Reviewer's report

**Title:** Different culture media affect growth characteristics, surface marker distribution and chondrogenic differentiation of human bone marrow-derived mesenchymal stromal cells

**Version:** 1  **Date:** 19 May 2013

**Reviewer:** Philip Kasten

**Reviewer's report:**

General statement

This is a first review of the manuscript “Different culture media affect growth characteristics, surface marker distribution and chondrogenic differentiation of human bone marrow-derived mesenchymal stromal cells” by Hagmann et al. The authors of this study aim to analyse the impact of the choice of different culture media on mesenchymal stromal cells growth potential and their differentiation, and more importantly their surface marker distribution in-vitro.

The authors present an interesting and well-designed study. While the media differ substantially regarding their composition, the authors clearly state that they wanted to emphasize on how important more standardized culture techniques may be, considering the increasing clinical application of MSCs. The study was performed accurately. The article is well structured and the posed questions are well defined. This is an article of some interest to all researchers and clinicians working on MSC applications. The study does adhere to ethical standards of scientific and medical research. The quality of written English is good and the read flow is nice and allows the reader to follow a red line.

Specific comments:

Research question

The authors chose to analyse the impact of four widely used expansion media (whereas Bernese media is the less frequently used in my opinion) on the growth of mesenchymal stromal cells, their surface marker distribution and their differentiation potential in-vitro. It has to be stated that two rather simple culture media are compared to two more elaborate media, containing a cocktail of growth factors. While one could consider that this does not allow determining the actual factors that caused the changes observed in surface marker distribution, the authors discuss this fact with a more “straight forward” approach. It is very interesting to see that basics choices such as the culture media in tissue engineering can significantly affect different populations of MSCs. This however does not allow any assumptions about functional properties, which is also mentioned by the authors. While the finding that growth parameters and differentiation potential may vary with different culture media is well known, there has been less emphasis on the actual effect on surface marker distribution. In
this regard, this study adds novel findings to an underestimated part of cell culture.

- Even though it is evident that it is not possible to analyse all culture conditions and growth factors, the authors should describe why these four media were chosen, especially Bernese medium, which has been described for chondrocyte cultures.

Title and abstract
The title and abstract are accurate and convey what has been found. I am aware of the fact that there is an ongoing discussion about the terms mesenchymal stem or stromal cells; I therefore would be interested why the cells that seem to fulfil all the ISCT criteria for mesenchymal stem cells were called mesenchymal stromal cells.

Introduction
Overall, it provides an adequate referenced review, and state purposes and hypotheses. The literature is represented in detail.

Methods:
In general the methods are appropriate and well-described and sufficient details are provided to replicate the work.

- Please name the used Isotype antibody controls.
- Please add the the parameters tested for each test in the statistics section.

Results:
The presented data are sound and well controlled and the study is able to address the raised question. The figures and tables are accurately structured and stand by their own. There is however an important variation in the results of the growth parameters, the differentiation results and the surface marker distribution, which seems to be donor related. This well known experience when working with MSCs is however addressed in the discussion (see below).

- The information on statistical tests should be given in the Methods section.

Discussion:
The discussion and conclusions are well balanced and adequately supported by the data. The drawn conclusions are supported by the data. As the authors mentioned it remains unclear what the functional impact of these findings is going to be. However, it is a relevant finding that the subpopulations of MSCs may be different in different laboratories.

- The heterogeneity of human BM-MSC preparation is a well-known problem. It would help if the literature considering this fact would be discussed (e.g. [1]).
- It is unclear why certain surface markers were chosen. Regarding the
surprisingly important differences in the expression of CD140b and CD146, for example, it would help the paper if some conclusions were drawn from the significance of these surface markers for tissue engineering applications.

- To this stage it is only an observation of surface marker changes but the functional impact needs to be studied. I would appreciate if the authors could mention their ideas and hypothesis about possible functional impacts.


The study does not need to be seen by a statistician.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests