

Format for ANSWERING REVIEWERS

Jan.9, 2015

Dear Editor,



Please find enclosed the edited manuscript in Word format (Microsoft Office Word 97-2003 .doc).

Title: A new model for cardiomyocyte sheet transplantation using a virus-cell fusion technique

Author: Yuto Takahashi, Tomotsune Daihachiro, Sakiko Takizwa, Fengming Yue, Mika Nagai, Tadayuki Yokoyama, Hirashima Kanji, Katsunori Sasaki

Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO:14651

I appreciate valuable comments of the reviewer very much.

I has responded to them and has improved our manuscript according to them.

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Major comments:

(1) *The merge of cardiomyocytes and skeletal muscle is quite partial and not convincing.*

His suggestion is right. The merge is partial. To simulate *in vivo* transplantation, we did not perform plate centrifugation, which the protocol recommended, because a centrifuge is not practical *in vivo*. But based on this data i.e. partial merge, we transplanted it under the latissimus dorsi, but on it, to press the sheet toward the muscle closely.

(2) *In page 14 lines 3-10, authors classified the beating pattern of the transplanted cell sheet, but the biological significance of classification is not clear.*

As he points out, they may be an excessive analysis. So we delete the lines 2-9. But the description of figure 4 is intact, because I think we should describe observations in detail as possible, even if it is unknown at that time whether it has a biological significance.

(3) *Although HVJ-E may facilitate the fusion of the cardiomyocytes and skeletal muscles, it is still not clear whether these two cells are just closely attached or fused. By EM observation, authors may observe gap junction, or adherence junction structures. EM observation is absolutely necessary for demonstrating the contact of these two sets of the cells in vivo.*

I agree with him completely. We expected the fused cells would show drastic cytoplasmic exchanges each other, but each muscle seemed to maintain its structure. Though we think it is due to tough, ordered cytoskeleton system (actin including myosin), as we could not prove it, we dared to use the sentence "...seemed to be maintained.... and seemed to be partially mingled..." as seen in page 18 lines 23-25. To prove it, as the referee points out, other techniques including TEM etc. are necessary, though it can not be proved only with a simple TEM. We have thought this observation leads to another new big theme. So we described in the previous text "However, to prove the interrelationship between the membrane fusion and the cytoskeleton, other cellular morphological methods, such as transmission electron microscopy and deep-etching, may be required. This complicated cell biological theme exceeds the present study and needs to be further examined in

future studies." I have nothing to add further now.

Minor comments:

(1) In page 3 lines 4-5, how many cells were used for the culture and cell sheet preparation?

The number of fetal cardiomyocytes used for the culture is 1.5×10^6 . The sheet area removed from the dish is about 3.5 cm^2 containing about 2.1×10^6 . We have added this information to Abstract and, if necessary, M&M.

(2) In page 3 lines 14-15, the official gene symbol should be italicized

We have revised them in Abstract, M&M, Results and Fig.6.

(3) In page 3 result section, the paragraph is not double-spaced

We have revised it.

(4) In page 9 lines 2-4, authors should give the brief procedures for the HJV-E. Did authors centrifuge the cells with HJV-E?

We did not centrifuge them, as we explained above. The sentence has been largely revised as followed: Subsequently, $5 \mu\text{L}/\text{mL}$ of the fusogen (HVJ Envelope Cell Fusion Kit GenomONE-CF; Ishihara Sangyo Kaisha Ltd., Osaka, Japan) was added to them. After then,....

(5) In page 9 lines 21-22, authors should give the concentration of NaOH. What did authors mean in 20% NaOH?

We dissolved 20g of NaOH into 100ml distilled water and called it 20%NaOH. We use 5M NaOH, though I do not know whether the usage is adequate.

(6) In page 13 lines 6-7, Figure 2a and 2b is interchanged?

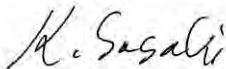
It is our mistake. We have interchanged them.

(7) In page 15 line 3, 'Figure 6d' is not correct

It is also our mistake. We have deleted 'Figure 6d'.

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

Sincerely yours,



Katsunori Sasaki, MD, PhD

Department of Histology and Embryology,

Shinshu University School of Medicine,

3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan. katsmd@shinshu-u.ac.jp

Telephone: +81-263-37-2589

Fax: +81-263-37-3093