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PEER-REVIEW REPORT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 74656

Title: Mesenchymal stem cell-derived exosomes: A novel and potential remedy for cutaneous wound healing and regeneration

Provenance and peer review: Invited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03712811
Position: Editor-in-Chief
Academic degree: MD, PhD
Professional title: Director, Full Professor
Reviewer's Country/Territory: Italy
Author's Country/Territory: China

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Reviewer performed review: 2022-01-01 21:49

Review time: 10 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [Y] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [ ] Major revision [Y] Rejection
Re-review	[ ] Yes [Y] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The issue the Authors decided to focus on in their review article is no doubt of high relevance for the potential future development of novel strategies for wound healing in clinical practice. Unfortunately, the way the Authors are presenting the overall field of the use of mesenchymal stem cell (MSC)-derived exosomes in wound healing is designed to give the Readers the impression that this is a pursuable strategy in clinical terms. Exosomes may indeed represent a ready-to-use alternative in wound healing, like in other regenerative medicine fields, but this will necessarily be unfolded within the context of an allogeneic setting, based upon the availability of pre-existing exosome preparationds amenable for immediate use. This is so far just a hope, hampered by many challenges that the Authors are not discussing at all. In particular: - Exosome content and type can be influenced by cell culture media. In addition, some cell culture media, which contains fetal bovine serum (FBS) may contain exosomes from the source, further complicating exosome analysis. - The implications of the different exosomes, even within a single cell type, create endless scientific questions. These questions are currently giving raise to additional studies in the fast-growing exosome scientific community and industry. In this regard, there are multiple challenging questions eluded in this review: - Why should we care about exosome subtypes? Although exosomes hold considerable promise, they provide much challenge, including devising consistent, reproducible methods within and among laboratories. The lack of standardizations is so far dramatically affecting not only the exosome content in signaling peptides, miRNA, long-chain RNA, DNA and lipids, but even the dynamics exosomes themselves perform the release of their cargos to the neighboring or distant cells, and modulate the recipient

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cells. - Why should we care about exosome purification and why do we need pure exosomes? The MSC secretome represents all of the materials a group of cells (or organism) secretes into the extracellular space, including all of the proteins, cytokines, growth factors, extracellular matrix proteins and regulators, shed receptors, EVs including exosomes, microvesicles and apoptotic bodies, peptides, cell free DNA or other portions of nucleic acids, viral particles, and cell waste products. When considering MSC exosomes only, these nanovescicles will necessarily "represent" the overall exosomal cargo from a given MSC source. The Authors should have been discussing the fact that while MSCs may be viewed as a "molecular biology lab" adapting their secretome, including the exosome composition, to the local needs within the hosting tissue after transplant, exosomes can only provide the recipient tissue with the repertoire they had embedded at the moment of isolation, with much poorer crosstalk with the hosting environment, as compared to intact MSCs. However, for some applications a mixture of all of the components in the exosomes is unacceptable, a problem which is more evident when considering regulatory requirements for a therapeutic application of exosomes themeselves. This issue has been totally ignored in this review, while it is of fundamental relevance. FDA, EMA and other regulatory agencies throughout the world require purity, potency, safety, and efficacy to grant approval. A pure product without contaminants such as peptides, proteins, cell free DNA and other cell debris is critically important. It is also important for exosome therapeutics, specifically. Although exosomes can be dosed, based on protein or nucleic acid content, the current state is to dose based on the number of exosomes (e.g., 1 X 1010). If foreign nanoparticles are present as contaminants, the dose could potentially contain a mixture of exosomes in addition to other nanoparticles which are not exosomes. In addition, unknown dilutions of the active ingredient create additional complexity. -

Challenges, Problems and Methods of Purification. The exosome field is

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experiencing exponential growth due to increased interest and research into exosome roles in disease pathology and potential treatment. Nonetheless, inconsistency in methodology for the collection, isolation, and analysis of exosomes has created a significant barrier to rapid advancement in the field. In fact, to address these issues, the International Society for Extracellular Vesicles (ISEV) has published a position statement offering guidelines to researchers in order to prevent variations across the studies of exosomes and EVs (Théry C, Witwer KW, Aikawa E, Alcaraz MJ, et al. . Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles. 2018 Nov 23;7(1):1535750. doi: 10.1080/20013078.2018.1535750). While the Authors don't give the proper emphasis to these major challenges, They also refer to MSC application in wound healing commenting that "A non-negligible restriction is biosafety caused by the occurrence of teratoma and immunogenicity, of which the incidence increases with the culture expansion or cryopreservation of cells" (page 6). The Authors ignore that more than 10 different methods and devices are currently available for harvesting and processing human fat tissue in 5-10 minutes (for a review see: Veronese S, Dai Prè E, Conti G, Busato A, Mannucci S, Sbarbati A. Comparative technical analysis of lipoaspirate mechanical processing devices. J Tissue Eng Regen Med. 2020 Sep;14(9):1213-1226. doi: 10.1002/term.3093). These methods/devices yield a tissue product, embedding MSCs within the context of an intact stromal-vascular niche, ready to use in an autologous fashion, an issue which has been shown to dramatically improve the outcome of difficult wound healing, as compared with expanded MSCs obtained after extensive (i.e. fat) tissue processing, and subsequent ex vivo expansion prior to transplantation. In this regard, the Authors continue to compare the potential easy way of exosome administration with the hurdles of a systemic administration of ex vivo expanded MSCs,

