Author's response to reviews

Title: Gene Therapy for Cardiovascular Disease Mediated by Ultrasound and Microbubbles

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Author's response to reviews: see over
Dear Professors,

Thank you for your time and thoughtful comments concerning our manuscript entitled “Gene Therapy for Cardiovascular Disease Mediated by Ultrasound and Microbubbles”. Those comments are all valuable and very helpful for revising and improving our paper. After carefully studying the reviewers’ advices, we have adopted the suggested revisions. Corresponding changes have been made in our original manuscript. The main corrections in the paper and the responds to the reviewer’s comments are as flowing:

Responds to the reviewer’s comments:

Reviewer 1 Comments:

1. Author should better discuss the biophysical effects of US that are crucial in US enhanced gene transfer (cavitation, microstreaming…).

Response: Thank you for your kind words. According with your advice, we have discuss the biophysical effects of US that are crucial in US enhanced gene transfer (cavitation, microstreaming…) in chapter 3 line 8-16 "The mechanism… (Fig.1)".

2. There is a lot of confusion in all the text in using due to the misuse of the abbreviations versus the full names. The authors should use the abbreviations/acronyms from the beginning.

Response: Thank you for your kind words. We have given out the proper full name when using the abbreviation.


Response: Thank you for your kind words. We are very sorry for our incorrect writing. And we have made correction according to your reminding.

Minor essential revision

1. Chapter 2 line 19: “occur” should be “occurs”

Response: Thank you for your kind words. We have made correction according to your comment.

2. Chapter 2 lines 27-29: the sentence is not clear. Please write this again

Response: Thank you for your kind words. We have rewrite the sentence as "In other hand, …of plasmid DNA."
3. Chapter 3 line 9 “in the lesions” should be “at the site of interest”

Responds: Thank you for your kind words. We have made correction according to your comment.

4. Chapter 3 14-15: “Thereby, UTMD…” It’s better link the two sentences

Responds: Thank you for your kind words. We have linked the two sentences as "Since UTMD can not only improve the efficiency, but also avoid the immunogenicity, it has been regarded as a new choice for gene therapy."

5. Chapter 4 line 2: please correct “microvessle” should be “microvessel”

Responds: Thank you for your kind words. We have made correction according to your comment.

6. Section 4.1 line14-17: this sentence is not clear. It needs explain better results. What’s WT-1?

Responds: Thank you for your kind words. We have revised the sentence, and the results were explain as "The result showed that WT1 started to express from the nucleus of epicardium layer cells one week after UTMD-TB4 treatment (Fig.2), and the level of WT1 mRNA of which treated with UTMD-TB4 group was 43.5-fold higher than in normal control or UTMD-DsRed groups in the 4th week, and c-kit mRNA level in UTMD-TB4 group was 52-fold higher after UTMD-TB4 treatment." WT1 is a biomarker for development of embryonic heart and is not normally expressed in adult rat heart, and it was described in our revised paper.

7. Section 4.2 line5: The advantages of UTMD have already been mentioned several times. Eliminate this sentence.

Responds: Thank you for your kind words. We have eliminate this sentence according to your comment.

8. Section 4.2: line 24: Please define USWF.

Responds: Thank you for your kind words. USWF means "ultrasonic standing wave fields". In order to make it easy to understand, we have replaced USWF by ultrasonic standing wave fields in our revised paper.

9. Section 4.2: “Cell regulatory or transcription factors...”. please explain better this sentence.

Responds: Thank you for your kind words. We are very sorry for our incorrect writing. And we
have rewritten this sentence as "It also presumably that ultrasound affected cell regulatory or transcription factors."

10. Section 4.3: “Definity microbubble” should be “commercially available Definity MB”
    
    Responds: Thank you for your kind words. We have made correction according to your comment.

Reviewer 2 Comments:

1. The authors may also discuss the limitation of UTMD, particularly how some of the experimental studies can be translated to real clinical applications.
    
    Responds: Thank you for your kind suggestion. We have discussed the limitation of UTMD and how some of the experimental studies can be translated to real clinical applications in last paragraph "UTMD is a promising technique…to move this field forward."

2. The authors need to go through all the cited pictures, consider the words limit and chose the representative pictures.
    
    Responds: Thank you for your kind words. We have gone through all the cited pictures carefully and deleted picture 1,3,7,14. And keep those were related to our theme.

3. The authors need to check the spelling and grammar carefully, provide full names when abbreviations is firstly introduced.
    
    Responds: Thank you for your kind words. We have carefully checked the spelling and grammar throughout the paper and given out the proper full name when using the abbreviation as suggested.

4. Page 3 "Nonviral systems is consist of chemical methods…protein (EGFP) plasmids could be delivered effectively into rabbit Achilles tendons by UTMD, causing no obvious injury". As the purpose of this paragraph is to introduce the "Current Status of Gene therapy of Cardiovascular Disease", it is necessary to cite some examples regarding nonviral systems related to cardiovascular disease.
    
    Responds: Thank you for your kind words. According to your comment, we have introduced reference 25 "Ko et al[25] conjugated… enhanced in the ischemic zone." as an examples regarding nonviral systems related to cardiovascular disease related to cardiovascular disease.

5. Page 4, the introduction of "Mechanisms of UTMD in Gene Therapy" is not comprehensive,
because the paper describe the mechanism as "Meanwhile, the destruction of MBs may transiently induce cell membrane penetrability to transfer drug the author better introduce it.

Response: Thank you for your kind words. According with your advice, we have discuss "Mechanisms of UTMD in Gene Therapy" in chapter 3 line 8-16 "The mechanism… (Fig.1)" in detail.

We acknowledge the reviewer’s comments and suggestions very much, which are valuable in improving the quality of our manuscript.