Round 1

Dear Editors and Reviewers:

Re: Manuscript reference No. 54910

Please find attached a revised version of our manuscript “Exosomes Derived from Stem Cells as an Emerging strategy for Intervertebral Disc Degeneration”, which we would like to resubmit for publication as a basic science in world journal of stem cells].

Your comments and those of the reviewers were highly insightful and enabled us to greatly improve the quality of our manuscript. In the following pages are our point-by-point responses to each of the comments of the reviewers as well as your own comments. Based on the instructions provided in your letter, we uploaded the file of the revised manuscript.

Revisions in the text are shown using yellow highlight for additions. In accordance with reviewer #1’s and #2’s suggestions, we revised the original manuscript. We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in world journal of stem cells.

We shall look forward to hearing from you at your earliest convenience.

Yours sincerely,

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Responses to the comments of Reviewer #1

Comment1: This is a very interesting review about stem-cell derived exosomes. Conclusion may be revised to include the detailed description about the further research needed. Proofreading is needed.

Response: The revised conclusion was "Exosomes are attracting increasing attention because of their unique structures and diverse properties. Exosomes have shown favorable possibilities during the repair of IVD, since they can promote the proliferation of NPCs, promote the homeostasis of the extracellular matrix, and inhibit cell apoptosis(Figure 1). However, the detailed mechanisms behind these activities are still unclear, so further research needed to explore the complex regulation mechanisms, optimize the culture and transplant conditions, perform more preclinical trials to verify the safety of exosomes."

Responses to the comments of Reviewer #2

Comment 1. The review by Hu et al., starts by presenting intervertebral disc (IVD) degeneration as a common health issue that may be alleviated by exosome-based therapy. The remaining follows by introducing basic features of exosomes and their cargo to follow with evidence of their therapeutic potential on different scenarios. The title should be adjusted to present exosomes as therapeutic entities rather than tools.

Response: The revised title was "Exosomes Derived from Stem Cells as an Emerging strategy for Intervertebral Disc Degeneration"

Comment 2. Although the value of the information gathered is appreciated, it seems that the authors fail to clearly communicate the level of evidence available for the

information provided. For example, it is not properly indicated whether the cited results come from animal experimentation or from human clinical trials. In either case, the number of individuals studied (N of the study) may also help readers understand if the data comes from pilot preliminary results or more statistically robust assays. Perhaps this information could be added to Table 2.

Response: It is not a long time for exosomes as new research objects for intervertebral disc degeneration regeneration.so, the existing articles on exosomes in this field are all cell and animal experiments, and no clinical trials have been conducted yet. We have reorganized Table 2 now.The revised table2 has been copy here below.

Studies on exosomes for IVD degeneration

Source	Experimental objective	Cargo analysis	Animal model	In vitro appraisement	In vivo appraisement	Inhibition test	Research type
HBMSCs Lu et al[26].	To detect the role of exosomes derived from (BM-MSCs) to	Not mentioned	None	1. Promoted proliferation 2. Increased synthesis of extracellular matrix and decrease in degradation	None	None	Cell experimentation

	NPCs.						
HBMSC Cheng et al[28].	To explore the protective effect of MSC-exosomes on NPCs in a cell and rat model.	Highly enrichment in miR-21	SD rat model of IVD degeneration by needle puncture	1. Decreased apoptosis rate 2. Decreased cleaved caspase-3	1. IVD degeneration score lower 2. Decreased apoptosis rate 3. Lower histologic score	1. MiR-21 antagonist enhanced cell apoptosis.	Cell and Animal experimentation
Rat Nucleus pulposus Moen et al[80].	To study the role of extracellular miRNA in lumbar radicular pain.	Increased miR-223	Lewis rat IVD herniation	None	1. MiR-223 increased after disc herniation.	None	Animal experimentation
Porcine notoch	To explore	None	None	Increased glycosamin	None	None	Cell experime

