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Manuscript Title: Mesenchymal stem cell-derived exosomes: a novel and potential remedy for cutaneous wound healing and regeneration
(**Original title:** Mesenchymal stem cell-derived exosomes: a novel and promising remedy for cutaneous wound healing and regeneration)

Dear Editors and Reviewers:
Thanks very much for your attention and evaluation regarding our manuscript. According to your kindly comments and suggestions, we have amended the related sections, which were **highlighted in red font** in our revised manuscript. Please find the point-by-point responses below.

RESPONSE to SCIENCE EDITOR COMMENTS to AUTHORS:
The manuscript by Hu et al reviews the application of MSC-derived exosomes from several sources for cutaneous wound healing. This review article is of high relevance for the potential future development of novel strategies for wound healing. The paper is well written, well-timed and is very interesting in focusing on cutaneous healing. However, there are some issues that authors need to address seriously.

1. *The references should follow the guidelines for reference listing and must be updated from 2019 to 2022. Also, some references did not list the authors correctly.*

Response: Thank you for the significant comments. We have amended the references following the guidelines for reference listing and updated them to 2022. Also, we have adjusted references to list the authors correctly. We added 18 references from 2020 to 2022 as fellows:

22 **Krampera M,** Le Blanc K. Mesenchymal stromal cells: Putative microenvironmental modulators become cell therapy. Cell Stem Cell 2021; **28**: 1708-1725 [PMID: 34624232 DOI: 10.1016/j.stem.2021.09.006]

30 **Liang Y,** Duan L, Lu J, Xia J. Engineering exosomes for targeted drug delivery. Theranostics 2021; **11**: 3183-3195 [PMID: 33537081 DOI: 10.7150/thno.52570]

31 **Tran PHL,** Xiang D, Tran TTD, Yin W, Zhang Y, Kong L, Chen K, Sun M, Li Y, Hou Y, Zhu Y, Duan W. Exosomes and nanoengineering: A match made for precision therapeutics. Adv Mater 2020; **32**: e1904040 [PMID: 31531916 DOI: 10.1002/adma.201904040]

32 **Aheget H,** Tristan-Manzano M, Mazini L, Cortijo-Gutierrez M, Galindo-Moreno P, Herrera C, Martin F, Marchal JA, Benabdellah K. Exosome: A new player in translational nanomedicine. J Clin Med 2020; **9**: [PMID: 32722531 DOI: 10.3390/jcm9082380]

33 **Chen J,** Li P, Zhang T, Xu Z, Huang X, Wang R, Du L. Review on strategies and technologies for exosome isolation and purification. Front Bioeng Biotechnol 2021; **9**: 811971 [PMID: 35071216 DOI: 10.3389/fbioe.2021.811971]

34 **Yang D,** Zhang W, Zhang H, Zhang F, Chen L, Ma L, Larcher LM, Chen S, Liu N, Zhao Q, Tran PHL, Chen C, Veedu RN, Wang T. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. Theranostics 2020; **10**: 3684-3707 [PMID: 32206116 DOI: 10.7150/thno.41580]

44 **Su N,** Hao Y, Wang F, Hou W, Chen H, Luo Y. Mesenchymal stromal

exosome-functionalized scaffolds induce innate and adaptive immunomodulatory responses toward tissue repair. Sci Adv 2021; **7**: [PMID: 33980490 DOI: 10.1126/sciadv.abf7207]
48 **Shen C,** Tao C, Zhang A, Li X, Guo Y, Wei H, Yin Q, Li Q, Jin P. Exosomal microrna rectangle93 rectangle3p secreted by bone marrow mesenchymal stem cells downregulates apoptotic peptidase activating factor 1 to promote wound healing. Bioengineered 2022; **13**: 27-37 [PMID: 34898374 DOI: 10.1080/21655979.2021.1997077]
56 **Zhang Y,** Pan Y, Liu Y, Li X, Tang L, Duan M, Li J, Zhang G. Exosomes derived from human umbilical cord blood mesenchymal stem cells stimulate regenerative wound healing via transforming growth factor-beta receptor inhibition. Stem Cell Res Ther 2021; **12**: 434 [PMID: 34344478 DOI: 10.1186/s13287-021-02517-0]
58 **Zhang Y,** Yan J, Liu Y, Chen Z, Li X, Tang L, Li J, Duan M, Zhang G. Human amniotic fluid stem cell-derived exosomes as a novel cell-free therapy for cutaneous regeneration. Front Cell Dev Biol 2021; **9**: 685873 [PMID: 34235150 DOI: 10.3389/fcell.2021.685873]
66 **Han ZF,** Cao JH, Liu ZY, Yang Z, Qi RX, Xu HL. Exosomal lncrna klf3-as1 derived from bone marrow mesenchymal stem cells stimulates angiogenesis to promote diabetic cutaneous wound healing. Diabetes Res Clin Pract 2022; **183**: 109126 [PMID: 34742784 DOI: 10.1016/j.diabres.2021.109126]
67 **Shi A,** Li J, Qiu X, Sabbah M, Boroumand S, Huang TC, Zhao C, Terzic A, Behfar A, Moran SL. Tgf-beta loaded exosome enhances ischemic wound healing in vitro and in vivo. Theranostics 2021; **11**: 6616-6631 [PMID: 33995680 DOI: 10.7150/thno.57701]
69 **Hu Y,** Tao R, Chen L, Xiong Y, Xue H, Hu L, Yan C, Xie X, Lin Z, Panayi AC, Mi B, Liu G. Exosomes derived from pioglitazone-pretreated mscs accelerate diabetic wound healing through enhancing angiogenesis. J Nanobiotechnology 2021; **19**: 150 [PMID: 34020670 DOI: 10.1186/s12951-021-00894-5]
70 **Wang J,** Wu H, Peng Y, Zhao Y, Qin Y, Zhang Y, Xiao Z. Hypoxia adipose stem cell-derived exosomes promote high-quality healing of diabetic wound involves activation of pi3k/akt pathways. J Nanobiotechnology 2021; **19**: 202 [PMID: 34233694 DOI: 10.1186/s12951-021-00942-0]
71 **Bailey AJM,** Li H, Kirkham AM, Tieu A, Maganti HB, Shorr R, Fergusson DA, Lalu MM, Elomazzen H, Allan DS. Msc-derived extracellular vesicles to heal diabetic wounds: A systematic review and meta-analysis of preclinical animal studies. Stem Cell Rev Rep 2022; **18**: 968-979 [PMID: 33893619 DOI: 10.1007/s12015-021-10164-4]
72 **Tieu A,** Hu K, Gnyra C, Montroy J, Fergusson DA, Allan DS, Stewart DJ, Thebaud B, Lalu MM. Mesenchymal stromal cell extracellular vesicles as therapy for acute and chronic respiratory diseases: A meta-analysis. J Extracell Vesicles 2021; **10**: e12141 [PMID: 34596349 DOI: 10.1002/jev2.12141]
73 **Liu C,** Wang J, Hu J, Fu B, Mao Z, Zhang H, Cai G, Chen X, Sun X. Extracellular vesicles for acute kidney injury in preclinical rodent models: A meta-analysis. Stem Cell Res Ther 2020; **11**: 11 [PMID: 31900218 DOI: 10.1186/s13287-019-1530-4]
74 **Kwon HH,** Yang SH, Lee J, Park BC, Park KY, Jung JY, Bae Y, Park GH. Combination treatment with human adipose tissue stem cell-derived exosomes and fractional co2 laser for acne scars: A 12-week prospective, double-blind, randomized, split-face study. Acta Derm Venereol 2020; **100**: adv00310 [PMID: 33073298 DOI: 10.2340/00015555-3666]

