 2: No, this haptic tissue consisted of fibroblasts and hepatic progenitor cells which can be differentiated to only hepatocytes and bile duct cells, not immune cells unfortunately. Some lymphocytes and monocytes might flow in the graft because some blood vessels could be observed after the implantation. However, we could not detect Kupfer cells in the grafts by the histological examination. These contents have been described in the Discussion Section, line 12 to 16 on page 14.

Comment 3: What is the rational that the author used only female mice as the recipients of 3-D liver tissue culture model, why not use the males?

Answer 3: Because hepatic progenitor cell line was established from the portal-ligated liver lobule of female mouse as same protocol of previous paper (H. Sakai, et al, BBRC, 2010 Dec 17;403(3-4):298-304.), the cells have no Y-antigen. The graft was transplanted into female mice. However, we grafted the hepatic progenitor cells with Matrigel in male mice also, and succeeded. These contents have been described in the Materials and methods Section, line 10 on page 6 and Discussion Section, Line 11 to 18 on page 12. In this samples, observations of angiogenesis in the transplant tissue model have been added in the supplement Figure 1.

Comment 4: The animal experiment is insufficient, more functional markers need to be investigated in the 3-D liver tissue.

Answer 4: Thank you for your comment. The results of another animal experiments were added in

the Discussion section, Line 11-18 on page12 as described in the Answer 3. However, we have succeeded in mass-culturing hepatic progenitor cells and constructing a liver tissue model. The main point of this study is that the constructed liver tissue had liver-specific functions. Finally, the tissue was succeeded to be and was engrafted into partially hepatectomized mice. We focus on portal branch-ligated hepatic lobe-derived hepatic progenitor cells because portal branch-ligated hepatic progenitor cells are expected to allow regenerative medicine to produce a cell source to provide an alternate source for transplantation. This sentence was added in Research motivation, line 12- 14, page, 13. We had compared to using Matrigel transplantation. In this samples, observations of angiogenesis in the transplant tissue model have been added in the supplement Figure 1C-F.

Comment 5: I suggest the author to perform a extended hepatectomy (86%) or lethal model (90%) to see if the survival rate differs when the 3-D liver tissue model is given.

Answer 5: We wanted to try such a severe partial hepatectomy, but operated mice would die in a few days. The graft is too large to start functioning immediately because vascularization is incomplete within a few days (Supplemental figure 1).