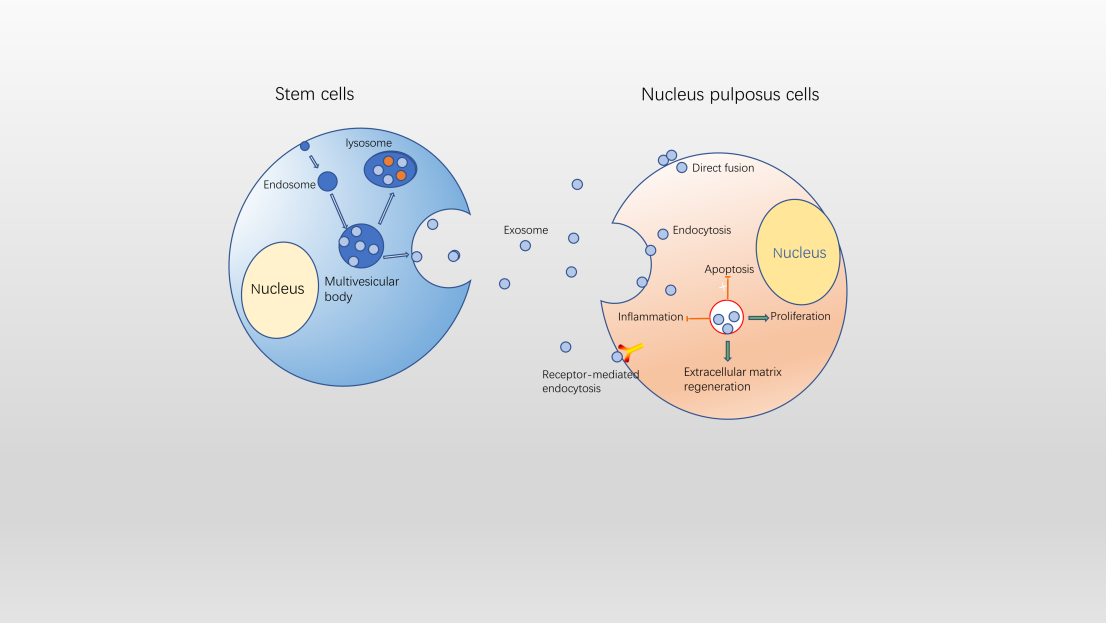
ordal cells Bach et al[81].	the biologic effect of the NCCM-derived EVs on canine and human CLCs from degenerated IVDs in vitro.			oglycan (GAG) deposition			ntation
HBMSCs Liao et al[27]	To prove that the delivery of MSC-exos could modulate ER stress and inhibit excessive NP cell	None	SD rat model of IVD degeneration by needle puncture	1. Western blot and TUNEL assays indicated decreased apoptosis rate 2. Western blot and qPCR data indicated decreased reticulum	1. Higher DHI 2. Lower Pfirrmann grade. 3. Lower histological grades 4. Decreased	Akt inhibitor LY294002 ERK inhibitor PD98059	Cell and Animal experimentation

	apoptosis during IDD.			stress	apoptosis rate		
C57BL/6 mice BMSCs Xia et al[69].	To investigate the therapeutic effect of exosomes for use as IVDD therapeutics.	None	Rabbit model of IVD degeneration by needle puncture	1. Decreased apoptosis rate 2. Western blot and qPCR data indicated recovery of matrix homeostasis 3. Decreased inflammatory marker expression 4. Suppressed inflammasome 5. Recovery of mitochondrial-related proteins	1. Higher DHI 2. Lower Pfirrmann MRI grade. 3. Lower Histological grades 4. Decreased apoptosis rate	None	Cell and Animal experimentation

	and attenuated mitochondrial dysfunction.

Comment 3: A drawing summarizing the potential mechanisms used by exosomes to improve IVD would be greatly appreciated by the readers.

Response: We summarized a picture for readers to understand the mechanisms of stem cell-derived exosomes regulating the activity of NPC.



Exosome-mediated mechanism of stem cells regulating nucleus pulposus cells

Comment 4: The first page including title and author information seems missing.

Response: The missing information has been added to the revised manuscript.

Comment 5: In addition, the document could benefit from some editing as advised below: Table 2 should be cited in the body of the text.

Response:Table2 has been cited in revised manuscript.

Comment6: The following statement: "..lipids, proteins, and nucleic acids are the three main substances that determine the specificity of exosomes" might not be correct if considering specificity comes from the surface and glycans reside on the outer leaflet of the vesicles.

Response: We have made a mistake for the sentence that pointed out by the reviewer #2 ,the revised one is "Among the components of exosomes, lipids, proteins, and nucleic acids are the three main substances that determine the biological function of exosomes"

Comment7: Please rephrase the sentence: "…because of the complex and harsh in vivo environment of the IVD, there have been different results in preclinical studies" . As no direct evidence to support the claim is provided.

Response: The revised sentence was" However, because of the complex and harsh in vivo environment of the IVD, there have been some obstacles to overcome for stem cells therapy for IVD degeneration".

Comment 8 Please try to avoid terms that can be confused with statistical calculations as in the following: "Among them, the role of exosomes is particularly significant..". How is the gradual attraction referred "..and has gradually attracted the interest of a growing number of researchers." documented?

Response: We have deleted this sentence which may cause unclear expression.

Comment 9: Cites should be provided at the end of the statements and not when the group is mentioned. As for example, cite 29, should appear after "..the noninjection

groups" on linen 212 and not on line 205, after "Cheng et al". Please review the whole document to consistently adhere to this rule.

Response: We have put the cited documents at the end of the sentence according to the opinions of the reviewers. Because of too many cites has changed, please refer to the revised manuscript.

Comment 10: Please clarify what the authors refer as "effective cells" (line 236).

Response: The "effective cells" expression here is not accurate, we have replaced it with "seed cell".

Comment 11: Line 18 and line 83, the word "will" in the sentence "This paper will mainly review .."should be removed as the review has been completed Line 30, "drop" by reduction Line 85, please review the sentence: will give a cautious outlook on their future applications in this field.

Response: We have replaced the wrong wording according to the reviewer's suggestion.