2. *More preclinical and clinical and evidences of exosomes in cutaneous wound healing must be added.*

Response: Thank you for the significant comments. We have added pre-clinical evidences of MSC-exosomes in cutaneous wound healing in the 2nd and 3rd paragraph of the "MECHANISMS OF MSC-EXOSOMES IN CUTANEOUS WOUND HEALING AND REGENERATION" section, as highlighted in red text:

And recently, studies have shown that local application of exosomes can regulate the innate and adaptive immune networks as a whole, and better promote wound healing[44]. These indicate that MSC-exosomes can exert multiple effects in inflammation phase of wound healing. (P2)

BMSC-derived exosomes could also repress apoptosis of HaCaT cells (human immortalized epidermal cells) induced by hydrogen peroxide via the miR-93-3p/APAF1 axis[48]. Also, research demonstrated that ADMSC-derived exosomes could prompt proliferation and migration of HaCaT cells via Wnt/β-catenin signaling[49]. These indicate that MSC-exosomes can accelerate the process of re-epithelization in proliferation phase. (P3)

And we have also added pre-clinical evidences of exosomes in diabetic and ischemic wound healing in the "EFFECTS OF MSC-EXOSOMES ON CUTANEOUS REGENERATION IN AGING AND DISEASE" section, as highlighted in red text:

And recently, Han et al reported that BMSC-derived exosomes contained lncRNA KLF3-AS1, which could induce angiogenesis to promote wound healing in diabetic condition (66). (Diabetic wound healing)

Chronic ischemic wounds are another challenging problem in trauma clinic with delayed wound healing and therapeutic difficulties. Due to ischemia and hypoxia, the healing process of ischemic wounds is inhibited, resulting in imperfect curative effect of conventional treatments. Thus, exosome-based therapies, with multiple therapeutic benefits, have been tentatively applied in this disease area. In the study by Shi et al (67), exosomes loaded with TGF-β have been proved to promote ischemic wound healing, which suggesting a promising regenerative therapy. And another study by Cooper et al (68) showed that human ADMSC-derived exosomes (hADMSC-exosomes) could stimulate human dermal fibroblasts migration and enhance ischemic cutaneous wound healing. All these results provide prospects and theoretical basis for clinical trials of exosomes in ischemic wounds. (Ischemic wound healing)

Still, we conclude that MSC-exosomes have a wider range of applications and ways of application, in the last paragraph of this section, as highlighted in red text:

Collectively, evidence shows that MSC-derived exosomes not only promote healing of cutaneous wounds in normal condition, but also promote healing of wounds in diabetic and ischemic conditions, as well as skin regeneration in aging condition. To make MSC-derived exosomes more effective in treating cutaneous wounds in special conditions, exosomes isolated from pretreated MSCs were studied. For instance, exosomes isolated from pioglitazone-pretreated BMMSCs and hypoxia ADMSCs were both confirmed to induce high-quality healing of diabetic wound(69, 70). These experiments expand the available scope of application of exosomes in cutaneous wounds, and suggest better sources of MSC-exosomes.

Additionally, we have enumerated preclinical meta-analyses and clinical studies to look

forward to the clinical application of MSC-exosomes, in the 1st paragraph of the "PERSPECTIVES FOR APPLICATION OF EXOSOMES IN CUTANEOUS WOUND HEALING AND REGENERATION" section, as highlighted in red text:

Notwithstanding a large body of evidence in the preceding sections that MSC-exosomes have positive effects on cutaneous wound healing in animal studies and preclinical trials, the data of exosomes in cutaneous wound healing from clinical studies is inadequate. Exhilaratingly, a lot of meta-analyses demonstrate MSC-exosomes to be potential and promising remedy for many acute and chronic diseases including cutaneous wounds in pre-clinical studies[71-73], revealing the therapeutic effect of MSC-exosomes on inflammation and injury. And these make successful clinical translation of MSC-exosomes more hopeful in cutaneous wound healing. Moreover, a randomized double-blind controlled clinical trial by Kwon et al demonstrated acne scars treated with human ADMSC-exosomes and fractional CO_2 laser exhibited better improvement than the control treated group, which gave a broad hint that ADMSC-exosomes provide synergistic therapeutic effects on atrophic acne scar clinical treatments[74]. Therefore, there are positive prospects of MSC-exosomes for a promising future in clinical translation.

