

Format for ANSWERING REVIEWERS



February 3, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7173-review.doc).

Title: Endothelial Progenitor Cells in Cardiovascular Diseases

Author: Poay Sian Sabrina Lee, Kian Keong Poh

Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO: 7173

The manuscript has been amended according to the suggestions of reviewers. The format has also been updated according to Editorial staff's instructions. We hope the Editor and Reviewers are satisfied with the improved version.

Reviewer 1

(1) The manuscript needs language service. For example, in page 4 line 13 "such as morphology such as the"

We thank the Reviewer for the pointing out this important aspect of article writing. We have extensively revised the manuscript, paying particular attention to the language. In addition, we have asked a senior member of the department to vet through and help us rectify any further language issues. The example given by the reviewer has been amended accordingly (refer to the edited manuscript).

Reviewer 2

(2) The undetermined effect that PAD has on EPC number or function seems confusing. It is unclear as to the effect of the disease on EPC numbers since multiple studies give contradicting results. Also the authors mention abdominal aneurysm in comparison to PAD and don't provide its relevance. It seems suitable for the authors to give their opinion about possible reasons for this discrepancy.

We thank the reviewer on the comments on EPC and PAD. We agree that studies investigating the effects of PAD on EPCs have yielded contrasting results. This may be due to the different studies recruiting patients with PAD of different severity and also the varying sample sizes. More studies are being conducted, including some from our group. Different methodological

differences in measuring EPC population may also complicate interpretation of data. The part on abdominal aortic aneurysm is now removed. The relevant amendments are made in the manuscript.

(3) The authors should elaborate more as to the effects of CVD on function of EPC such as the colony forming property they mention.

We have now elaborated more on the effects of CVD on functions of the EPC. The number of colony forming units indicates the angiogenic capacity of the cells cultured. Generally, cardiovascular disease has been shown to reduce EPC functions including the colony forming properties. In addition, angiogenic-related function of EPCs such as tubule formation, proliferation and apoptosis are also downregulated in the presence of cardiovascular risk factors (diabetes, hypertension and hypercholesterolemia) and CVD (stable CAD, PAD). Treatment with cardiovascular-related medicine to improve these risk factors has also improved EPC function. Several more studies have been added as references into the manuscript.

(4) The authors have a separate section for “Endothelial Progenitor Cells as a Prognostic Marker for Cardiovascular Diseases”. It is not clear why the authors separate this section from the first part where they talk about the different CVD and the EPC indication.

We initially wanted to separately discuss EPCs as a prognostic marker, rather than its utility as a biomarker for disease severity. However, we have now moved the sections so that they are sequential.

(5) The authors mentioned the types of EPC; early and out-growth EPCs. The differences between the two kinds of cells should be mentioned at the genetic and functional level beside the cell morphology, and the relevant references should be cited. For Fig.1 and Fig.2, the detailed information should be offered, such as where the cells come from and what is the passages they are. Fig 2 doesn't show typical cobblestone EC.

We thank the Reviewer for the comments on the types of EPCs. We have clarified the differences between early and out-growth EPCs at the functional level as well as cell morphology. However, we could not find studies on genetic differences between these cells. If we miss this out and the Reviewer can enlighten us, we will be delighted to include those references.

Early EPCs are cells that can form colony forming units (CFU). They possess many endothelial characteristics such as CD31, TIE2 and VEGFR2 (Asahara *et al.*, 1997). Functionality of CFU has been demonstrated in a study by Hill and colleagues,.. Outgrowth EPCs also possess endothelial characteristics such as VE-cadherin and von Willebrand factor in addition to CD31, CD133, CD34

and VEGFR2 (Shantsila *et al.*, 2008). These outgrowth EPCs can differentiate into mature endothelial cells for angiogenesis and vasculogenesis.

Details on Figures 1 and 2 have been updated in the manuscript.

(6) Why do the cardiovascular- related medicines induce the increase of EPCs number? The reason should be summarized and/or discussed.

Reviewer's comment is noted. These drugs improved EPC number and function via various mechanisms, such as through the PPAR- γ signaling pathway, Akt pathway and reduction in oxidative stress via NAD(P)H oxidases. These contributed to increase in mobilization of EPCs from the bone marrow. Summary of mechanisms in improvement of EPC number and function for each category of drugs is now included in the manuscript. Improvements in cardiovascular health in patients on medical therapy also correlate with EPC number and functions.

(7) The authors state "Recent study also reported lower CD34+ count in patients with peripheral vascular disease compared to abdominal aortic aneurysm". What is the importance of this? Why is this relevant for PAD

We thank the Reviewer for the comment. There is a study that compared EPCs in PAD to that in AAA, which we cited. However, we agree that inclusion of abdominal aortic aneurysm in the PAD section was not justified and that has been removed from the section.

(8) The authors state "There is also contrasting evidence from other studies which reported increased or no difference in number of EPCs in CAD patients compared to controls. Notably, in patients with CAD, the circulating numbers of EPCs seem to predict cardiovascular outcome". Based on the results from the different groups cited by the authors the correlation between the EPC numbers and cardiovascular outcome is unclear.

We appreciate the reviewer's comment. Majority of the studies reported decreased EPC in patients with CAD. Some studies did report contrasting results. The differences in the methodologies may account for the different results. In addition, the low frequency of EPCs in circulation and types of EPCs harvested may also contribute to some of the differences. Some studies look at patients with different severity of CAD and this may also affect EPC results. On prognostic significance, overall EPC numbers does correlate to outcomes, as most studies support the association between the level of circulating EPCs and the risk of cardiovascular events among patients with angiographically documented CAD. The relevant references have been added into the manuscript.



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3 References and typesetting were corrected

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

Sincerely yours,

A handwritten signature in black ink, appearing to read 'Kian Keong Poh'.

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