Comment 12: Lines 88 and 89: please review the sentence "..the other two types of vesicles" as there may be more than 3 types of vesicles. It would be more appropriate to mention that the other two main types of extracellular vesicles described are…to contemplate that classifications are subjectively imposed by selective criteria.

Response: We have revised the sentence as "Exosomes are a type of extracellular vesicle; the other two main types of extracellular vesicles are microvesicles and apoptotic bodies" according to the reviewer's suggestion.

Comment 13: Line 114 and 115: please avoid term repetition in: “The function of exosomes mainly depends on the contents they contain” and Line 119: “Lipids in exosomes are mainly located in the membrane of exosomes… and other types of lipids” and other sentences.

Response: We have revised the sentence as “The function of exosomes mainly depends on their contents.” and “Lipids in exosomes are mainly located in the membrane, include cholesterol, phosphatidylserine, sphingomyelin, etc.”.

Comment 14: Please review the whole manuscript. Line 135: replace long by longer in the sentence: “Mature miRNAs are produced from long primary transcripts” Line 140: replace degrees by degree in the sentence: “..based on the degrees of complementarity[65]”

Response: We have revised the sentence as “Mature miRNAs are produced from longer primary transcripts that undergo a series of nuclease-mediated cleavages;” and “based on the degree of complementarity”.

Comment 15: Line 198: review the meaning of reversed in the following sentence: “while the degradation-related genes MMP-1 and MMP-3 were reversed”

Response: We have revised the sentence as” Additionally, the expression of the extracellular matrix synthesis and protection genes ACAN, COL2A1, SOX-9, and TIMP-1 increased with incubation time, while the degradation-related genes MMP-1 and MMP-3 were decreased.”

Comment 16: Line 200: “Therefore, MSC-derived exosomes have the ability..” perhaps should be rephrased with more appropriate terms like “seems to indicate” “it suggests” rather than using a rotund affirmation with a questionable level of evidence.

Response: We have revised the sentence as" Therefore, it seems to indicate MSC-derived exosomes promote the proliferation and extracellular matrix homeostasis of NPCs".

Comment 17: Line 204: please add the term "their" in the sentence: "proliferation of NPCs but also inhibit apoptosis"

Response: We have revised the sentence as " Stem cell-derived exosomes not only promote the proliferation of NPCs but also inhibit their apoptosis.".

Round 2

Dear Editors and Reviewers: Re: Manuscript reference No. 54910 Please find attached a re-revised version of our manuscript "Exosomes Derived from Stem Cells as an Emerging strategy for Intervertebral Disc Degeneration", which we would like to resubmit for publication as a basic science in world journal of stem cells. Your comments and those of the reviewers were highly insightful and enabled us to greatly improve the quality of our manuscript. In the following pages are our point-by-point responses to each of the comments of the reviewers as well as your own comments. Based on the instructions provided in your letter, we uploaded the file of the revised manuscript. In accordance with re-reviewer's suggestions, we revised the manuscript. We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in world journal of stem cells. We shall look forward to hearing from you at your earliest convenience. Yours sincerely, Changqing Li, MD, PhD Address: Department of Orthopedics, Xinqiao Hospital, Army Military Medical University, Xinqiao Main Street, Shapingba District Chongqing, Chongqing 400037, China. E-mail: changqli@163.com

Responses to the comments of 2nd round Reviewer Comment1: The answers to the points raised in the previous review seem satisfactory, with the exception of Comment 7. The sentence provided should be replaced by the following: " However, because of the complex and harsh in vivo environment of the IVD, there are obstacles to be overcome by IVD degeneration stem cell therapy approaches".

Response: we have revised the original sentence as" However, because of the complex and harsh in vivo environment of the IVD, there are obstacles to be overcome by IVD degeneration stem cell therapy approaches" in accordance with re-reviewer's suggestions. Comment2: The manuscript still needs English native review. For example in the last sentence of the abstract both verbs appear in plural, and they should be third person of the singular. Namely, "reviews" and "highlights" instead of

"review" and "highlight" for the subject. Response: we have revised the last sentence of the abstract as" This paper mainly reviews the biological characteristics of exosomes and highlights the current status of exosomes in the field of IVD degeneration, as well as future developments regarding exosomes." And this paper has been edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at AJE.

Responses to the comments of Editor Comment 1: You need to provide the grant application form(s) or certificate of funding agency for every grant, or we will delete the part of "Supported by the National Natural Science Foundation of China, No. 81572208".

Response: We would like to provide Approval of NSFC funded projects in the compressed file。

Comment 2: Repeated references: 29 and 75. Please delete the reference 75 and rearrange the references in numeral order.

Response: We have deleted the reference 75.

Comment 3: The requirements for the figures and figure legends include: (A) All submitted figures, including the text contained within the figures, must be editable. Please provide the text in your figure(s) in text boxes; (B) For line drawings that were automatically generated with software, please provide the labels/values of the ordinate and abscissa in text boxes; (C) Please prepare and arrange the figures using PowerPoint to ensure that all graphs or text portions can be reprocessed by the editor; and (D) In consideration of color-blind readers, please avoid using red and green for contrast in vector graphics or images.

Response: We have rearranged the fig1 to be editable.