

Format for ANSWERING REVIEWERS

May 20, 2015

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 17502-review.doc).

Title: Hepatocyte selection medium eliminating induced pluripotent stem cells among primary human hepatocytes

Author: Minoru Tomizawa, Fuminobu Shinozaki, Yasufumi Motoyoshi, Takao Sugiyama, Shigenori Yamamoto, and Naoki Ishige

Name of Journal: *World Journal of Methodology*

ESPS Manuscript NO: 17502

The manuscript has been improved according to the suggestions of reviewers:

In title, "to eliminate" has been changed to "eliminating" to reduce 13 words to 12.

"Abstract" and "core tip" have been changed to reduce word count to follow the "format for editorial".

Reviewer 1

In this review, Tomizawa M et al introduced their work on creating a hepatocyte selection medium (HSM) that lacks glucose and arginine but is supplemented with galactose, ornithine and proline, can efficiently eliminate undifferentiated iPS after differentiation induction. It would be better if the authors included some information concerning:

1. the successful induction of hepatocytes from iPS

Response: We have not yet succeeded in induction of hepatocytes from hiPS cells because the cells die within three days in HSM. "no reports exist on successful induction of fully differentiated hepatocytes from hiPS cells. Our HSM has not succeeded in induction of hepatocytes from hiPS cells because the cells die in HSM in three days." is added in "Elimination of unwanted hiPS cells among primary human hepatocytes" section. In abstract, "When primary human hepatocytes are co-cultured with hiPS cells, successful differentiation occurs." has been deleted because the sentence was confusing.

2. the specific killing of HSM to iPS not the differentiated and primary hepatocytes

Response: HiPS cells die and primary human hepatocytes survive in HSM as reported in reference 42, Tomizawa et al., PLoS One 8, e71897, 2013. Figure 3 C and D are added.

This review is well organized and written. It could be accepted for publication consideration after minor revision.

Reviewer 2

The authors hypothesized that the culture medium without glucose and arginine, and supplemented with galactose and ornithine, could eliminate iPSC but it could enable the growth of primary human hepatocytes. It is an interesting idea, but the proposal should be supported by experimental data. The manuscript is without such support. The authors should prepare a mixture of iPSCs and hepatocytes (or primary hepatocytes) and to culture such cell mixture in the proposed hepatocyte selection medium. If hepatocytes will survive and iPSC are eliminated, everything is OK. Without such demonstration, the manuscript is only a theoretical proposal.

Response: HiPS cells die and primary human hepatocytes survive in HSM as reported in reference 42, Tomizawa M et al., PLoS One 8, e71897, 2013. Figure 3 C and D are added.

Thank you again for publishing our manuscript in the *World Journal of Methodology*.

Sincerely yours,

A handwritten signature in cursive script that reads "Minoru Tomizawa".

Minoru Tomizawa, MD, PhD
Department of Gastroenterology
National Hospital Organization Shimoshizu Hospital
934-5 Shikawatashi,
Yotsukaido City, Chiba 284-0003, Japan
Phone: +81-43-422-2511
Fax: +81-43-421-3007
e-mail: nihminor-cib@umin.ac.jp