Name of Journal: *World Journal of Diabetes*

Manuscript NO: 80473

Manuscript type: REVIEW

Title: Mesenchymal stem cell-derived exosomes: the dawn of diabetic wound healing

Dear Editor-in-Chief and Reviewers:

We thank the Editor and Reviewers for their time and constructive critiques and greatly appreciate the opportunity to resubmit our manuscript, Manuscript NO: 80473 entitled " Mesenchymal stem cell-derived exosomes: the dawn of diabetic wound healing". Our point-by-point replies to your comments are provided below. All the authors have reviewed and approved the revisions.

Reviewer #1:

1. Figure 1 is untidy and has too much information. Although schemas representing the cutaneous wound healing on the lower side of the figure are well drawn and easy to understand, signaling pathways on the upper side of the figure should be better reorganized and likely split in new figures to improve the reading.

Response:

Thank you for your valuable comments on Figure 1. We reorganized the contents listed in Figure 1. The specific changes are as follows:

(1) We divided the whole figure into three parts;

(2) Figure 1A draws the biogenesis of exosomes and the way in which exosomes exchange information with wound healing-related cells. The information regulation mechanism covers the PTEN/PI3K/AKT, NF-κB, TGF-β/Smad and RAS/Raf/MAPK/ERK signaling pathways. These signaling pathways had been studied most frequently in the diabetic acute wound models, and the

RAS/Raf/MAPK/ERK pathway had been proposed in the only diabetic chronic wound model but have not been verified at the cellular level. The regulation mechanisms need to be further confirmed in more studies of exosomes in treating diabetic chronic wounds in the future. Other signaling pathways involved in the treatment of diabetic wounds with MSC-Exos can be found in Table 1;

(3) We placed schemas representing the cutaneous wound healing on the lower side of previous Figure 1 into Figure 1B (inflammatory phase) and Figure 1C (proliferative phase), respectively, in order to show the pathophysiological changes more clearly.

(80473-Figure 1.pptx)

2. The author should mention more specific barrier for clinical application of MSC-Exos in the section of "Current status and prospects of clinical applications of exosomes in diabetic chronic wounds".

Response:

Thank you for your advice. That also compensates for our lack of more specific barrier for clinical application of MSC-Exos in diabetic chronic wounds. We added the corresponding contents in the section of "CURRENT STATUS AND PROSPECTS OF CLINICAL APPLICATIONS OF EXOSOMES IN DIABETIC CHRONIC WOUNDS", as below:

(1) We added the specific barriers of transformation of using exosomes in treating diabetic chronic wounds from preclinical research to clinical application. The specific changes are as follows:

"*Exosome research is still in its infancy, and the realization of the transformation from preclinical research to clinical application still has great exploration value. The problems of optimal preparation, extraction, isolation, and storage of exosomes on a large scale and their production efficiency have not yet been determined; preparation and identification of components due to different source cells and the high heterogeneity of exosome components have not yet been solved; specific regulatory mechanisms in*

DCWs have not yet been fully elucidated; efficacy and safety of different cell sources and/or administrations have not been proven, and reasonable and effective methods of fusing exosomes with other biomaterials have not yet been implemented, all these issues are barriers that limit the clinical application of exosomes."

(Main Text - CURRENT STATUS AND PROSPECTS OF CLINICAL APPLICATIONS OF EXOSOMES IN DIABETIC CHRONIC WOUNDS, paragraph 4)

(2) We also increased the need for toxicological analysis of exosomes derived from different cell sources and the need to focus on short- and long-term safety assessments in future clinical applications, as below:

"Nevertheless, toxicological analysis of different tissue-derived MSCs-Exos and more evidence of short and long-term health safety assessments are required to confirm their safety."

(Main Text - CURRENT STATUS AND PROSPECTS OF CLINICAL APPLICATIONS OF EXOSOMES IN DIABETIC CHRONIC WOUNDS, paragraph 3)

3. The author should modify and soften the expression that stem cell-based therapies have risks and that exosome-based therapies are replacement for them. Various types of stem cell have been already applied for the clinical phase in other areas without any adverse events.

Response:

We thank the Reviewer for this insightful suggestion. We do not deny the fact that stem cell therapies have clinical benefits, but rather we suggest that with the increasing number of stem cell clinical studies being conducted, there is a need to be concerned about possible problems and strict regulation.

