We thank the reviewers and editors for their comments, which have allowed us to generate a new improved version of the manuscript, as indicated below.

REVIEWER 1:

In the present study, Pulido-Escribano et al. summarized role of hypoxia preconditioning in therapeutic potential of mesenchymal stem cell-derived extracellular vesicles. Although it is interesting, there are some comments for the authors.

1. **Language needs to be improved. For example, "Among the conditions used to induce a better therapeutic response of MSC is the culture of cells in low O2 concentrations (hypoxia)" is wrong expression.**

   Answer (A). Sentence removed and information integrated in previous one: "Therefore, preconditioning MSC under conditions that increase their regenerative power, like hypoxia, may induce production of EV with enhanced regenerative potential". On the other hand, the new version of the manuscript has been proofread by a native English speaker, expert in the topic.

2. **In the page 5, MSC have been isolated from different tissues, such as fatty one, placenta, umbilical cord, synovium, periodontal ligament and bone marrow. What is fatty one? Additionally, the sources of menstruation (PMID, 31864423) and Wharton's jelly (PMID, 32557631) can be added.**

   A. Corrected as adipose tissue. Also, following your suggestions, we have included menstrual blood and umbilical cord Wharton's jelly as sources of MSC isolation.

3. **In the page 6, Exosomes are vesicles generating after fusion of multivesicular bodies with plasma membranes, ranging between 40 to 100 nm, I suggest authors provide some references.**

   A. Following your suggestions we have added two references (doi: 10.1126/science.aau6977; and doi:10.3389/fbioe.2020.00146).

4. **The part "CELL THERAPY VS. CELL-FREE THERAPY" is not clear.**

   A. The objective of such section was to describe the concept of extracellular vesicles, their isolation and potential use in regenerative medicine, as a alternative to cell therapy. However, we agree with the reviewer that the title can be confusing. Therefore, we have changed its title in the new revision to read "MSC-DERIVED EXTRACELLULAR VESICLES AS A NOVEL APPROACH TO CELL-FREE THERAPIES".

5. **In the page 8, When oxygen concentrations decrease to less than 5% in tissues, cells have to adapt their metabolism and functions to such hypoxic conditions. Less than 5% is the hypoxic condition, is there any criterion. 4.9% or 0.1% are same one for the hypoxic condition? Please make some detail explanation.**

   A. Under physiological conditions, depending on the type of tissue, O_2 concentration may vary. Between 5 and 8% O_2 can be considered close to physiological conditions. However, below 5% there are different levels of hypoxia, generally defined as moderate (<5 - >2% O2), severe (≤2 - ≥0,1% O2) and anoxia (<0.1% O2) . Different levels of hypoxia can range from substantial adaptation to cell death, so there are a wide variety of preconditioning protocols that have been used, depending on cell type. In fact, there are discrepancies depending on culture conditions and percentage and duration of hypoxia, to achieve maximum paracrine effects. We have included this in the new revision.

6. **Figure 2 should be improved. The basic description for the elements is lacked.**

   A. The Figure legend has been improved, to read "Clinical potential of extracellular vesicles from preconditioned mesenchymal stem-cells under hypoxia. MSC exposed to hypoxia secrete EV that can be isolated and used for clinical purposes, such as treatment of wound healing and bone fractures, as well as cardiovascular, neurodegenerative and renal diseases, among others. Isolation of EV is made from MSC culture medium, which can be carried out in different ways. In this case, the use of a size-exclusion column is shown. EV secreted under hypoxia were enriched in various proteins, nucleic acids like miRNA, as well as grow factors that are implicated in modulation and improvement of different biological processes, related to tissue regeneration in different pathologies". On the other hand, we have observed a loss of information in the figure when it was added to the manuscript, which has also been corrected.

7. **Table 3. EV used in medicine therapies derived from hypoxic MSC. I suggest authors added more references.**

A. Six more references have been added, five of which have been published in the last three months.

8. **The challenge of MSC-EV in regenerative medicine should be added.**

A. The aim of the review was to address one aspect of the use of MSC-EV in regenerative medicine. Specifically, the preconditioning of MSC in hypoxia, to produce EV with enhanced regenerative capacity. Therefore, we have not focused on challenges of MSC-EV in regenerative medicine, as we believe that this would be part of a more general review on this topic. However, although some of the challenges to be met for the clinical use of MSC-EV in regenerative

medicine were already included in the conclusions, we have expanded them and added a new reference on this topic, following the reviewer's suggestions.

REVIEWER 2:

The authors titled the paragraph 3 as: ''CELL THERAPY VS. CELL-FREE THERAPY''. However, in this section mainly a description of EV was made. Based on the title, the authors should described the therapeutic applications of both cells and their products, list the strengths and weaknesses of each therapy and, finally, compare them. Otherwise the authors should change the title.

Answer (A). As indicated above for reviewer 1, the objective of such section was to describe the concept of extracellular vesicles, their isolation and potential use in regenerative medicine, as a alternative to cell therapy. However, we agree with the reviewer that the title can be confusing. Therefore, we have changed its title in the new revision to read "MSC-DERIVED EXTRACELLULAR VESICLES AS A NOVEL APPROACH TO CELL-FREE THERAPIES".

SCIENCE EDITOR:

The role of hypoxia preconditioning in the therapeutic potential of mesenchymal stem cell-derived extracellular vesicles. The implementation of cell-free therapy for MSC derived EV is an interesting field. The manuscript is well, concisely and coherently organized and presented and the style. Relevant clinical trials? Or basic experiments can be summarized in a table. It is unacceptable to have more than 3 references from the same journal. To resolve this issue and move forward in the peer-review/publication process, please revise your reference list accordingly.
Language Quality: Grade C (A great deal of language polishing)
Scientific Quality: Grade B (Very good)

Answer (A). Table 3 summarizes the main studies related to the subject. As for relevant clinical trials, we have only identified two, so we believe it is more convenient to cite them in the text. On the other hand, references have been checked and the most convenient ones have been selected in case there were more than three from the same journal. Finally, the new version of the manuscript has been proofread by a native English speaker, expert in the topic.

COMPANY EDITOR-IN-CHIEF:

Answer (A). Tables have been edited and Figures have been submitted in PowerPoint format, with the corresponding copyright credit. All figures are self-made by ourselves.