3. *Exosomes are originated from different sources and conditions, and the effect of exosomal heterogeneity on cutaneous wound healing needs to be discussed.*

Response: Thank you for the precious comments. Regarding the issue on heterogeneity of MSC-exosomes, we have further argued about it its influence on application exosomes in clinical practice, in the 4th paragraph of the "TRANSLATIONAL POTENTIAL AND REGULATORY ASPECTS OF MSC-EXOSOMES" section, as highlighted in red text:

Homogeneity and quality control are also important considerations or challenges in regulatory aspect. Exosome homogeneity cannot be certain as chemically defined drugs, even exosomes from one cell are heterogeneous. However, exosome heterogeneity does not preclude adoption of exosome products in clinical use. A variety of experimental techniques can be used to determine the mechanism of action of exosomes in therapy. And then we can regulate the major active ingredients within exosomes related to the mechanism of action to assure quality and potency[32]. With a better understanding of the mechanism of action, we can identify the exact active ingredients and overexpress them, through which to improve homogeneity and determine the quality control strategy of manufacturing. In addition, screening exosomes with biomarkers such as surface receptors is also a method to obtain more homogenous exosomes, and to enrich exosomes with higher efficacy[33]. Although the lack of standardizations in the methodology for the collection, isolation, and analysis of exosomes can affect the exosome contents and potency, we can still determine the mainly active contents responsible for therapeutic efficacy by inactivation assay. And once active contents identified, we can use them to make quality control as described above and even in turn determine the best methodology for the collection, isolation and purification of exosomes[34].

4. *The lack of standardizations in the methodology for the collection, isolation, and analysis of exosomes is affecting the exosome content and their effect, and need to be discussed.*

Response: Thank you for the valuable comments. From our point of view, the lack of

standardizations in the methodology for the collection, isolation and analysis of exosomes can result in issues on safety, homogeneity and quality of exosome products, which can affect the exosome contents and their effects. Therefore, we have further discussed about these issues in the 3th and 4th paragraph of the "TRANSLATIONAL POTENTIAL AND REGULATORY ASPECTS OF MSC-EXOSOMES" section, as highlighted in red text:

Nevertheless, when we consider exosomes as biological agents in clinic application, there are a series of nonnegligible challenges in the regulatory and quality control aspects of exosome manufacturing. Due to the lack of standardizations in the methodology or procedures for the collection and isolation of exosomes, exosome products often differ in safety and quality aspects. To the challenge of safety considerations, exosome manufacturing should follow clinical good manufacturing practice (cGMP) protocols like other pharmaceutical preparations to obtain clinical-grade exosome preparations. Besides, with the successful development and use of various serum-free media, the medium that do not contain animal serum is recommended for MSC culturing to avoid mixing of exogenous exosomes derived from animal serum. Also, bioengineering technology may be applied to modify exosome phenotypes or contents, which can add or subtract specific biological molecules possessed by exosomes to increase efficacy or reduce undesirable effects during therapeutic course[30, 31].

Homogeneity and quality control are also important considerations or challenges in regulatory aspect. Exosome homogeneity cannot be certain as chemically defined drugs, even exosomes from one cell are heterogeneous. However, exosome heterogeneity does not preclude adoption of exosome products in clinical use. A variety of experimental techniques can be used to determine the mechanism of action of exosomes in therapy. And then we can regulate the major active ingredients within exosomes related to the mechanism of action to assure quality and potency[32]. With a better understanding of the mechanism of action, we can identify the exact active ingredients and overexpress them, through which to improve homogeneity and determine the quality control strategy of manufacturing. In addition, screening exosomes with biomarkers such as surface receptors is also a method to obtain more homogenous exosomes, and to enrich exosomes with higher efficacy[33]. Although the lack of standardizations in the methodology for the collection, isolation, and analysis of exosomes can affect the exosome contents and potency, we can still determine the mainly active contents responsible for therapeutic efficacy by inactivation assay. And once active contents identified, we can use them to make quality control as described above and even in turn determine the best methodology for the collection, isolation and purification of exosomes[34].

5. *The authors should discuss the fact that the conditions in which the MSCs are found when exosomes are isolated can affect the composition of the exosomes, and how this can affect wound healing treatment.*

Response: Thank you for the precious comments. We have amended discussions about this. Admittedly, the exosomes in the studies or preclinical trials are usually derived from normal two-dimensional cultured MSCs, but they are shown to have exact positive effects on cutaneous wound healing. Although the contents and composition of exosomes depending on the cellular states, there are no essential differences in these exosomes that derive from MSCs in different conditions. Optimistically, exosomes derived from MSCs in certain

conditions have better effects on wound healing. We discussed some conditions in which exosomes are isolated from pre-treated MSCs can enhance therapeutic effects of MSC-exosomes on cutaneous wound healing, in the last paragraph (Line 3-9) of the "EFFECTS OF MSC-EXOSOMES ON CUTANEOUS REGENERATION IN AGING AND DISEASE" section, as highlighted in red text:

To make MSC-derived exosomes more effective in treating cutaneous wounds in special conditions, exosomes isolated from pretreated MSCs were studied. For instance, exosomes isolated from pioglitazone-pretreated BMMSCs and hypoxia ADMSCs were both confirmed to induce high-quality healing of diabetic wound[69, 70]. These experiments expand the available scope of application of exosomes in cutaneous wounds, and suggest better sources of MSC-exosomes.

In addition, the problem of different composition of exosomes equals to exosome heterogeneity, which has a certain impact on the therapeutic effect. Therefore, we also addressed the issue of heterogeneity and treatment, in the 4th paragraph (Line 3-10) of the "TRANSLATIONAL POTENTIAL AND REGULATORY ASPECTS OF MSC-EXOSOMES" section, as highlighted in red text:

However, exosome heterogeneity does not preclude adoption of exosome products in clinical use. A variety of experimental techniques can be used to determine the mechanism of action of exosomes in therapy. And then we can regulate the major active ingredients within exosomes related to the mechanism of action to assure quality and potency[32]. With a better understanding of the mechanism of action, we can identify the exact active ingredients and overexpress them, through which to improve homogeneity and determine the quality control strategy of manufacturing.

6. *The authors compare the possible simple way of administration of exosomes with the obstacles of a systemic administration of ex vivo expanded MSCs, while the use of exosomes in clinic is still far from being applied and they need to make this issue clear.*

Response: Thank you for the significant comments. We have added arguments to make it clear that the use of exosomes in clinic is still far from being applied, in the 3rd paragraph of the "PERSPECTIVES FOR APPLICATION OF EXOSOMES IN CUTANEOUS WOUND HEALING AND REGENERATION" section, as highlighted in red text:

Despite many exciting prospects, we also need to recognize that actually the clinical use of exosomes is still hampered by many safety concerns and consistent regulatory issues. The clinical translation process of MSC-exosomes is still in a long way and far from the foreseeable prospect. Thus the use of exosomes in clinic is still far from being applied, until these problems are better solved and perfected.