Current researches reveal that Various types of stem cell have been already applied for the clinical phase in various disciplines, and evidence shows their benefits in treating these diseases. However, there are still some studies

reporting problems with stem cell therapies such as allogeneic immune rejection, and adverse events such as tumorigenesis or vitreous injection leading to blindness with autologous stem cell transplantation. In addition, stem cell therapies are still in the early clinical stage and longer follow-up is needed to confirm their long-term health effects, and the need of further attention and supervision of stem cell therapies has been raised by both the FDA and Chinese clinical research authorities. We added these arguments and supporting references about the current benefits and risks of stem cell therapies in Introduction and Main Text, as below:

(1) We added these arguments and supporting references in Introduction. The specific changes are as follows:

"Clinical trials of MSCs for treating various types of cutaneous wounds are currently in full swing, and their efficacy and safety in promoting wound regeneration have been initially demonstrated. As clinical trials continue to progress, further attention and supervision need to be paid to their potential safety issues of proliferative lesion formation, abnormal organ reaction and unknown long-term health effects after transplantation[18-20]*."*

(INTRODUCTION, paragraph3)

Reference 18 ***Hyun I****, Lindvall O, Ahrlund-Richter L, Cattaneo E, Cavazzana-Calvo M, Cossu G, De Luca M, Fox IJ, Gerstle C, Goldstein RA, Hermerén G, High KA, Kim HO, Lee HP, Levy-Lahad E, Li L, Lo B, Marshak DR, McNab A, Munsie M, Nakauchi H, Rao M, Rooke HM, Valles CS, Srivastava A, Sugarman J, Taylor PL, Veiga A, Wong AL, Zoloth L, Daley GQ. New ISSCR guidelines underscore major principles for responsible translational stem cell research. Cell Stem Cell 2008;* ***3****: 607-609 [PMID: 19041777 DOI:10.1016/j.stem.2008.11.009]*

Reference 19 ***Lovell-Badge R****, Anthony E, Barker RA, Bubela T, Brivanlou AH, Carpenter M, Charo RA, Clark A, Clayton E, Cong Y, Daley GQ, Fu J, Fujita M, Greenfield A, Goldman SA, Hill L, Hyun I, Isasi R, Kahn J, Kato K, Kim JS, Kimmelman J, Knoblich JA, Mathews D, Montserrat N, Mosher J, Munsie M, Nakauchi H, Naldini L, Naughton G, Niakan K, Ogbogu U, Pedersen R, Rivron N, Rooke H, Rossant J, Round J, Saitou M, Sipp D, Steffann J, Sugarman J, Surani A, Takahashi J, Tang F, Turner L, Zettler PJ, Zhai X. ISSCR Guidelines for Stem Cell Research and Clinical Translation: The 2021*

update. Stem Cell Reports 2021; ***16****: 1398-1408 [PMID: 34048692 DOI:10.1016/j.stemcr.2021.05.012]*

Reference 20 ***Marks PW****, Witten CM, Califf RM. Clarifying Stem-Cell Therapy's Benefits and Risks. N Engl J Med 2017;* ***376****: 1007-1009 [PMID: 27959704 DOI:10.1056/NEJMp1613723]*

(2) We changed the name of section "STEM CELL-BASED THERAPIES CARRY RISKS, COMING EXOSOMES INTO BEING" to "STEM CELL-BASED THERAPIES BECOME HOT TOPICS, COMING EXOSOMES INTO BEING", and we also added arguments and supporting references in this section. The specific changes are as follows:

"Stem cells have the potential for self-renewal and multidirectional differentiation with great research and application value in life sciences, clinical trials and disease research. Stem cell-based therapies are now approved by several countries, and have been widely used in various disciplines. MSCs are currently the main experimental cell sources and have shown their excellent therapeutic potential and value in clinical trials in the field of regenerative medicine[16, 54]*."*

(Main Text - STEM CELL-BASED THERAPIES BECOME HOT TOPICS, COMING EXOSOMES INTO BEING, paragraph 1)

Reference 16 ***Kanji S****, Das H. Advances of Stem Cell Therapeutics in Cutaneous Wound Healing and Regeneration. Mediators Inflamm 2017;* ***2017****: 5217967 [PMID: 29213192 DOI:10.1155/2017/5217967]*

Reference 54 ***Margiana R****, Markov A, Zekiy AO, Hamza MU, Al-Dabbagh KA, Al-Zubaidi SH, Hameed NM, Ahmad I, Sivaraman R, Kzar HH, Al-Gazally ME, Mustafa YF, Siahmansouri H. Clinical application of mesenchymal stem cell in regenerative medicine: a narrative review. Stem Cell Res Ther 2022;* ***13****: 366 [PMID: 35902958 DOI:10.1186/s13287-022-03054-0]*

