February 27, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15863-review.docx).

Title: Contemporary perspective on endogenous myocardial regeneration

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Name of Journal: *World Journal of Stem Cells*

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

1 Format has been updated and abstract was expanded to include over 200 words.

2 We thank reviewers for their comments aimed at improving the manuscript.
Revisions have been made according to the suggestions of the reviewers.

Reviewer 1:
The manuscript deals with an interesting and important topic. It provides a good overview and is well-written.

We thank Reviewer 1 for his/her comments.

Reviewer 2:
The manuscript "Contemporay perspective on endogenous myocardial regeneration" addresses in a review the complex field of cardiac regeneration including clinical aspects like stem cell application, growth factor injection and coronary sinus occlusion. As the mansucript adresses many aspects, it is not easy to follow and needs some more concretization (focus on clinical implication). The first part is very long and needs shortening. Furthermore the use of figures or tables would be helpful to understand signal pathways.

We thank Reviewer 2 for his/her comments.

(1) *As the mansucript adresses many aspects, it is not easy to follow and needs some more concretization (focus on clinical implication)*

- in the introductory paragraphs we clarify the structure of the manuscript:
On page 5, lines 25-30
We proceed in three subsequent steps by examining 1) the evidence for the self-regenerative capacity of the heart, 2) the initiation of endogenous myocardial repair mechanisms in a clinical setting such as acute or chronic myocardial ischemia and 3) the results of the hitherto conducted clinical studies that investigated different methods of triggering endogenous myocardial regeneration in failing human hearts.;

- we have revised and expanded sections 4 and 5 on the hitherto conducted clinical studies on endogenous myocardial regeneration and added the section on cell reprogramming.
The following paragraphs were added:

On page 14, lines 14-19

Principal limitations of the hitherto conducted trials are the small number of included patients and the non-standardized cell therapy that varied both in respect to the cell type (cardioshpere-derived cells versus isolated c-kit+ cells) and the route of delivery (intracoronary versus intramyocardial). In addition, one study (ALCADIA) used bFGF to boost the retention of the transplanted cells.

AND lines 23-32

However, the endogenous pathways of regeneration still remain largely enigmatic. As has been presented in the above sections of this review, there is still controversial evidence regarding the origin of the newly formed cardiac myocytes (myocardium-specific progenitor cells versus self-replicating cardiomyocytes). The ambivalence in respect to the key factors behind the process of endogenous myocardial regeneration hampers clinical research, in as much as it makes the standardization of the therapeutic approach virtually impossible – the variety of scientific theories that are aiming at deciphering the endogenous signals is mirrored by the variety of clinical interventions and techniques (different cell types, routes of delivery, target populations etc., Table 1).

AND on page 16, lines 4-8

Growth factors such as VEGF, fibroblast growth factors (FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), erythropoietin (EPO), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), placental growth factor (PIGF), stem cell factor (SCF) and angiopoietin (Ang) have been postulated as activators of endogenous stem cells within the myocardium and so as initiators of its self-regeneration.

AND on page 16, lines 16-32 AND on page 17, lines 1-19

Initial trials on the effects of FGF in patients with coronary artery disease (CAD), via intracoronary delivery of adenoviruses, showed potential of this method to improve regional blood flow. However, further clinical studies failed to produce reliable favorable clinical outcomes in CAD patients, albeit the effect of FGF seemed to have been gender-specific with improvement in outcomes in women.

Experimental studies have demonstrated the potential of G-CSF to improve LV function after MI, mainly by fostering arteriogenesis. Initial clinical application of G-CSF in MI patients indicated the potential to improve echocardiographic LV functional parameters, but subsequent randomized trials did not show improvement in clinical outcomes. Similarly, in patients with chronic occlusive CAD, subcutaneous injection of G-CSF improved neither myocardial perfusion nor LV function, albeit it appeared to reduce anginal symptoms .

GM-CSF has been associated with mobilization of endothelial progenitor cells (EPCs) and ultimately neoangiogenesis. A clinical trial showed enhanced collateral flow following the subcutaneous injection of GM-CSF in CAD patients . However, the study raised a safety concern due to occurence of acute coronary syndrome in patients on treatment with GM-CSF.

The use of VEGF as a potential mediator of neovascularization has been extensively studied both experimentally and in clinical trials. Animal models of myocardial ischemia indicated the potential of VEGF to induce angiogenesis. Subsequent trials showed tendency toward improvement of functional parameters such as regional myocardial perfusion or wall motion, but with no significant effect on clinical outcomes .

EPO is secreted in tissues under hypoxia and is posited to foster angiogenesis and progenitor cell development, while inhibiting apoptosis. Whereas animal studies indicated that EPO administration was associated with the decrease in infarct size, a clinical trial in patients with acute MI did not show improvement of heart's function.