Besides, we have changed some wordings that was too optimistic about the use of exosomes in clinical practice, as highlighted in red text:

Mesenchymal stem cell-derived exosomes: a novel and potential remedy for cutaneous wound healing and regeneration. (Title)

Intriguingly, exosomes that are cell-secreted granular vesicles with lipid bilayer membrane structure and contain specific components of the source cells, may emerge to be an excellent substitute for MSCs. ("Abstract", P1, Line7-9)

Therefore, the application of MSC-exosomes may be a promising alternative to cell therapy in

the treatment of cutaneous wounds. ("Abstract", P1, Line14-15)
Thus, the application of MSC extracts may be a more feasible and practical paradigm than direct cellular delivery treatment. ("INTRODUCTION", P2, Line26-27)
The application of exosomes has become a novel and cell-free therapeutic paradigm and been given high expectations due to their convenience in clinical use. ("INTRODUCTION", P2, Line30-32)

RESPONSE to COMPANY EDITOR-IN-CHIEF COMMENTS to AUTHORS:

I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Stem Cells, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2022. 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 ×). A: Control group; B: Model group; C: Pioglitazone hydrochloride group; D: Chinese herbal medicine group. 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.

Response: Thank you for the precious comments. We have undertaken a revision of the manuscript according to the constructive suggestions offered by the editors and reviewers. A detailed explanation of our revisions in response to the reviewers' comments is provided. And decomposable Figure organized into a single PowerPoint file is provided with copyright information.

RESPONSE to SPECIFIC COMMENTS to AUTHORS by REVIEWER 1:

1. *Add new classification of extracellular vesicles.*

Response: Thank you for the valuable comments. We have described the classification of extracellular vesicles in our original manuscript, in 1st paragraph (Line7-12) of the "EXTRACELLULAR VESICLES AND EXOSOMES" section, as highlighted in red text:

According to their diameters or biogenesis, EVs are usually divided into three main subtypes, *i.e.* exosomes, microvesicles and apoptotic bodies. Microvesicles and apoptotic bodies are vesicles derived from budding and pinching out of the surface of plasma membrane, while exosomes are vesicles derived from intracellular endosomes. Within recent years, exosomes as a special category of EVs, are more widely and deeply studied.

Because this review focuses on the role of exosomes in cutaneous wound healing, we described exosomes in more detail rather than other extracellular vesicles such as

microvesicles and apoptotic bodies.

2. *Depiction of how EVs/Exos help in wound healing - Image to be drawn .*

Response: Thank you for the valuable comments. We depicted how MSC-exosomes help in cutaneous wound healing in Figure 1. The figure and its legend are shown as below:

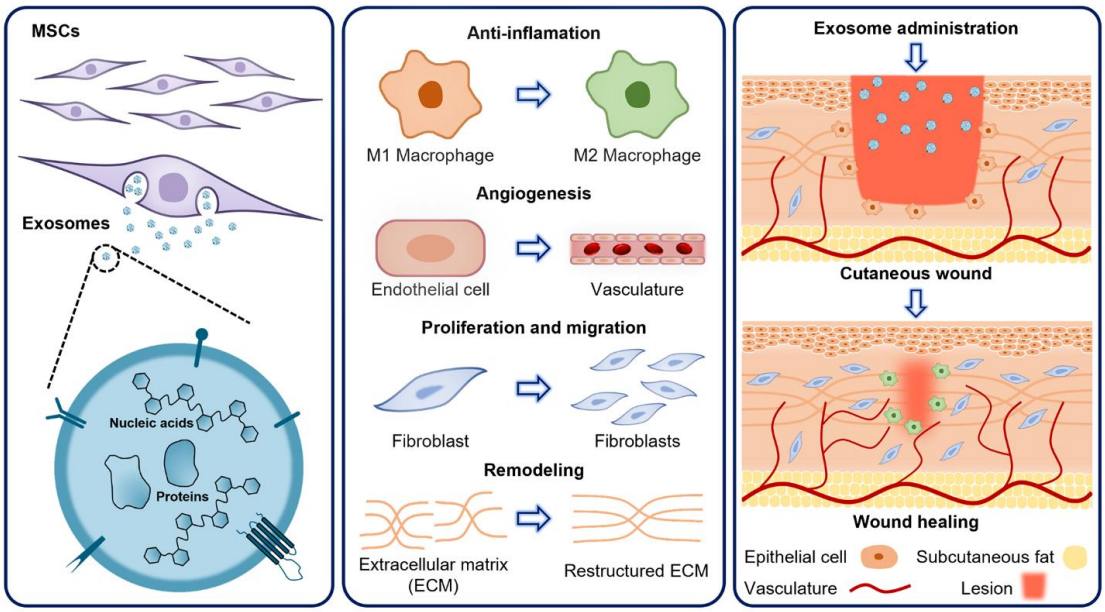

Figure 1. Mechanisms underlying the therapeutic effects of MSC-derived exosomes on cutaneous wound healing.

MSC-derived exosomes which contain a variety of proteins and nucleic acids hold great potential for promoting cutaneous wound healing. In specific, MSC-derived exosomes exert therapeutic effects through multiple mechanisms. They can inhibit inflammation via modulating macrophage polarization. Besides, during the proliferation phase, exosomes promote angiogenesis as well as proliferation and migration of fibroblasts. Furthermore, exosomes can improve the remodeling of the extracellular matrix (ECM). As a result, MSC-derived exosomes have offered a new paradigm in the treatment of cutaneous wound.