"Previous clinical studies have demonstrated that MSC transplantation in patients with DFUs is safe and feasible with the properties of improving microcirculation, wound healing, ulcer recurrence, and amputation[61-63]*. However, stem cell therapies are still in their early clinical stage, further attention and supervision are required of declined performance during production and application as*

cellular senescence and loss of multipotency during ex vivo expansion and from variable donors[64, 65]*, decreased survival rate caused by advanced glycosylation end products*[66]*, potential safety issues as proliferative lesion formation and abnormal organ reaction*[20]*, and unknown long-term health effects after transplantation. Basic and clinical researches related to allogeneic/autologous stem cells are subject to the International Society for Stem Cell Research Guidelines for Clinical Translation of Stem Cells and national ethical guidelines and related guidelines/regulations*[20, 67]*."*

(Main Text - STEM CELL-BASED THERAPIES BECOME HOT TOPICS, COMING EXOSOMES INTO BEING, paragraph 3)

Reference 61 ***Kirana S****, Stratmann B, Prante C, Prohaska W, Koerperich H, Lammers D, Gastens MH, Quast T, Negrean M, Stirban OA, Nandrean SG, Götting C, Minartz P, Kleesiek K, Tschoepe D. Autologous stem cell therapy in the treatment of limb ischaemia induced chronic tissue ulcers of diabetic foot patients. Int J Clin Pract 2012;* ***66****: 384-393 [PMID: 22284892 DOI:10.1111/j.1742-1241.2011.02886.x]*

Reference 62 ***Lu D****, Jiang Y, Deng W, Zhang Y, Liang Z, Wu Q, Jiang X, Zhang L, Gao F, Cao Y, Chen B, Xue Y. Long-Term Outcomes of BMMSC Compared with BMMNC for Treatment of Critical Limb Ischemia and Foot Ulcer in Patients with Diabetes. Cell Transplant 2019;* ***28****: 645-652 [PMID: 30917698 DOI:10.1177/0963689719835177]*

Reference 63 ***Moon KC****, Suh HS, Kim KB, Han SK, Young KW, Lee JW, Kim MH. Potential of Allogeneic Adipose-Derived Stem Cell-Hydrogel Complex for Treating Diabetic Foot Ulcers. Diabetes 2019;* ***68****: 837-846 [PMID: 30679183 DOI:10.2337/db18-0699]*

Reference 64 ***Rombouts WJ****, Ploemacher RE. Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. Leukemia 2003;* ***17****: 160-170 [PMID: 12529674 DOI:10.1038/sj.leu.2402763]*

Reference 65 ***Siddappa R****, Licht R, van Blitterswijk C, de Boer J. Donor variation and loss of multipotency during in vitro expansion of human mesenchymal stem cells for bone tissue engineering. J Orthop Res 2007;* ***25****: 1029-1041 [PMID: 17469183 DOI:10.1002/jor.20402]*

Reference 66 ***Wang Z****, Li H, Zhang D, Liu X, Zhao F, Pang X, Wang Q. Effect of advanced glycosylation end products on apoptosis in human adipose tissue-derived stem cells in vitro. Cell Biosci 2015;* ***5****: 3 [PMID: 25973170 DOI:10.1186/2045-3701-5-3]*

Reference 67 ***Jin J****. Stem Cell Treatments. Jama 2017;* ***317****: 330 [PMID: 28114555 DOI:10.1001/jama.2016.17822]*

(3) Exosomes do not have a cellular component and they have several advantages over direct stem cell transplantation, and we have added these perspectives and supporting references in the section of "STEM CELL-BASED THERAPIES BECOME HOT TOPICS, COMING EXOSOMES INTO BEING". The specific changes are as follows:

"Compared to direct cell transplantation, MSC-Exos avoid the immune rejection because of low immunogenicity; allow to cross various biological barriers and avoid the risk of embolism from intravenous injection based on their smaller sizes[70]*; the dose and fraction can be adjusted artificially and genetic modifications are easier and safer*[71]*; avoid the problem of malignant transformation; and allow to repair diabetic complications through multiple actions*[72]*."*

(Main Text - STEM CELL-BASED THERAPIES BECOME HOT TOPICS, COMING EXOSOMES INTO BEING, paragraph 4)

Reference 70 ***Nair S****, Salomon C. Extracellular vesicles and their immunomodulatory functions in pregnancy. Semin Immunopathol 2018;* ***40****: 425-437 [PMID: 29616307 DOI:10.1007/s00281-018-0680-2]*

