

Point-by-point responses to Reviewer #1

The authors would like to thank Reviewer #1 for the comments and suggestions provided.

Reviewer Comments to Author:

- 1) *Authors have to review more about the characteristics of Embryonic Stem Cells and versus pluripotency versus phenotype, since I suggest that these concepts are not accurate addressed in the review and it can make confusion to the readers.*

Answer: We thank you for your relevant comments and we agreed that in some parts of the text these concepts were not clear enough. We made changes in the text in all relevant parts as you may see below, in the comment #4.

- 2) *In the following paragraph, is not true that MSC have pluripotency: “The periodontal ligament stem cells (PDLSCs), also known as periodontal ligament mesenchymal cells, are a unique cell population that are easily obtained and exhibit important characteristics of MSCs, such as self-renewal, pluripotency, and immunomodulation [13]” The MSC have multi plasticity cell differentiation potential, bur they are not able to induce cell differentiation to all cells of the body (ecto, meso and endoderm layer).*

Answer: We agree with the reviewer and the use of the word “pluripotency” was a mistake in this case. Our intention was to say “multipotency”. The sentence was reformulated in the revised manuscript according to the reviewer’s suggestion, as follows:

‘The periodontal ligament stem cells (PDLSCs), also known as periodontal ligament mesenchymal cells, are a unique cell population that are easily obtained and exhibit important characteristics of MSCs, such as self-renewal, multipotency, and immunomodulation [13].’

- 3) *I suggest to add the reference that support the following paragraph: “For all these reasons, PDLSCs have been extensively studied over the years and showed potential to regenerate not only the periodontal complex but also other dental and non-dental tissues.”*

Answer: The following references were added in this paragraph in the revised manuscript according to the reviewer’s suggestion:

- Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004;364:149–55.
- Trubiani O., Pizzicannella J., Caputi S., et al. Periodontal ligament stem cells: current knowledge and future perspectives. Stem Cells and Development. 2019;28(15):995–1003.

- 4) *There are data that shown the expression of OCT-4/SOX2 and stemness in no pluripotent cells. So, I suggest to be careful to describe this information through the manuscript: -- “In a recent study, we reported that about 10% of PDLSCs could present double positivity for SOX2 and OCT-4”. --- “Populations of*

PDLCS with an embryonic stem cell phenotype also expressed genes related to cardiomyogenesis after treatment with low concentrations.” --- “this basis, we recently showed that inflammatory conditions in periodontal tissues also alter the pluripotency state of PDLSCs,” --- “Additionally, the in vitro expansion of PDLSCs cultures induce morphological changes as well, by bursting their myofibroblastic phenotype, which up-regulate their contractile activity meanwhile reduce their pluripotency state [52]”.

Answer: It is correct to say that these markers have been previously detected in cells other than pluripotent ones. We accepted your suggestion and changed the text in order to make it clearer. Below, we address each of the changes:

-- *“In a recent study, we reported that about 10% of PDLSCs could present double positivity for SOX2 and OCT-4”.*

The sentence was reformulated in the revised manuscript according to the reviewer’s suggestion, as follows:

‘In a recent study, we reported that about 10% of PDLSCs could present double positivity for SOX2 and OCT-4 [38], two transcription factors important for the pluripotency of embryonic stem cells, but which are also detected in somatic stem cells, such as those derived from the periodontal ligament (Peng et al., 2021).’

--- *“Populations of PDLCS with an embryonic stem cell phenotype also expressed genes related to cardiomyogenesis after treatment with low concentrations.”*

The sentence was reformulated in the revised manuscript according to the reviewer’s suggestion, as follows:

‘Populations of PDLCS positive for well-known markers of embryonic stem cells and neural crest markers also expressed genes related to cardiomyogenesis after treatment with low concentrations of hydrogen peroxide [33].’

--- *“this basis, we recently showed that inflammatory conditions in periodontal tissues also alter the pluripotency state of PDLSCs,”*

The sentence was reformulated in the revised manuscript according to the reviewer’s suggestion, as follows:

‘On this basis, we recently showed that inflammatory conditions in periodontal tissues also alter the expression of typical pluripotent embryonic stem cell markers in PDLSCs.’

--- *“Additionally, the in vitro expansion of PDLSCs cultures induce morphological changes as well, by bursting their myofibroblastic phenotype, which up-regulate their contractile activity meanwhile reduce their pluripotency state [52]”*

The sentence was reformulated in the revised manuscript according to the reviewer's suggestion, as follows:

'Additionally, the in vitro expansion of PDLSCs cultures induce morphological changes as well, by bursting their myofibroblastic phenotype, which up-regulate their contractile activity meanwhile reduce the expression of NANOG, SOX2 and OCT-4, factors associated with pluripotency of embryonic stem cells, but that are also expressed in MSCs.'

Point-by-point responses to Science Editor

The authors would like to thank Science Editor for the comments and suggestions provided.

Reviewer Comments to Author:

- 1) *Summary of the Peer-Review Report: The paper is a fluently written manuscript which provides enough information regarding of potential of periodontal ligament stem cells into regenerative medicine. However, the authors should describe the right and accurate characteristic of the stem cells, pluripotency, embryonic stem cells, mesenchymal stem cells and their PDLSCs. The questions raised by the reviewer should be answered;*

Answer: We thank you for your comments and we agreed that in some parts of the text these concepts were not clear enough. We made changes in the text according to all the questions raised by the reviewer #1.

- 2) *The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);*

Answer: The grant from FAPESP funding agency is not valid anymore. Therefore, we would like to delete the previous supportive foundation information.

- 3) *The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;*

Answer: A copy of the original figure in power point format has being uploaded in this reviewed version.

- 4) *Please obtain permission for the use of picture(s). If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published; and correctly*

indicating the reference source and copyrights. For example, “Figure 1 Histopathological examination by hematoxylin-eosin staining (200 ×). Citation: Yang JM, Sun Y, Wang M, Zhang XL, Zhang SJ, Gao YS, Chen L, Wu MY, Zhou L, Zhou YM, Wang Y, Zheng FJ, Li YH. Regulatory effect of a Chinese herbal medicine formula on non-alcoholic fatty liver disease. World J Gastroenterol 2019; 25(34): 5105-5119. Copyright ©The Author(s) 2019. Published by Baishideng Publishing Group Inc[6]”. And please cite the reference source in the references list. If the author fails to properly cite the published or copyrighted picture(s) or table(s) as described above, he/she will be subject to withdrawal of the article from BPG publications and may even be held liable.

Answer: The authors attest that Figure 1 is original and was designed solely for this article with the purpose of synthesize the knowledge pertinent to the topic. It was drafted using the Inkscape and Microsoft power point software. So, no copyright nor refence source is required.