3. *Remove basic biology of EVs.*

Response: Thank you for the valuable comments. We moderately compressed the content of basic biology of exosomes. As understanding of basic biology of exosomes is very important to comprehension on therapeutic effects of exosomes, we reserved this part of contents. And we added the following statement on basic biology of exosomes to avoid abruptness of this part, as highlighted in red text:

Thus, the basic biology of exosomes indicates that MSC-exosomes contain MSC-specific components to exert specific effects on recipient cells, which somewhat equivalent to the therapeutic effects of MSCs. ("EXTRACELLULAR VESICLES AND EXOSOMES" section, P2, Line15-17)

4. *Add more clinical and preclinical evidences of EVs in cutaneous wound healing.*

Response: Thank you for the significant comments. We have added pre-clinical evidences of MSC-exosomes in cutaneous wound healing in the 2nd and 3rd paragraph of the

"MECHANISMS OF MSC-EXOSOMES IN CUTANEOUS WOUND HEALING AND REGENERATION" section, as highlighted in red text:

And recently, studies have shown that local application of exosomes can regulate the innate and adaptive immune networks as a whole, and better promote wound healing[44]. These indicate that MSC-exosomes can exert multiple effects in inflammation phase of wound healing. (P2)

BMSC-derived exosomes could also repress apoptosis of HaCaT cells (human immortalized epidermal cells) induced by hydrogen peroxide via the miR-93-3p/APAF1 axis[48]. Also, research demonstrated that ADMSC-derived exosomes could prompt proliferation and migration of HaCaT cells via Wnt/β-catenin signaling[49]. These indicate that MSC-exosomes can accelerate the process of re-epithelization in proliferation phase. (P3)

And we have also added pre-clinical evidences of exosomes in diabetic and ischemic wound healing in the "EFFECTS OF MSC-EXOSOMES ON CUTANEOUS REGENERATION IN AGING AND DISEASE" section, as highlighted in red text:

And recently, Han et al reported that BMSC-derived exosomes contained lncRNA KLF3-AS1, which could induce angiogenesis to promote wound healing in diabetic condition[66]. ("Diabetic wound healing")

Chronic ischemic wounds are another challenging problem in trauma clinic with delayed wound healing and therapeutic difficulties. Due to ischemia and hypoxia, the healing process of ischemic wounds is inhibited, resulting in imperfect curative effect of conventional treatments. Thus, exosome-based therapies, with multiple therapeutic benefits, have been tentatively applied in this disease area. In the study by Shi et al [67], exosomes loaded with TGF-β have been proved to promote ischemic wound healing, which suggesting a promising regenerative therapy. And another study by Cooper et al [68] showed that human ADMSC-derived exosomes (hADMSC-exosomes) could stimulate human dermal fibroblasts migration and enhance ischemic cutaneous wound healing. All these results provide prospects and theoretical basis for clinical trials of exosomes in ischemic wounds. ("Ischemic wound healing")

Still, we conclude that MSC-exosomes have a wider range of applications and ways of application, in the last paragraph of this section, as highlighted in red text:

To sum up, evidence shows that MSC-derived exosomes not only promote healing of cutaneous wounds in normal condition, but also promote healing of wounds in diabetic and ischemic conditions, as well as skin regeneration in aging condition. To make MSC-derived exosomes more effective in treating cutaneous wounds in special conditions, exosomes isolated from pretreated MSCs were studied. For instance, exosomes isolated from pioglitazone-pretreated BMMSCs and hypoxia ADMSCs were both confirmed to induce high-quality healing of diabetic wound[69, 70]. These experiments expand the available scope of application of exosomes in cutaneous wounds, and suggest better sources of MSC-exosomes.

Additionally, we have enumerated preclinical meta-analyses and clinical studies to look forward to the clinical application of MSC-exosomes, in the 1st paragraph of the "PERSPECTIVES FOR APPLICATION OF EXOSOMES IN CUTANEOUS WOUND HEALING AND REGENERATION" section, as highlighted in red text:

Notwithstanding a large body of evidence in the preceding sections that MSC-exosomes have positive effects on cutaneous wound healing in animal studies and preclinical trials, the data

of exosomes in cutaneous wound healing from clinical studies is inadequate. Exhilaratingly, a lot of meta-analyses demonstrate MSC-exosomes to be potential and promising remedy for many acute and chronic diseases including cutaneous wounds in pre-clinical studies[71-73], revealing the therapeutic effect of MSC-exosomes on inflammation and injury. And these make successful clinical translation of MSC-exosomes more hopeful in cutaneous wound healing. Moreover, a randomized double-blind controlled clinical trial by Kwon et al demonstrated acne scars treated with human ADMSC-exosomes and fractional CO2 laser exhibited better improvement than the control treated group, which gave a broad hint that ADMSC-exosomes provide synergistic therapeutic effects on atrophic acne scar clinical treatments[74]. Therefore, there are positive prospects of MSC-exosomes for a promising future in clinical translation.

5. *Tabulate the results in the studies cited inside the manuscript.*

Response: Thank you for the precious comments. We have tabulated the results in the studies cited inside the manuscript, as shown in Table 1:

Table1 Preclinical studies of MSC-exosomes in cutaneous wound healing phases

Wound Healing Phase	Exosome cellular origin	Model	Functional effects	Pathways	Reference
Inflammation	hBMMSCs hJMMSCs	Mice dorsal skin defects	Macrophage M2 polarization	miR-223 via pknox1	38
	hUCMSCs	Rat severe burn	M2 polarization Inflammation alleviation	miR-181c via TLR4	39
	LPS-pretreated hUCNSCs	Rat diabetic cutaneous wound	M2 polarization	Let-7b via TLR4/NF-κB/STAT3/AKT	40
	mBMMSCs	Mice skin excision wound	Promote beneficial regulatory T cell responses and M2 polarization	M2/Th2/Treg responses	44
Proliferation	hADMSCs	Mice full-thickness incision wound	Promote fibroblast proliferation and migration; optimize collagen deposition	PI3K/Akt	47
	hUCMSCs	Rat skin burn	Enhance re-epithelialization and cell proliferation; reduce heat stress-induced apoptosis	Wnt/β-catenin AKT	51
	hiPSC-MSCs	Rat skin wound	Accelerate skin cell proliferation and migration; promote collagen synthesis and angiogenesis	ERK1/2	52 53
Remodeling	hADMSCs	Mice skin incisional wound	Mitigating scar formation; promote ECM reconstruction	ERK/MAPK	55
	hUCMSCs	Mice full-thickness skin defects	Suppress myofibroblast differentiation and scar formation	TGF-β/SMAD2	56 57
	hAFSCs	Rat full-thickness skin wound	Anti-fibrotic scarring; suppress the excessive aggregation of myofibroblasts and ECM	TGF-β	58