Reference 71 ***Lamichhane TN****, Sokic S, Schardt JS, Raiker RS, Lin JW, Jay SM. Emerging roles for extracellular vesicles in tissue engineering and regenerative medicine. Tissue Eng Part B Rev 2015;* ***21****: 45-54 [PMID: 24957510 DOI:10.1089/ten.TEB.2014.0300]*

Reference 72 ***Newton WC****, Kim JW, Luo JZQ, Luo L. Stem cell-derived exosomes: a novel vector for tissue repair and diabetic therapy. J Mol Endocrinol 2017;* ***59****: R155-r165 [PMID: 28835418 DOI:10.1530/jme-17-0080]*

4. In the abstract section authors also should soften their comments "However, the preparation process of cell therapy is cumbersome, and there are problems of malignant differentiation, immune rejection, and embolization after transplantation, making clinical implementation difficult"

since it is not right and the safety of cell therapy is not at risk. The exosomes are parts of new biotherapies in addition to cell-bases ones however, nothing yet demonstrated that this is a better treatment.

Response:

(1) We thank the reviewer for this comment. Similarly, we support the clinical benefits of stem cell therapies, but some potential safety concerns and the need for further attention and regulation of stem cell therapies still remain. We modified the previous one-sided description in the Abstract, as below:

"Stem cell-based therapies have achieved specific efficacy in various fields, with mesenchymal stem cells (MSCs) being the most widely used. Although MSCs have achieved good feedback in preclinical studies and clinical trials in the treatment of cutaneous wounds or other situations, the potential safety concerns associated with allogeneic/autologous stem cells and unknown long-term health effects need further attention and supervision."

(Abstract)

"MSC-derived exosomes inherit the powerful inflammation and immune modulation, angiogenesis, cell proliferation, and migration promotion, oxidative stress alleviation, collagen remodeling imbalances regulation of their parental cells, and can avoid the potential risks of direct stem cell transplantation to a large extent, thus demonstrating promising performance as novel "cell-free" therapies in chronic wounds."

(Abstract)

5. The preparation process of exosomes is difficult due to the large heterogeneity of their components. This point should be better highlighted for future clinical trials and routine practice.

Response:

We agree with the Reviewer's comment. We added this content, as below:

"More research is required in future clinical trials and routine practice to determine the most effective cell sources for diabetic wounds; to establish optimal large-scale culture conditions of MSCs; to solve the preparation problem of huge heterogeneity of exosome components; to explore standardized isolation, quality control, purification, and characterization techniques of MSC-Exos; and to determine the best approach for long-term storage[162]*."*

(Main Text - CURRENT STATUS AND PROSPECTS OF CLINICAL APPLICATIONS OF EXOSOMES IN DIABETIC CHRONIC WOUNDS, paragraph 5)

Reference 162 ***Varderidou-Minasian S****, Lorenowicz MJ. Mesenchymal stromal/stem cell-derived extracellular vesicles in tissue repair: challenges and opportunities. Theranostics 2020;* ***10****: 5979-5997 [PMID: 32483432 DOI:10.7150/thno.40122]*

6. Check syntaxes and errors

Response:

We apologize for the syntaxes and errors. We modified these issues and sent our revised manuscript to a professional English language editing company to polish the manuscript.

Reviewer #2:

1. Grammar: Need Some revision. (Check The Paper Comments).

Response:

We apologize for the errors. We modified these issues and sent our revised manuscript to a professional English language editing company to polish the manuscript.

2. Please provide and edit the following information in the Paper

1) Conflict of Interest.

2) Source of Funding.

3) Some references without DOI.

4) Writing references according to the terms of the journal.

Response:

We verified the contents to ensure that the information was included in the revised manuscript. We also strictly verified references according to the terms of the journal, however, some references do not have DOI.

5) The result and discussion must be in one paragraph.

Response:

Since our manuscript type is REVIEW, the results and discussion sections are not covered.

Science editor and Company editor-in-chief：

1. Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file.

2. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content.

3. 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.

Response:

Thank you for your time and constructive critiques and greatly appreciate the opportunity to resubmit our manuscript. We revised the format of the figure and tables as required and provided the appropriate documentation for review.

4. Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript.

Response:

We supplement and improve the highlights of the latest cutting-edge research results, Study No. 27 in Table 1 and Study No.14 in Supplementary Table S1 are the latest research we added.

