Author’s response to reviews

Title: Dissolved molecular hydrogen (H2) in Peritoneal Dialysis (PD) solution preserve mesothelial cells and peritoneal membrane integrity

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Author’s response to reviews:
Reply to comments (Masaaki Nakayama)

Editor Comments:
1. We did not feel that you fully addressed comment 9 from reviewer 1 (It would be helpful to know why the authors assessed the expression of the chosen genes, especially aSMA and cytokeratin) so please indicate what changes were made to the manuscript in response to this point.

Reply:
Thank you very much for pointing out this crucial point. We chose these genes because we originally hypothesized that H2 could ameliorate activation of the process of EMT resulting from exposure to PD solutions by changing the expression of related genes, e.g. increase in aSMA and decrease in cytokeratin.
We revised the following sentences in the revised manuscript:
(Original sentences: Discussion; p10, line 11-)
In regards to the mechanism of H2 for peritoneal preservation, the exact mechanism also remained to be elucidated. But interestingly, as mentioned above, there were no differences in gene expressions, such as, EMT related genes (e.g. SNAIL, ECADHERIN aSMA), and anti-apoptotic and apoptotic gene (e.g. BCL2, BAX, BAD) between the PD and the H2PD groups (Fig. 4). This fact may indicate the potential of indirect action of H2 in terms of cellular protection by H2.

⇒Revised sentences

With regard to PCR analysis, we chose EMT and its related genes, and anti-apoptotic and apoptotic genes, because we originally hypothesized that H2 could ameliorate activation of the process of EMT and the oxidative cellular injury resulting from exposure to PD solutions. We expected differences in gene expressions between the PD and H2PD groups, e.g. increases in SNAIL, vimentin, aSMA and VEGF, and decreases in ECADHERIN and CYTOKERATIN in the former group, and increases in BAX and BAD, and decreases in BCL2 in the latter group. However, unexpectedly, there were no differences in gene expressions between the two groups (Fig. 4). Therefore, we suppose other potential mechanisms for the effect of H2 on membrane protection.

2. The quality of the English language should be improved in the revised manuscript. You should have your manuscript reviewed by someone who is fluent in English.

Reply:
I am very sorry for the poor quality of the language in the previous version of our paper. The revised manuscript has been edited thoroughly by a native English-speaking scientist (Forte Inc.).

3. Please consider the list of authors as it currently stands with reference to our guidelines regarding qualification for authorship. Currently, the contributions of authors AMS, SK and SI do not automatically qualify them for authorship. Please provide clarification on their contributions, or remove their names from the list of authors and place them in the “Acknowledgements” section instead.

4. In the Authors' contributions section please add a statement confirming that all the authors read and approved the final version of the manuscript.
All authors listed in the original manuscript were substantially involved with the study. Therefore, we revised the section on author contributions as below:

(Original manuscript)
Authors’ contributions MN designed and supervised the whole study, and finalized manuscript. WZ, KW, and AG carried out the experimental study. WZ, KW, and MN wrote the draft manuscript. AMS, SK, and SI discussed the results for manuscript preparation.

(Corrected (Revised manuscript))
Authors’ contributions MN designed and supervised the entire study and finalized the manuscript. WZ, KW, and AG carried out the experimental study. WZ, KW, and MN wrote the draft manuscript and created the figures. AMS, SK, and SI contributed to study conception and analysis and interpretation of data. All the authors read and approved the final version of the manuscript.

5. Please add an 'Ethics approval and consent to participate' section to the Declarations.

Reply:
We added the following sentences in the revised manuscript: ‘All the study procedures and protocols were approved by the Animal Committee of Fukushima Medical University (approval number: 25017).’

6. In addition, please change the title of the Acknowledgements section to 'Funding' and please add a separate Acknowledgements section where you should acknowledge anyone who contributed towards the article who does not meet the criteria for authorship including anyone who provided professional writing services or materials.

Reply:
We changed ‘Acknowledgement’ to ‘Funding’, as recommended.
Further, we added the following sentences in the Acknowledgement section:
Our special thanks to Ms. Ohashi and Ms. Hashimoto for their technical assistance with the experiments.

7. On re-submission, please select section 'Pre-dialysis care and dialysis'.
8. When you have completed all the requested revisions please upload an entirely clean version of the manuscript with no revisions indicated.

Reviewer reports:
Carl Öberg (Reviewer 2): While the manuscript is much improved, there are still some minor issues that need to be dealt with.
First, it is stated both in the Background and in the Discussion that hydrogen gas is inert. Hydrogen gas is however not chemically inert. By contrast, hydrogen reacts readily with many oxidizing agents such as oxygen and other electronegative elements. In my opinion this factual error must be corrected before the article can be accepted.
Reply:
Thank you very much for pointing out this error in our manuscript. We revised the sentences in order to make the logic scientifically acceptable as follows:
In the Introduction:
(Original) Dihydrogen (H2) is an inert gas with no known side effects.
(Revised) delete
In the Discussion:
(Original) H2 is inert and is very easy to apply clinically.
(Revised) To date, no critical adverse effects of H2 have been reported in humans, making it seem like a very good candidate for clinical application, provided there is scientific rationale for its use.

Secondly, as I pointed out in my first review, the manuscript is not paginated. This makes it much more difficult to review. Please, for future reference, make sure all your manuscripts have page numbers. Lastly, there are several misspelled words and grammatical errors in the manuscript that need to be corrected.
Reply:
Thank you for the advice and recommendations. All misspelled words have been corrected and pages have been numbered in the revised manuscript, as recommended.
In addition, the revised manuscript has been thoroughly edited by a native English speaking scientist (Company Forte).