RESPONSE to SPECIFIC COMMENTS to AUTHORS by REVIEWER 2:

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.*

Response: Thank you for the significant comments. We have amended the references and updated them to 2022. We added 18 references from 2020 to 2022 as fellows:

22 **Krampera M,** Le Blanc K. Mesenchymal stromal cells: Putative microenvironmental modulators become cell therapy. Cell Stem Cell 2021; **28**: 1708-1725 [PMID: 34624232 DOI: 10.1016/j.stem.2021.09.006]

30 **Liang Y,** Duan L, Lu J, Xia J. Engineering exosomes for targeted drug delivery. Theranostics 2021; **11**: 3183-3195 [PMID: 33537081 DOI: 10.7150/thno.52570]

31 **Tran PHL,** Xiang D, Tran TTD, Yin W, Zhang Y, Kong L, Chen K, Sun M, Li Y, Hou Y, Zhu Y, Duan W. Exosomes and nanoengineering: A match made for precision therapeutics. Adv Mater 2020; **32**: e1904040 [PMID: 31531916 DOI: 10.1002/adma.201904040]

32 **Aheget H,** Tristan-Manzano M, Mazini L, Cortijo-Gutierrez M, Galindo-Moreno P, Herrera C, Martin F, Marchal JA, Benabdellah K. Exosome: A new player in translational nanomedicine. J Clin Med 2020; **9**: [PMID: 32722531 DOI: 10.3390/jcm9082380]

33 **Chen J,** Li P, Zhang T, Xu Z, Huang X, Wang R, Du L. Review on strategies and technologies for exosome isolation and purification. Front Bioeng Biotechnol 2021; **9**: 811971 [PMID: 35071216 DOI: 10.3389/fbioe.2021.811971]

34 **Yang D,** Zhang W, Zhang H, Zhang F, Chen L, Ma L, Larcher LM, Chen S, Liu N, Zhao Q, Tran PHL, Chen C, Veedu RN, Wang T. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. Theranostics 2020; **10**: 3684-3707 [PMID: 32206116 DOI: 10.7150/thno.41580]

44 **Su N,** Hao Y, Wang F, Hou W, Chen H, Luo Y. Mesenchymal stromal exosome-functionalized scaffolds induce innate and adaptive immunomodulatory responses toward tissue repair. Sci Adv 2021; **7**: [PMID: 33980490 DOI: 10.1126/sciadv.abf7207]

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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.*

Response: Thank you for the precious comments. Regarding the issue on heterogeneity of MSC-exosomes, we have further argued about it its influence on application exosomes in clinical practice, in the 4th paragraph of the "TRANSLATIONAL POTENTIALS AND REGULATORY ASPECTS OF MSC-EXOSOMES" section, as highlighted in red text:

Homogeneity and quality control are also important considerations or challenges in regulatory aspect. Exosome homogeneity cannot be certain as chemically defined drugs, even exosomes from one cell are heterogeneous. However, exosome heterogeneity does not preclude adoption of exosome products in clinical use. A variety of experimental techniques can be used to determine the mechanism of action of exosomes in therapy. And then we can regulate the major active ingredients within exosomes related to the mechanism of action to assure quality and potency[32]. With a better understanding of the mechanism of action, we

can identify the exact active ingredients and overexpress them, through which to improve homogeneity and determine the quality control strategy of manufacturing. In addition, screening exosomes with biomarkers such as surface receptors is also a method to obtain more homogenous exosomes, and to enrich exosomes with higher efficacy[33]. Although the lack of standardizations in the methodology for the collection, isolation, and analysis of exosomes can affect the exosome contents and potency, we can still determine the mainly active contents responsible for therapeutic efficacy by inactivation assay. And once active contents identified, we can use them to make quality control as described above and even in turn determine the best methodology for the collection, isolation and purification of exosomes[34].

3. *A few references did not list authors correctly.*

Response: Thank you for the significant comments. We have adjusted references to list the authors correctly following the guidelines for reference listing.

4. *A table listing exosome applications on cutaneous wound treatment should be helpful.*

Response: Thank you for the precious comments. We have tabulated the results in the studies cited inside the manuscript, as shown in Table 1:

Table1 Preclinical studies of MSC-exosomes in cutaneous wound healing phases

Wound Healing Phase	Exosome cellular origin	Model	Functional effects	Pathways	Reference
Inflammation	hBMMSCs hJMMSCs	Mice dorsal skin defects	Macrophage M2 polarization	miR-223 via pknox1	38
	hUCMSCs	Rat severe burn	M2 polarization Inflammation alleviation	miR-181c via TLR4	39
	LPS-pretreated hUCNSCs	Rat diabetic cutaneous wound	M2 polarization	Let-7b via TLR4/NF-κB/STAT3/AKT	40
	mBMMSCs	Mice skin excision wound	Promote beneficial regulatory T cell responses and M2 polarization	M2/Th2/Treg responses	44
Proliferation	hADMSCs	Mice full-thickness incision wound	Promote fibroblast proliferation and migration; optimize collagen deposition	PI3K/Akt	47
	hUCMSCs	Rat skin burn	Enhance re-epithelialization and cell proliferation; reduce heat stress-induced apoptosis	Wnt/β-catenin AKT	51
	hiPSC-MSCs	Rat skin wound	Accelerate skin cell proliferation and migration; promote collagen synthesis and angiogenesis	ERK1/2	52 53
Remodeling	hADMSCs	Mice skin incisional wound	Mitigating scar formation; promote ECM reconstruction	ERK/MAPK	55
	hUCMSCs	Mice full-thickness skin defects	Suppress myofibroblast differentiation and scar formation	TGF-β/SMAD2	56 57
	hAFSCs	Rat full-thickness skin wound	Anti-fibrotic scarring; suppress the excessive aggregation of myofibroblasts and ECM	TGF-β	58

RESPONSE to SPECIFIC COMMENTS to AUTHORS by REVIEWER 3:

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?*

Response: Thank you for the valuable comments. We have further discussed about the safety issue in the 3th paragraph of the "TRANSLATIONAL POTENTIALS AND REGULATORY ASPECTS OF MSC-EXOSOMES" section, as highlighted in red text:

