

## Format for ANSWERING REVIEWERS

June 3, 2014



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: BAER PC WJSC 2014 R1.doc).

**Title:** Adipose-derived mesenchymal stromal/stem cells: An update on their phenotype in vivo and in vitro

**Author:** Patrick C. Baer

**Name of Journal:** *World Journal of Stem Cells*

**ESPS Manuscript NO:** 8404

The manuscript has been improved according to the suggestions of reviewers (please find my answers on the following pages). Revised parts of the manuscript are highlighted in red.

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

A handwritten signature in blue ink, appearing to read 'Patrick C. Baer'.

P. C. Baer

A handwritten signature in blue ink, appearing to read 'Patrick C. Baer'.

**REVIEWER 1:**

The review article discusses the most recent findings concerning Adipose-derives stem cells. This review article is well-written, and the reviewer has only a few minor comments below.

I thank the reviewer for his complaisant comments. I revised the manuscript due to the comments.

1: In page 7, the author abbreviates smooth muscle actin as smA, and the reviewer would like to make sure that the author wants to abbreviate it as such, not as SMA, which is the common abbreviation.

I changed the abbreviation to SMA.

2: In page 11, is "CD" missing in "haematopoietic stem-like cells (34+/45+)?"

I added "CD" in this sentence.

3: In page 15, is the abbreviation "aSC" correct? NOT "ASC"?

I corrected the abbreviation.

**REVIEWER 2:**

In this manuscript, the author has well updated the isolation, culture, both in vitro and in vivo phenotype characterization of adipose-derived mesenchymal stromal/stem cells. Overall, this is a nicely written review article on ASCs and this reviewer does not have further comment.

I thank the reviewer for his complaisant comment.

**REVIEWER 3:**

This is a very interesting and well written review on the MSCs derived from adipose tissues. It is very clearly presented that the presence of CD34 marker on the ADMSCs is a questionable subject. Since the CD34+ cells are only present during the intitial stages of MSC separation, the problem may hinder the proper characterization of clinically applicable MSC preparations.

I thank the reviewer for his complaisant comment.

#### REVIEWER 4:

The Ms. 8404 attempts to review literature on concerning ASCs' subpopulations, heterogeneity and culture standardization. The manuscript is well researched with 83 references and well written with clarity of subheadings dealing with critical questions and issues. As the field is chaotic in a phase of collecting data with limited knowledge on all these three issues, the author's insight, if offered, will be greatly appreciated. Some specific comments below may help the author revise for better logic flow of the intertwined narrative on ASCs' subpopulations, heterogeneity and culture standardization. Specific comment:

I thank the reviewer for his helpful and comprehensive comments. I answered all comments directly in this letter.

Furthermore, I revised some parts of the manuscript.

1. Rewrite the abstract to focus on the issues about ASCs' subpopulations, heterogeneity and culture standardization. The current form seems like an introduction.

I revised the abstract. Some sentences are needed to introduce the cells (the article deals with), especially the first three sentences. Nevertheless, I know that these sentences sound like an introduction, but I think the sentences are essential. The following sentences were added, and I think that the abstract focuses now clearer on the issues of the review.

2. Better revise with an intertwined narrative on ASCs' subpopulations, heterogeneity and culture standardization, which is inter-related. Schematic diagrams or tables may help the readability.

I agree with the reviewer that a diagram or a table may increase readability. Nevertheless, I didn't add a diagram or a table because I found no setup (e.g. for a table) with an additional benefit for the reader.

3. Definition of MSCs is as follows: 1) Culture definition - "MSCs are isolated by their capacity to adhere to cell culture plastic surfaces" (culture-pressured selection), and 2) marker expression - (positive for CD73, CD90, CD105, and negative for CD11b or CD14, CD19 or CD79?, CD34, CD45, and HLA-DR). While CD34+ is controversial, how can we define ASCs based on BM-MSCs or HSC?

I added an additional sentence on page 4 (MSCs definition).

The CD34 expression of BM-MSC is discussed on page 11 (I know this feature of BM-MSC is not widely accepted, but there are several manuscripts describing CD34 expression of BM-MSC in early culture). On the other hand, BM-MSCs can distinguished from ASCs by their expression of CD106 (or by ASC's expression of CD36) (References see Page 6).

In contrast, HSCs can be defined by their CD34/CD45 expression and are therefore clearly confined from MSCs/ASCs.

4. Reconcile the followings, consider how we can sort them out. P.5: native ASC - CD45-/CD235a-/CD31-/CD34+ cells P.6 cultured ASCs are characterized as CD73+/CD90+/CD105+/CD44+/CD45-/CD31- cells p.6: the in vivo counterpart(s) of the ASC population(s).

Yes, this is an interesting question (how we can sort out the in vivo counterparts). Native ASCs can be sorted by their CD34 expression (but do we really sort all ASCs or only a subpopulation? I think we only sort a subpop !!). Then, the researcher needs to deplete CD31-positive cells. Nevertheless, recently this question is absolutely not resolved in a satisfying manner, especially due to several subpopulations with different phenotype.

5. What's the ASCs niche? In adipose? In perivascular? Or multiple niches? p.6: Any other biomarkers beside these ("CD34+/CD31- cells (ASCs) in a perivascular location using immunofluorescence staining" "CD34+/CD31+ capillary endothelial cells") can be used in the context of adipose? That's the main issue: If we cannot define the ASC niche, we cannot fine tune the culture method, and then we cannot get the right ASCs in culture.

That is absolutely correct. If we don't know the exact location, it is hard to fine tune the best culture conditions. Therefore, more in situ characterization studies are needed to identify the *in vivo* counterpart(s) of the ASC population(s) and their in vivo microenvironment.

6. p.6: "This is a complicated attempt because no marker has been described recently which unambiguously identifies native ASCs." It's catch-22, what's solution? Your insight?

In my opinion, there is recently no satisfiable answer and therefore no real solution. My insight is to rely on the most plausible characterization of native ASCs (CD34<sup>+</sup>/CD90<sup>+</sup>/CD31<sup>-</sup>/CD45<sup>-</sup>/CD146<sup>-</sup>) and their perivascular origin, but maybe subsets with another phenotype exist (as speculated at the end of the chapter (on page 8)).

7. p13 – Well-written section "that single-cell-derived clonal MSC populations are also highly heterogeneous and contain undifferentiated stem/progenitors and lineage-restricted precursors with varying capacities to proliferate and differentiate" – Is it culture-driven or inherited? How can we sort it out?

That's a good question. I don't know any study showing if it is culture-driven or inherited. My opinion is that it is culture-driven, but it's only my opinion.

8. P.16: "the lack of standardization in the isolation methods and culture protocols needs to be overcome in order to eliminate the significant variability in cell quality (if not solely based on donor-specific variabilities)." That's problematic in the field, what standard does the author propose?

In my opinion, the minimal standard should be the use of low-glucose DMEM (or even  $\alpha$ MEM) and a maximum of 10% FCS (maybe with an additional supplementation with  $\beta$ -FGF). Nevertheless, the minimal standards need to be discussed (and then the society (e.g. ISCT) should define these standards – as done for MSCs minimal criteria).

9. A list of abbreviations used in text is appreciated.

I added a list of frequently used abbreviations.