To
Lian-Sheng Ma
Founder and Chief Executive Officer
Baishideng Publishing Group Inc

Manuscript NO.: 55683

"Response to Reviewer"

Dear Dr. Ma,

Thank you for the review of our revised manuscript, which we have now carefully revised according to the remarks and suggestions of the Reviewer, and which we think has further improved the manuscript.

We resubmit the revised manuscript to "**World Journal of Stem Cells**". Please find attached our point-by-point response to the referee's comment in blue, with the added sections highlighted in the manuscript in yellow:

Reviewer #1:

This is a very important study about transplantation of human adipose-derived MSCs into porcine left ventricular free wall. The difference between native haMSCs and differentiated haMSCs after transplantation in terms of marker expression may be explained more in detail. The mechanism of BMP4-induced differentiation of haMSCs into pacemaker cells may be added.

Thank you for these valuable comments. We have now added a description of pacemaker-related marker expression in haMSC, before and after *in vitro* differentiation (which we called pre-conditioning in our *in vivo* paper). We have further explained the effect of *in vivo* differentiation on these cells in more detail, which we have observed in pre-conditioned cells, only. Accordingly, we have added the following section to the discussion in the revised manuscript:

"Immunohistochemical analyses of sections derived from the site of cell injection indicated abundant expression of pacemaker-specific proteins in dhaMSC, including HCN1 and HCN4, both important for spontaneous diastolic depolarization in the human sinus node[22,24], the L-type calcium channel alpha subunit $Ca_v1.2$, implicated in the upstroke of action potential in cardiac pacemaker cells[25], and the pacemaker-specific connexins Cx31.9 and Cx45, which play a crucial role in impulse propagation of nodal tissue[26]. As shown previously [17], these markers were only

poorly expressed or even absent in undifferentiated nhaMSC. Of note, differentiation medium RPMI-B27 supplemented with BMP4 caused a significant upregulation of these markers and other pacemaker-related genes, while HCN4 was still lacking[17]. Interestingly, in vivo transplantation of nhaMSC was not associated with increased membrane surface expression of pacemaker-related markers. In contrast, preconditioning with RPMI-B27/BMP4 medium resulting in so-called dhaMSC induced expression of multiple pacemaker-related genes and further improved expression of these markers, including HCN4, after in vivo myocardial integration. Thus, in vitro pre-conditioning of MSC may act as prerequisite for further pacemaker-lineage differentiation after in vivo integration."

Moreover, in the revised manuscript, we elaborate on the putative mechanism that may underlie BMP4-induced differentiation of haMSCs into pacemaker cells, as suggested by the Reviewer:

"Efficiency of MSC differentiation may be importantly regulated by BMP4, which is one of the major components of the differentiation medium. As previously shown[17], supplementation of the differentiation medium by BMP4 resulted in pronounced upregulation of the sinus node transcription factor SHOX2, and of BMP4 itself, considering that SHOX2 activates BMP4 during early sinoatrial development [29]. This mechanism may activate a positive feedback-loop, driving cell differentiation towards a cardiac pacemaker phenotype."

We look forward to your assessment of our revised manuscript.

Yours truly,

Patrick A. Schweizer
Corresponding author