**Response to reviewers**

**Reviewer #1:**

The title of this review is very attractive, but the text is relatively simple and needs to be supplemented.

1. In the INTRODUCTION section, there is almost no introduction to hPSC and hPSC-EVs. In addition, paragraphs 2 and 3 may be deleted.
2. In the second part, "HUMAN PLURIPOTENT STEM CELLS-DERIVED EXCELLELLAR VESICLES", it is suggested to add three subtitles, focusing on the separation and identification of EVs, the applications and mechanisms of disease treatment, and adjust and supplement the relevant contents.

***Response (items 1 and 2):*** *We understand the reviewer's concern and greatly appreciate the suggestions. In fact, in this mini-review, our objective was to bring an overview of works that use human PSC-EV. In the Introduction, the idea was mainly to present general concepts of EVs, the advances in the use of stem cell-derived EV, what are the concerns in the use of MSC-EV and that the hPSC-EV can be an alternative. Considering the proposed suggestions, we deleted paragraphs 2 and 3 of the Introduction, divided the second section of the manuscript ("HUMAN PLURIPOTENT STEM CELLS-DERIVED EXCELLELLAR VESICLES") into subtopics, where we included some references about the PSC. In addition, we include and explore some more works throughout the text, to enrich the review.*

**Reviewer #2:**

Bruno et al. highlight the incipient status of the studies in the significance of hPSC-EVs prospective applications as PSC-derived cell-free therapy products. It is interesting, but there are some comments for the authors.

1. "There are several advantages to using EVs instead of stem cells", I suggest authors provide some practical examples. More details should be performed to investigate the important differences. Discussion should be further improved.

**Response:** *We appreciate the reviewer's suggestion. The main focus of this mini-review is PSC-EVs, so the advantages of EV-based therapies are briefly mentioned in the introduction. In order not to dwell on the subject and considering the existence of very nice works in the area with this purpose, we indicated in the manuscript references for more details on the subject.*

**2.** "Despite the high potential of MSC-derived EVs (MSC-EVs), several factors limit their use", what limits the applications of MSC-EVs? What is the purpose of the review.

**Response:** *Thank you for questioning. The primary purpose of the review is to enlighten the current status of human PSC-EVs research. We understand the reviewer's concerns and the various biases through which this issue may be addressed, but our goal is highlight methodologies used for the culture of hPSCs for isolating EVs, their characteristics, and potential applications. So, to quickly address this issue and make it clear to readers, we added in the manuscript some lines shedding light on the factors that limit the use of MSC-EVs.*

**3.** "Figure 1. Overview of studies on hPSC-EV published between 2015 and 2022." I suggest listed published between 2012 and 2022.

***Response:*** *Thank you for your suggestion. We changed the figure 1A to represent the years between 2012-2022 (from 2012-1014 we couldn't find any studies with human PSC-EV). In addition, we have included some other articles that were published in 2022, but that were not available at the time we submitted the first version of the manuscript. So, the table 1 was also modified.*

**4.** MSC-EVs should be presented more contents.

***Response:*** *We appreciate the reviewer's suggestion. The main focus of our mini-review is human PSC-EVs, indicating why they would have equal or better potential than MSC-EVs. However, considering the importance of the subject, we cite some current references that discuss MSC-EVs specifically to direct the reader.*

**5.** The differences of hESC-, hiPSC-, and hMSC-EXOs should be presented.

***Response:*** *Thanks for your suggestion. In fact, this is a very relevant issue, but still little explored in the literature. Our review focused on works that used EVs isolated from human PSCs, and we noticed very few works that compared them with EVs from MSCs. Those who did it, were included in the manuscript in more details. As an exemple, the work from Bi and coworkers (2022) evidenced that in hPSC, exosomal protein content was related development, metabolism, and anti-aging properties, whereas in hMSC, it was related to immune regulation. Despite that, considering the actual state of the art, few differences are generally observed between MSC-EVs and PSC-EVs - mainly slight differences in the molecular composition and similar in vitro effects. However, it should be noted that there are many more studies with MSC-EV than with PSC-EV. So further studies are needed to verify the potential of PSC-EV and clearly demonstrate the differences between hPSC-, and hMSC-EXOs.*

**6.** "EVs generated from KO cells" should have the CRISPR/Cas9 description.

***Response:*** *Thank for your comment. We included the information that for the generation of overexpressing or knockout cells were used lentiviral transduction and CRISPR-Cas9 system respectively.*

Dear Journal Chief Editor,

Thank you very much for your considerations. We evaluated and corrected the manuscript according to the suggestions. Below are the responses and indications of changes made to the manuscript:

**1) Pages 5-6: "It was shown that the hiPSC-derived EVs (hiPSC-EV) contains a variety of microRNAs (miRNAs) (such as miR-382, miR-611, and others) related to pathways such as focal adhesion, Wnt, PI3K-Akt, and MAPK signaling, as well as proteins related to processes involved in signal transduction." [use contain, not contains].**
*Response: Thank you. We corrected the sentence.*

**2) Page 8: "It was also demonstrated that MVs, but not EXOs, retrodifferentiated Müller cells into retinal progenitor cells in vitro[71]." It is infrequent to see "retrodifferentiated Müller cells." Thus, the authors need to elaborate on the definition of the term.**
*Response: Thank you for your suggestion. We changed the sentence to make it clearer and exclude the use of "retrodifferentiation" term to avoid misinterpretations.*

3) Page 12: "The advantages of PSC-EVs could be related to a higher level of EV production since the cells have the greater proliferative capacity, along with the fact that we can isolate EVs from a single source, possibly reducing the variability between batches." Neither PSCs nor PSC-EVs are homogeneous in production but heterogenous, implying neither contents are consistent. Revise the statement to reflect on the heterogeneity of both PSCs and PSC-EVs.
*Response: Thank you for your comment. The production of EVs may vary according to cell culture conditions and hPSC lineage. However, if we maintain the same culture conditions and the same cells, we believe that EVs with very similar efficiency and content would be produced. Among the greatest advantages of hPSC-EV over other stem cell EVs, mainly MSC-EV, is the fact that the cell culture is homogeneous (we have a pure population of PSC with the same characteristics), the cells proliferate more,*

*the same PSC can be used for a long period of time (with all controls) and, despite some variations in hPSC lineages, their characteristics are the same: pluripotent cells with a high rate of proliferation. So, considering these possibilities and the question raised by the Journal Chief Editor, we changed the sentence to include some more discussion about hPSC lineages and the possibility of different EV composition/ potential:*
*"hPSC can be obtained from different sources (embryonic or reprogrammed from adult cells) and, despite showing some heterogeneity between lineages, they are highly similar in their main characteristics: they are pluripotent and with a high proliferative capacity. The latter makes it possible to obtain a large number of EVs. It should be noted that PSC-EV derived from different hPSC lineages may show some variability in their content. But considering the fact that we can isolate EVs from a single source (a homogenous culture), this can possibly bring less variability between batches compared to other common EV sources."*