Nevertheless, when we consider exosomes as biological agents in clinic application, there are a series of nonnegligible challenges in the regulatory and quality control aspects of exosome manufacturing. Due to the lack of standardizations in the methodology or procedures for the collection and isolation of exosomes, exosome products often differ in safety and quality aspects. To the challenge of safety considerations, exosome manufacturing should follow clinical good manufacturing practice (cGMP) protocols like other pharmaceutical preparations to obtain clinical-grade exosome preparations. Besides, with the successful development and use of various serum-free media, the medium that do not contain animal serum is recommended for MSC culturing to avoid mixing of exogenous exosomes derived from animal serum. Also, bioengineering technology may be applied to modify exosome phenotypes or contents, which can add or subtract specific biological molecules possessed by exosomes to increase efficacy or reduce undesirable effects during therapeutic course[30, 31].

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?*

Response: Thank you for the valuable comments. We discussed about the further possible exosome administration methods for other less severe wound models in the 2nd paragraph of the "PERSPECTIVES FOR APPLICATION OF EXOSOMES IN CUTANEOUS WOUND HEALING AND REGENERATION" section, as highlighted in red text:

Also, the surface of exosome can be modified with some functional molecules such as aptamers to enable the transfer of engineered exosomes to target sites when administered systematically or locally, which can improve therapeutic efficiency. (Line16-19)

Also, we expounded about exosome administration for other less severe wound models such as ischemic wound healing, in the 3rd paragraph of the "EFFECTS OF MSC-EXOSOMES ON CUTANEOUS REGENERATION IN AGING AND DISEASE" section, as highlighted in red text:

Chronic ischemic wounds are another challenging problem in trauma clinic with delayed wound healing and therapeutic difficulties. Due to ischemia and hypoxia, the healing process of ischemic wounds is inhibited, resulting in imperfect curative effect of conventional treatments. Thus, exosome-based therapies, with multiple therapeutic benefits, have been tentatively applied in this disease area. In the study by Shi et al [67], exosomes loaded with TGF-β have been proved to promote ischemic wound healing, which suggesting a promising

regenerative therapy. And another study by Cooper et al [68] showed that human ADMSC-derived exosomes (hADMSC-exosomes) could stimulate human dermal fibroblasts migration and enhance ischemic cutaneous wound healing. All these results provide prospects and theoretical basis for clinical trials of exosomes in ischemic wounds.

RESPONSE to SPECIFIC COMMENTS to AUTHORS by REVIEWER 4:

I would like to congratulate the authors for this manuscript. It is interesting and can bring new perspective. I have some comments:

1. *Please check for grammatical errors and mistyped words.*

Response: Thank you for the sincere comments. We have checked for grammatical errors and mistyped words and modified these errors or words.

2. *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.*

Response: Thank you for the sincere comments. We have replaced 'In conclusion' with 'Collectively', to avoid ambiguity.

3. *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).*

Response: Thank you for the significant comments. We have amended references following the guidelines for reference listing.

4. *Please check the figure legend note of figure 1: …'proliferation phage' or proliferation phase?*

Response: Thank you for the significant comments. We have changed 'proliferation phage' into 'proliferation phase'.

RESPONSE to SPECIFIC COMMENTS to AUTHORS by REVIEWER 5:

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 preparations 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.

Response: Thank you for the precious comments. We have changed the statement in the title that exosomes are promising remedy into that exosomes are potential remedy. Meanwhile, in this review, we have more rigorously elucidated the status and prospect of MSC-exosomes in

the treatment of cutaneous wounds. As for the possible contamination of exosomes by FBS, we have discussed about this in 3rd paragraph in the "TRANSLATIONAL POTENTIALS AND REGULATORY ASPECTS OF MSC-EXOSOMES" section, as highlighted in red text:
Besides, with the successful development and use of various serum-free media, the medium that do not contain animal serum is recommended for MSC culturing to avoid mixing of exogenous exosomes derived from animal serum. (Line8-11)

- 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 cells.
Response: Thank you for the precious comments. Admittedly, the MSC-exosomes in most preclinical studies are the whole exosomes that are isolated from conditioned MSCs culture medium. This leads to multiple exosome subtypes of a certain kind of MSCs being used in those studies. However, this does not affect our confirmation of the effectiveness of MSC-exosomes in promoting cutaneous wound healing. Although the exosomes used in studies are a mixture of multiple subtypes, we are still able to identify the key components with therapeutic function in MSC-exosomes by inactive assay or other methods. Based on the in-depth insights of these understandings, we can better apply exosomes into practice and improve the therapeutic efficiency of MSC-exosomes by screening specific subtypes of exosomes or regulating the contents of MSC-exosomes. These are the directions for future researches, and also alternative solutions to the above problems.

- 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 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. 2018Nov23;7(1):1535750. doi: 10.1080/20013078.2018.1535750). While the Authors don't give the proper emphasis to these major challenges.

Response: Thank you for the precious comments. Firstly, unlike single-component pharmaceutical preparations, exosomes contain a mixture of active ingredients, which hindering the approval of exosome drug licensing, but also allowing MSC-exosomes to produce combined effects on wound healing. The complexity of exosome composition results in a series of problems as mentioned above, yet, with the advancement of technology and further research, these problems are expected to be solved. By means of enrichment of active components, removal of ineffective components and exosome engineering, the complexity of exosome composition will become a controllable variable. Secondly, the above considerations can be summarized as the safety, heterogeneity and quality control aspects of exosome products in practical application. We have discussed about these aspects in 3rd, 4th and 5th paragraphs of the "TRANSLATIONAL POTENTIALS AND REGULATORY ASPECTS OF MSC-EXOSOMES" section, as highlighted in red text:

Nevertheless, when we consider exosomes as biological agents in clinic application, there are a series of nonnegligible challenges in the regulatory and quality control aspects of exosome manufacturing. Due to the lack of standardizations in the methodology or procedures for the collection and isolation of exosomes, exosome products often differ in safety and quality aspects. To the challenge of safety considerations, exosome manufacturing should follow clinical good manufacturing practice (cGMP) protocols like other pharmaceutical preparations to obtain clinical-grade exosome preparations. Besides, with the successful development and use of various serum-free media, the medium that do not contain animal serum is recommended for MSC culturing to avoid mixing of exogenous exosomes derived from animal serum. Also, bioengineering technology may be applied to modify exosome phenotypes or contents, which can add or subtract specific biological molecules possessed by exosomes to increase efficacy or reduce undesirable effects during therapeutic course[30, 31].