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while such a strategy has been by far avoided by the aid of the above-mentioned tissue products, especially within the context of cutaneous wound healing. On the whole, the Authors should clearly report that actually the clinical use of exosomes is also hampered by many safety concerns, as I have summarized above, and also by consistent regulatory issues. The Authors should clearly avoid serious misunderstandings on the feasibility of the exosome clinical usage, and report that: so far "The FDA has not approved any exosome products for any uses" (cited from: Stem Cell and Exosome Products, Warning about unapproved therapies: https://www.cdc.gov/hai/outbreaks/stem-cell-products.html It should be highlighted that all the analyzed studies in this review have been performed in vitro or in vivo animal models. To this end, conclusive statements like: "Notwithstanding strong evidence in the preceding sections that exosomes derived from various MSCs have therapeutic effects on cutaneous wound healing" (page 17), are particularly misleading.

To this end, the Authors refer to challenges in exosome use mainly regarding the combination of exosomes with biomaterials, or hydrogels to further improve their actions, thus giving the impression that exosomes are closed to become consolidated strategies, while the challenges are well ahead from such further refinement.

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Manuscript NO: 74656

Title: Mesenchymal stem cell-derived exosomes: A novel and potential remedy for cutaneous wound healing and regeneration

Provenance and peer review: Invited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05935626

Position: Peer Reviewer

Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: Indonesia

Author's Country/Territory: China

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Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

I would like to congratulate the authors for this manuscript. It is interesting and can bring new perspective. I have some comments: Please check for grammatical errors and mistyped words. In the section of Therapeutic mechanisms of MSC-exosomes in cutaneous wound healing and regeneration, please rephrase 'in conclusion....' because the conclusion is separate and supposed to be at the end of your manuscript. Regarding the references: please follow the guidelines for reference listing (the names of authors from reference no 2-9, 13, 14, 22, 25-28, 30, 32, 34, 36, 38-51, 53-60). Please check the figure legend note of figure 1: …'proliferation phage' or proliferation phase?

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Title: Mesenchymal stem cell-derived exosomes: A novel and potential remedy for cutaneous wound healing and regeneration

Provenance and peer review: Invited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03812042
Position: Editorial Board
Academic degree: MSc, PhD
Professional title: Assistant Professor, Professor, Research Associate
Reviewer's Country/Territory: Italy
Author's Country/Territory: China

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Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

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Peer-reviewer statements	Peer-Review: [ ] Anonymous [Y] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

In this mini-Review the authors recapitulate the applications of MSC-derived exosomes in cutaneous regeneration and explain the underlying cellular and molecular mechanisms. Finally, they clarify the future prospective for their application in clinic and latent problems to be solved. The paper is well written, is quite comprehensive and is very interesting in focusing on cutaneous healing. Improvement and completeness elements may be: 1) What are the safety procedures to isolate exosomes from MSC to use in cutaneous regeneration? 2) The authors at the end of the review described some possible methods to applicate exosomes to severe wound models under diabetic conditions. What are further possible exosome administration methods for other less severe wound models?

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Provenance and peer review: Invited Manuscript; Externally peer reviewed

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Reviewer's code: 03017535
Position: Peer Reviewer
Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: South Korea

Author's Country/Territory: China

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Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The manuscript by Hu et al reviews results of application of exosomes derived from MSCs of various source to cutaneous wound healing. The manuscript is well-timed and written well enough to provide useful information on the subject of rising interest. However, there are a few issues that should be taken care of by the authors seriously. 1. The majority of articles referred in the manuscript are published before 2019. I wonder if there are any articles published since 2020 in the reference. This area is relatively hot and attract interests of many researchers and industry recently. Therefore, there must be lots of recent developments in 2020s, which should be included in this review. 2. The effect of exosomal heterogeneity originated from different sources and conditions on cutaneous wound healing is also required to be discussed, probably in 'Perspectives' section. 3. A few references did not list authors correctly. 4. A table listing exosome applications on cutaneous wound treatment should be helpful.

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Title: Mesenchymal stem cell-derived exosomes: A novel and potential remedy for cutaneous wound healing and regeneration

Provenance and peer review: Invited Manuscript; Externally peer reviewed

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Academic degree: MS, PhD

Professional title: Academic Research, Assistant Professor, Research Associate

Reviewer's Country/Territory: India

Author's Country/Territory: China

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Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

1. Add new classification of extracellular vesicles 2. Depiction of how EVs/Exos help in wound healing - Image to be drawn 3. Remove basic biology of EVs 4. Add more clinical and preclinical evidences of EVs in cutaneous wound healing 5. Tabulate the results in the studies cited inside the manuscript