ANSWERING REVIEWERS

October 22nd, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12926-review.doc).

Title: Cardiac disease modeling using iPSC-derived human cardiomyocytes

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Name of Journal: *World Journal of Stem Cells (special issue)*

ESPS Manuscript NO: 12926

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

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

1. Authors reviewed more than 10 types of experimental protocols including differentiation protocols and from spontaneously cells to mature cardiomyocytes. Then, they reviewed some cardiac disease models using iPSC to cardiomyocyte and more than 12 inheritable cardiac disease models from recent published literatures. However, through the MS, too many literature-reviews are summarized, but almost no any author's comments and suggestions.

We mentioned our data in two points (p. 6 and p.7), and we add a figure of our cells (Fig. 1)
p.6
We tested several of these procedures using iPSC derived from patients' fibroblasts and adapted to grow on Matrigel-coated dishes; in our experience, we observed beating areas only when cells were treated with modulators of the Wnt pathway, while the general *TNNT2* expression was achieved in most protocols (Fig. 1). Of particular interest for therapeutic purposes the recent setup by Burridge and colleagues that employs a chemically defined medium consisting of just three components on a dish covered by synthetic biological matrices [8]. Indeed, using this protocol we strongly increased the number of beating cells in our culture up to 50%, but the best result, around 70%, was recently obtained using the PSC Cardiomyocyte Differentiation Kit (Life Technologies).
p.7
Our data indicate that, after 16 days of *in vitro* differentiation, iPSC-derived CMs start to segregate in the various subtypes, showing pronounced sarcomeric structures that indicate a certain degree of maturation (Fig. 1C).

2. Using iPSC-derived human cardiac related models to mimic patients' symptoms including lethal arrhythmias is a new landmark for determining the mechanisms of some inheritable cadiac diseases including sudden death. However, author did not mention this topic. Relative literatures indicated some negative points of iPSC, such as its, copy number variation, or aberrant epigenomic reprogramming and coding mutation in human iPSC.

We believe that the iPSC model will hardly replicate patient's symptoms, but surely the cellular electrophysiological findings, as reported by many authors and as provided in the review, will help in designing new therapeutic strategies. For the genomic and epigenetic aberrations of iPSC, we believe that most of the "work" is done by the use of recombinant viruses carrying the reprogramming factor. Thus we add the last phrase suggesting the use of a Sendai-based technology.

3. Table 2 and Table 3 may combine to one table, or divide into two parts, one is *in vitro* cell culture part and anther is *in vivo* in mice part. **We changed it**

4. Through the review, authors have not mentioned the possible prospects or limitation on using iPSC for a personalized therapy.

We believe that, especially for cardiac therapies, the use of iPSC-derived cardiomyocytes is not an immediate and easy issue. Nevertheless, we add a sentence at page 5th, last raw "can be used, in a close future, as a continuous source of differentiated cells for therapeutic purposes".

5. P4, Introduction, line 1, Bizzozzero may Bizzozero. **We changed it**

6. P19, line 13: "proband" and line14 "[50]" need to combine to as: "proband[50]." **We changed it**

3 References and typesetting were corrected

4 All the authors have agreed to add two new authors after revision.

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

Sincerely yours,

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