Homogeneity and quality control are also important considerations or challenges in regulatory aspect. Exosome homogeneity cannot be certain as chemically defined drugs, even exosomes from one cell are heterogeneous. However, exosome heterogeneity does not preclude adoption of exosome products in clinical use. A variety of experimental techniques

can be used to determine the mechanism of action of exosomes in therapy. And then we can regulate the major active ingredients within exosomes related to the mechanism of action to assure quality and potency[32]. With a better understanding of the mechanism of action, we can identify the exact active ingredients and overexpress them, through which to improve homogeneity and determine the quality control strategy of manufacturing. In addition, screening exosomes with biomarkers such as surface receptors is also a method to obtain more homogenous exosomes, and to enrich exosomes with higher efficacy[33]. Although the lack of standardizations in the methodology for the collection, isolation, and analysis of exosomes can affect the exosome contents and potency, we can still determine the mainly active contents responsible for therapeutic efficacy by inactivation assay. And once active contents identified, we can use them to make quality control as described above and even in turn determine the best methodology for the collection, isolation and purification of exosomes[34].

The regulatory and quality control of exosome products need further development, so there is still a long way to go before they can be authentically used in clinical practice. Yet this needs to be based on in-depth exploration of the mechanism of action. Thus, in the following part of this review, we will elaborate on the underlying mechanisms of MSC-derived exosomes in cutaneous wound healing and regeneration.

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, 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.

Response: Thank you for the precious comments. The above mentioned application scheme of autologous MSCs in wound healing has its corresponding advantages. However, the limitations of this scheme are that obtaining autologous MSCs requires additional invasive operations, the time required for the MSC tissue product construction prevents timely implementation of the treatment, and the scheme does not take shape to a commercial product that can be transported, stored, and used immediately. Therefore, exosomes as the novel application form of MSCs still have irreplaceable advantages, as explained in the review:

However, the application of exosomes as therapeutic biologics takes on many advantages over the whole MSCs[19, 23]. Firstly, exosomes can be stored and transported at low temperature for a longer time without significant loss in bioactivity than whole cells.

Secondly, exosomes have better penetrating abilities to cross biological barriers such as blood brain barrier and avoid entrapment in filter organs or tissues. Also, their lipid bilayer membranes can protect the bioactivity of content molecules in sophisticated physiological environment. Thirdly, exosomes can be engineered to obtain specific properties and can be quantitatively administered to patients in clinic to obtain better clinical effects. ("TRANSLATIONAL POTENTIALS AND REGULATORY ASPECTS OF MSC-EXOSOMES"section, P1, Line2-11)

Exosomes as natural bi-layered lipid spheres possess high skin penetration efficiency, similar to liposomal nanoparticles[26, 27]. This enables topical administration of exosomes, rendering wound areas more receptive to the therapeutic exosomes[28]. Furthermore, delivered exosomes can also be chemotactic to the inflammatory or injured site when a distance exists between the administered area and the lesion center[29]. Additionally, with a variety of bioactive molecules inside, exosomes can exert their curative benefits through many different therapeutic mechanisms simultaneously, which leads to better biological effects than small molecular compounds. ("TRANSLATIONAL POTENTIALS AND REGULATORY ASPECTS OF MSC-EXOSOMES"section, P2)

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.

Response: Thank you for the precious comments. We have altered the statement "Notwithstanding strong evidence in the preceding sections that exosomes derived from various MSCs have therapeutic effects on cutaneous wound healing" to "Notwithstanding a large body of evidence in the preceding sections that MSC-exosomes have positive effects on cutaneous wound healing in animal studies and preclinical trials, the data of exosomes in cutaneous wound healing from clinical studies is inadequate" ("PERSPECTIVES FOR APPLICATION OF EXOSOMES IN CUTANEOUS WOUND HEALING AND REGENERATION" section, P1, Line1-4). We have made it clear that the use of exosomes in clinic is still far from being applied, in the 3rd paragraph of the "PERSPECTIVES FOR APPLICATION OF EXOSOMES IN CUTANEOUS WOUND HEALING AND REGENERATION" section, as highlighted in red text:

Despite many exciting prospects, we also need to recognize that actually the clinical use of exosomes is still hampered by many safety concerns and consistent regulatory issues. The clinical translation process of MSC-exosomes is still in a long way and far from the

foreseeable prospect. Thus the use of exosomes in clinic is still far from being applied, until these problems are better solved and perfected.

And concluded at the end that:

Taken together, MSC-derived exosomes, as a cell-free therapeutic paradigm, provides a novel promising option for cutaneous regeneration. Yet, more researches are needed so as to further excavate the curative potentials of exosomes and make them more suitable for clinical use. ("CONCLUSION" section, P1, Line1-4)

Besides, we have changed some wordings that was too optimistic about the use of exosomes in clinical practice, as highlighted in red text:

Mesenchymal stem cell-derived exosomes: a novel and potential remedy for cutaneous wound healing and regeneration. (Title);

Intriguingly, exosomes that are cell-secreted granular vesicles with lipid bilayer membrane structure and contain specific components of the source cells, may emerge to be an excellent substitute for MSCs. ("Abstract", P1, Line7-9);

Therefore, the application of MSC-exosomes may be a promising alternative to cell therapy in the treatment of cutaneous wounds. ("Abstract", P1, Line14-15);

Thus, the application of MSC extracts may be a more feasible and practical paradigm than direct cellular delivery treatment. ("INTRODUCTION", P2, Line26-27);

The application of exosomes has become a novel and cell-free therapeutic paradigm and been given high expectations due to their convenience in clinical use. ("INTRODUCTION", P2, Line30-32).

In summary:

Thank you again for the valuable comments and suggestions of our manuscript. We would be glad to respond to any additional questions and comments you may raise.

Best regards,
Sincerely yours,
Yan Jin