Response to reviewers

May 25, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name:18188-EDITED.doc).

Title: An easily-handled method to isolate mesenchymal stem cells from coagulated human bone marrow samples

Authors: Heng-Xiang Wang, Zhi-Yong Li, Zhi-Kun Guo, Zi-Kuan Guo

Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO: 18188

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

Response: The manuscript has been revised according to the suggestions of reviewers.
2 Revision has been made according to the suggestions of the reviewer

(1) Put spaces between values and units (e.g., 8 hr vs. 8hr or 8 mm vs. 8mm), this was not done consistently in the manuscript.

Response: Spaces have been put between values and units.

(2) Also, a space between "Fig. and the number" (e.g., "Fig. 1" rather than "Fig.1" In statistical analysis section, use "A p-value" rather than "A P value".

Response: The errors have been corrected.

(3) Each of the graphs in figure 2 lack a label on the Y-axis. Labels should be included for clarity.

Response: The labels have been added in the revised manuscript.

(4) Data in figure 2A is depicted as the number of CFU-F's. However, according to the methods colonies were counted in plates charged with 2 million bone marrow cells after 12 days of expansion. Normally, the CFU-F assay is performed on purified MSC populations plated at low density (100 cells in 10 cm dish). While it is possible to count colonies by the method described in the paper it is atypical of the standard CFU-F assay. Consequently, it would be beneficial if the authors included as part of Figure 3 lower power images of the Geimsa stained plates so that the overall number of colonies per plate is discernable. The high powered images in Figure 3 are valuable since they show the fibroblast-like morphology of the isolated cells.

Response: Lower images of the Gimsa-stained plates have been added in the revised version.

(5) Data in figure 2b-d would be more informative if each time point (0, 8, 16h) were plotted individually as a function of passage. This would reveal whether populations isolated by different methods (urokinase, etc.) show similar growth kinetics, despite the fact that initial cell yields were significantly different. This information is more important than the effect of time left at 4 degrees. Since the data in figure 2 includes multiple cell aliquots from multiple donors, it would be appropriate to analyze the data using ANOVA to see differences between treatment groups. For example, analyses can be performed to evaluate differences in yield based on isolation method (urokinase, etc.), and a similar analysis can be used to evaluate effect of time at four degrees. There is allot of data represented here and the authors should take time to perform some multivariate analysis to extract as

much information as possible. This approach will provide more valuable information than a simple Students t test.

Statistical analysis has been re-performed.

(6) The authors state that prolonged storage at four degrees reduced the viability of the cells used in the study. Were any of the CFU-F data normalized to cell viability? If not how would this affect the data? The authors should at a minimum report the differences in viability between the stored samples.

Indeed, the decrease of CFU-F numbers after storage was due to the cell viability.

(7) Data in figure 5 are qualitative in nature. It would be useful if these data were quantifiable, which would allow the authors to compare the bi-lineage differentiation potential of MSCs prepared by the different treatments. Is it possible to quantify the data?

The samples have been used and quantitative data could not be collected.
3 References and typesetting were corrected

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

Sincerely yours,

Zikuan Gu, MD, PhD
Dept. of Experimental Hematology
Beijing Institute of Radiation Medicine
27# Taiping Road
Fax: +86-10-68182778
E-mail: guozk@bmi.ac.cn