5. Before final acceptance, the author(s) must provide the English Language Certificate issued by a professional English language editing company.

Response:

We sent our revised manuscript to a professional English language editing company to polish the manuscript and provide the English Language Certificate.

Other revisions were shown below and changes were highlighted in the revised manuscript.

1. We modified some expressions in Core tip, as below:

"Diabetic chronic wounds are one of the most serious chronic complications of diabetes, and the efficacy of stem cell therapy for refractory chronic wounds has been studied previously. Stem cell-derived exosomes are one of the important active components of stem cell paracrine secretion, which inherit the wound repair capacity of parental cells as parts of novel cell-free therapies in addition to cell-bases ones. Herein we discuss the mechanism and latest progress of mesenchymal stem cell-derived exosomes in promoting diabetic chronic wound healing."

(Core tip)

2. We adjusted the logic between some paragraphs and sentences to make the text more coherent, as below:

"MSCs provide assistance in all phases of wound healing by exerting their functions of regulating skin homeostasis and wound healing through migration into the skin damage site and interaction with skin cells and can influence the function of these cells by paracrine secretion of bioactive factors and differentiation into them[55, 56]*. As MSCs have exhibited wound healing in many preclinical studies as powerful tools for regulating inflammation, promoting cell proliferation and migration, angiogenesis, and collagen synthesis*[57-60]*, the application of MSCs for DCWs contributes to progress toward clinical trials. Twenty-five clinical trials of MSCs for diabetic ulcers have been conducted or are recruiting subjects, which are recorded in the ClinicalTrials.gov database (clinicaltrials.gov)."*

(Main Text - STEM CELL-BASED THERAPIES BECOME HOT TOPICS, COMING EXOSOMES INTO BEING, paragraph 2)

"According to the search results in ClinicalTrials.gov, no clinical trials of MSC-Exos and exosomes from other sources for diabetic cutaneous wound healing have been registered. Therefore, we expanded the scope of clinical trials to search for exosomes

derived from any sources and exosome-enriched stem cell-conditioned medium in various wound types (Table 2). None of the included four registered clinical trials had related results published, while they were all non-randomized one-arm pilot studies. Thus, more high-quality randomized controlled trials are required to further confirm these research results. Of note, the application of cell-free therapies in clinical patients requires special attention to security, although no adverse reactions of exosomes have been reported in preclinical studies. Moreover, ADSC-Exos has been confirmed to not induce any irritation or toxicity in skin sensitization, irritation, or oral toxicity tests[161]*; therefore, they can be considered in clinical practice to promote wound healing in combination with basic wound care measures. Nevertheless, toxicological analysis of different tissue-derived MSCs-Exos and more evidence of short and long-term health safety assessments are required to confirm their safety."*

(Main Text - CURRENT STATUS AND PROSPECTS OF CLINICAL APPLICATIONS OF EXOSOMES IN DIABETIC CHRONIC WOUNDS, paragraph 3)

"Thus, efficient, stable, safe, and mass-producible stem cells and related products for the treatment of diabetic wounds are yet to be explored and developed. More research is required in future clinical trials and routine practice to determine the most effective cell sources for diabetic wounds; to establish optimal large-scale culture conditions of MSCs; to solve the preparation problem of huge heterogeneity of exosome components; to explore standardized isolation, quality control, purification, and characterization techniques of MSC-Exos; and to determine the best approach for long-term storage[162]*. Researchers also need to fully understand the abilities, loss, distribution, diffusion efficiency, and clearance efficiency of exosomes after transporting them to target areas. Physical, chemical, or biological methods for preconditioning, genetic engineering, and transfection are used to specifically enhance a certain therapeutic potential to achieve relatively better wound healing than native exosomes, thus becoming new treatment directions*[163]*. Additionally, combining exosomes with biomaterials is possible to create bioactive dressings to enhance or*

combine repair ability, provide local microenvironment stability, and achieve sustained release of exosomes[74]. Additionally, starting clinical trials as soon as possible is necessary to verify the optimal dosages, administration methods, and efficacy evaluation of MSC-Exos in clinical patients, looking forward to its broad application prospects in promoting DCW healing in clinical practices[162]*."*

(Main Text - CURRENT STATUS AND PROSPECTS OF CLINICAL APPLICATIONS OF EXOSOMES IN DIABETIC CHRONIC WOUNDS, paragraph 3)

Sincerely thanks for your valuable comments and suggestions. Hope to have the opportunity to publish on *World Journal of Diabetes*.

Looking forward to hearing from you.

Best regards,

Sincerely,

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