Further growth factors such as PIGF, Ang and the ligand of c-kit, SCF, which have been proposed to induce neovascularization and mobilization of progenitor cells, showed their potential to regenerate damaged myocardium in animal models, but solid clinical evidence is still lacking.

A common characteristic of the described growth factors and cytokines is that their potential to induce myocardial regeneration, mainly via paracrine effects, has been established in experimental models, but

with no direct translation in the improvement of clinical outcomes.

AND on page 17, lines 21-32 AND on page 18, lines 1-7

The idea of cell reprogramming to achieve structural and functional renewal of the damaged myocardium has been substantialized through the concept of induced pluripotency, whereby fibroblasts could be reprogrammed to assume pluripotency and further directed into the state of cardiac lineage cells. However this process implies transplantation of such induced cells into the damaged myocardium (Figure 1). On the other hand, direct reprogramming of the scarred myocardium involves conversion of fibroblasts into functioning cardiomyocytes by delivery of the following transcription factors into the cells of heart tissue: Gata4, Mef2c, Tbx5 (GMT). Despite its luring perspective cardiomyocyte replenishment via GMT reprogramming has been hampered by a purported lack of efficiency, relating primarily to the degree of molecular and electrophysiological development of the newly formed cardiomyocytes.

Cell reprogramming has been enhanced by the delivery of microRNAs only or in combination with transcription factors. Furthermore, the delivery of sets of microRNAs that have been associated with cardiac developmental pathways may present another option of initiating myocardial regeneration without the need for cell transplantation.

(2) *The first part is very long and needs shortening.*

- We have revised the introduction, sections 2 and 3 to simplify and eliminate any redundancy. The following paragraphs have been removed:

On page 5, lines 30-32

~~We also investigated novel, clinically applicable strategies for inducing endogenous myocardial regeneration in ischemic heart without relying on stem-cell transplantation.~~

AND, on page 6, lines 5-8

~~, a contention based on the inability to experimentally detect DNA synthesis and consequently mitosis in postnatal cardiomyocytes and the pioneering studies showing that cardiac myocytes do not reenter cell cycle and proliferate.~~

AND lines 11-16

~~Hence, cardiomyocytes respond to internal and external stimuli such as ischemia and aging by growing in size and leading in most cases to heart enlargement. If hypertrophy of cardiac muscle cells were heart's exclusive answer to pathologic stimuli, such as ischemia, then myocardial regeneration would only be possible by means of repopulation with transplanted cardiomyocytes or progenitor cells capable of reclaiming the functional role of cardiomyocytes.~~

AND on page 8, lines 10-14

~~Despite the presented evidence some questions remained unanswered and some of the stated hypotheses about the proliferation capacity of cardiac myocytes under stress are still controversially debated. Most importantly it must be noted that the observed processes of DNA synthesis and mitosis alone do not guarantee that the actual cellular division occurs.~~

AND on page 9, line 10

~~of which several types have been identified within heart tissue~~

AND on page 11, lines 15-18

~~The mitotic index (ratio of the nuclei undergoing mitosis over those not undergoing mitosis) after acute myocardial infarction was found to be 520 cardiomyocytes out of 1 million, thus accounting for approximately 2 million cardiac myocytes in the mitosis within the left ventricle wall early after AMI~~

AND on page 12, lines 1-11

~~The argument for generation of new heart muscle cells in response to acute ischemia was construed upon the following observations. Mitotic spindle as a product of a spatial arrangement of microtubules was microscopically detected. Sacromeric α-actin antibody was used to successfully identify the contractile ring, which itself delineated the division line and emergence of two daughter cells. Finally, both images of mitotic divisions as well as of cytokinesis were obtained. It may thus be assumed that the visible evidence of mitosis did correlate with the mentioned up-regulation of the cell cycle antigen Ki-67. Accordingly, border zones of infarcted myocardium had 70 times more mitotic myocytes as compared to the healthy tissue, whereas remote myocardial zones harbored 24 times more dividing myocytes than healthy myocardium.~~

AND further on page 12

~~termed ischemic cardiomyopathy or chronic IHD~~ ~~AND~~ ~~The question thus remained why the calculated myocyte loss appeared to be not so dramatic, despite~~ ~~AND~~ ~~Interestingly, quantitative analyses have not confirmed the expected cardiomyocyte loss in patients with vast collagen deposition.~~ ~~AND~~ ~~The fact that cardiomyocytes proliferate as an answer to ischemia might provide a logical explanation for the observed phenomena.~~

(3) *the use of figures or tables would be helpful to understand signal pathways.*

We have added Table 1 that summarizes the hitherto conducted clinical trials in the field of endogenous myocardial regeneration, the Figure 1 to summarize two basic approaches to myocardial regeneration, transplantation of different types of stem cells versus activation of self-repair pathways without cell transplantation and finally the Figure 2 to visualize the posited hypothesis of "embryonic recall".

3 References and typesetting were corrected

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

Sincerely yours,

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