

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (General priority)

Specific Comments to Authors:

The authors reviewed different papers that were dealing with the generation of MSCs from iPSCs and compared different techniques. The paper may be considered as a one of the references for those who are working in the field or would like to start producing MSCs from iPSCs. However, there was not much information about limitations and advantages of each technique.

***Response:** Thank you for the recommendation. Additional text has been added to the Discussion to highlight this important point. Please see lines 425 and following of the clean version of the manuscript ("These methods presented their own advantages, depending on the application....").*

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Moreover, although the MSCs isolation from different tissues have limitations, the generation of iMSCs from iPSCs has also different complications which limit their use in clinical practice.

***Response:** Thank you. This has been addressed on lines 472 and following to explain the phenotype of the obtained cells might not fit the clinical demand ("According to Frobel et al., [71] and Mc Grawth et al., [54] iMSCs obtained with safe platelet lysate methods, however, might limit their use as immunomodulators"); on lines 491-511 to highlight different aspects, such as ethics, safety etc.*

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Minor revision

Specific Comments to Authors:

In this review, the authors summarize the current protocols used to obtain Mesenchymal Stem Cells (MSCs) from induced pluripotent stem cells (iPSCs) and describe the markers commonly used to identify them.

The paper contains useful information regarding the topic, but the English language should be revised.

Response: *English language has been revised.*

Some sentences are not well written, such as lines 102-105 ("Among the Indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), transforming growth factor beta (TGF- β), sHLA-G5, interleukin-10 (IL-10) and interleukin-6 (IL-6) are a few of these soluble factors allowing MSCs and immune cells cross-talk"),

Response: *Thank you for noticing: The words "Among the" have been removed to give sense to the sentence.*

lines 256-258 ("The only other uncommon supplement worth mentioning for EB protocols being ITS and SB431542, with 2 studies out of the 13 (15%) each"),

Response: *Thank you for noticing: The sentence has been rephrased, and the meaning of ITS detailed.*

lines 306-311 ("In addition to the thirty-two studies selected as the main representatives for the five categories to produce iMSCs by non-commercial methods, twelve additional studies, using the protocols described by these thirty-two original studies, were included in our analysis, as they described characterization of the obtained iMSCs (Table 6 and Supplementary Table S4) by the evaluated protocols")

Response: *In addition to the thirty-two studies selected as the main representatives for the five categories to produce iMSCs by non-commercial methods, twelve additional studies were included in this section. The reason for their inclusion was that they describe the characterization of iMSCs obtained by one of the five protocol categories studied in this review (Supplementary Table S1, and Supplementary Table S4).*

and others. I suggest having a native English speaker proofread the text.

Response: *This has been done. The track changes show all the newly introduced edits.*

Moreover, I have some comments for the authors: 1) in the background, I would suggest citing the articles that reported the concepts described in the

original contexts rather than review, so that the reader can identify the topic presented in the original scientific article. For example, in the sentence in line 95-96 ("but later have been found in many other tissues: adipose tissue, umbilical cord, neural crest cells, dental tissues") I would suggest inserting the references regarding the identification of MSCs in each of the tissue cited.

Response: *Thank you. This has been done. Please see added references 4-12.*

Moreover, I think that the background section should be enriched explaining the potential uses of MSCs in clinic (regenerative medicine? How these cells could practically be used to treat inflammatory disease?)

Response: *The following text has been added: "Still, their use for the treatment of inflammatory and autoimmune diseases is becoming recognized[33,34]. Readers are referred to recent systematic reviews summarizing the results of MSC clinical trials for the treatment of inflammatory disorders and for regenerative therapies[35,36]."*

2) Line 41 and line 122: it is not totally correct to affirm that iPSCs have no ethical issue. Indeed, they have their own ethical issue, that are different from those of ESCs.

Response: *Thank you, we have amended this with the corresponding edits. A reference dealing with this important point has been added [80].*

3) Lines 117-118: I suggest correcting the sentence "it was shown that during in vitro culture expansion MSC cells rapidly senescence, limiting the amounts obtained from donors", senescence is not a verb.

Response: *The word has been replaced by "senesce".*

4) Lines 143-150: I would suggest expressing the number of studies that used a particular method only as a fraction. The sum of percentages is not 100% (because some papers described more than one method) and, in my opinion, this could be confusing.

Response: *Thank you. This has been corrected to keep only fractions as suggested.*

5) Tables 1-2-3-4-5: I would add a caption for the tables. For example, what represent the "Time" column? The required days to reach the MSCs state from induction of differentiation? I can not understand why some "time" have a plus and other not, please explain. Moreover, what is the meaning of "NA" in "iPSC origin" column?

Response: *Yes, the "Time" column refers to the required minimum number of days to obtain iMSCs.*

Table foot legends have been added to explain this and other, including the meaning of NA, as follows: "iPSC ORIGIN refers to the cell type used for reprogramming; TIME is indicated as the minimum number of days required

to obtain iMSCs; NA (information not available); CITATIONS show numbers on March 2020; () in references indicate the study includes methods in more than one protocol category."*

6) Figure 1-2: The sum of percentage of some pie charts is not 100 because some media and coating have been used in more than one article. I think that the pie chart is not the best to graphically represent these data. I would suggest using bar charts.

Response: *Thank you. Bar charts and ratios are now replacing pie diagrams and percentages.*

7)Lines 398-400: I would suggest correcting the sentence "Their relationship with iMSCs properties is yet another underexplored sector of high priority to set protocols in accordance to therapeutic demands" because it is not clear.

Response: *Thank you. We have rephrased the sentence as follows:*

"Relationships between iMSCs surface markers and cell properties is yet another underexplored, but high priority issue towards the development of protocols meeting therapeutic demands."

Reviewer #3:

Scientific Quality: Grade C (Good)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Major revision

Specific Comments to Authors:

This manuscript provides a review about the different methods used to differentiate iPSCs into MSCs. this is an interesting topic to the scientific community given the therapeutic potential of MSCs. However, there are some aspects that need to be addressed by the authors before this work can be accepted.

1. Cite appropriately the latest, important and authoritative references in the introduction and discussion sections. there are hardly any citations in the introduction as well as the discussion section which is unacceptable.

***Response:** This has been addressed. Please note additional references have been added to each of the pointed sections.*

2. the English needs extensive editing. herein some examples , the paragraph (lines 327-line 330) should be rewritten.

***Response:** this has been addressed. The whole manuscript has been revised in this sense.*

no need for line 41 (you can delete it);

***Response:** We respectfully disagree with the reviewer. However, the sentence has been rephrased to contemplate that the use of iPSCs is not free of ethical concerns and reference 80 added.*

paragraph (line 100-line 103) should be rewritten.

***Response:** Thank you for noticing. The words "Among the" have been removed now to prevent sentence truncation.*

line 116: cells rapidly senesce not senescence,

***Response:** Corrected, thank you.*

line 120: subject to ethical concerns.

***Response:** Corrected, thank you.*

line 122: adult MSCs face for clinical .

***Response:** Corrected, thank you.*

Line 128-130: the paragraph is not clear, rewrite.

Response: Corrected, thank you.

line 132: describes ;

Response: Corrected, thank you.

line 149, consists of ;

Response: Corrected, thank you.

line 209: to replace FBS and therefore produce ..

Response: Corrected, thank you.

line 229: media not mediums

Response: Corrected, thank you.

3. no explanation is provided for the MSC switch strategy as well as EB formation. Provide examples from papers and elaborate.

Response: *The basis of the switch strategy is described on lines 149 and the EB on lines 157 and following. Paper examples are tabulated on Tables 1 and 2. Elaboration includes analysis of basic components, cells used to originally obtain the iPSCs in the cited studies, and the surface markers used by the different studies. The intention of this review is to orientate the reader by providing an objective list of available methods to produce iMSCs. We have not empirically compared these protocols and therefore cannot provide an opinion on them, although we agree with the reviewer that it is an important point to be addressed in the future.*

4. try to draw comparison between the different strategies by stating the limitation and strength of each.

Response: *Thank you. This has been addressed on lines 472 and following to explain the phenotype of the obtained cells might not fit the clinical demand ("According to Frobel et al., [71] and Mc Grawth et al., [54] iMSCs obtained with safe platelet lysate methods, however, might limit their use as immunomodulators"); on lines 491-511 to highlight different aspects, such as ethics, safety etc.*

Please see lines 425 and following of the clean version of the manuscript for strenghts ("These methods presented their own advantages, depending on the application....").

4. all figures , delete the word evaluates

Response: Corrected, thank you.