Dear Editor:

Thank you for your insightful comments on our manuscript titled: “Characterization and sequencing analysis of inflammatory factor-induced changes to mesenchymal stem cell exosomes and exosomal microRNA” (Manuscript Number: 46087).

Based on the reviewers’ comments, we have made modifications to our manuscript and have also supplemented extra test, figures and data（**highlight main changes in yellow in the revised manuscript**）. Point-by-point responses to the comments are listed in this response letter.

We hope that given our revisions and accompanying responses, you will find our manuscript suitable for publication in **World Journal of Stem Cells**.

Sincerely,

Chen Huang

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Note: In the annotated version of the revised manuscript,
R1: indicates a response to suggestions of Reviewer #1
R2: indicates a response to suggestions of Reviewer #2
R3: indicates a response to suggestions of Reviewer #3
R4: indicates a response to suggestions of Reviewer #4
R5: indicates a response to suggestions of Reviewer #5

Reviewer #1(Reviewer’s code: 03551035): The manuscript by Huang et al. discusses the effects of inflammatory cytokines on the morphology and quantity of mesenchymal stem cells exosomes, but more importantly, the differential expression of microRNAs in the exosomes. This subject worth deep studying, considering its wide clinical application. The article is well written in general, although I have some minor suggestions to be included in the manuscript. 1. A careful editing is needed to correct minor spelling mistakes, for example on page 3 "diseasesl" should be read as

"diseases". Frequent repetitions should be also corrected (such as "impact"/ "impacted" on page 3-4). 2. The numbers showing the cell purity (85% to 95%) (page 4) should be displayed under chapter "Results" instead of chapter "Materials & Methods". 3. Chapter "Results" (page 7): the concentration of MSCs-exo in the IL-6 group should be also presented (data showed only for the other 3 groups). 4. Data (including figures) should be presented as "Experimental vs. Control" instead of "Control vs. Experimental" (e.g., "VCAM-1 vs. Control" instead of "Control vs. VCAM-1" and so on….) (Figure 6, 8, 9…). 5. I think that the impact would increase if the authors could show comparatively the number of relative angiogenesis gene distributions in the VCAM-1/ TNFα/IL-6 group into one graph (bars with different color for each group) (Figure 10 a, c, e). 6. I suggest the authors to comment on the similarities and differences with other similar studies in the literature (for instance, Domenis, Rossana, et al. "Pro inflammatory stimuli enhance the immunosuppressive functions of adipose mesenchymal stem cells-derived exosomes." Scientific reports 8 (2018).

Response: Thank you for your comment. We have corrected some Spelling and form mistakes, as shown below.

⑴ Original page 3 "diseasesl" has been corrected as "diseases". Frequent repetitions have been also corrected（R1.1，page 5）

⑵ The cell purity (85% to 95%) (original page 4) have been displayed under chapter "Results" instead of chapter "Materials & Methods".（R1.2，page 6,9）

⑶ Chapter "Results" (original page 7): the concentration of MSCs-exo (3.01×10^8/ml) in the IL-6 group has been presented. （R1.3，page 10）

⑷Data (including figures) have been presented as "Experimental vs. Control" instead of "Control vs. Experimental" (e.g., "VCAM-1 vs. Control" instead of "Control vs. VCAM-1" and so on….) (Figure 6, 8, 9…).（R1.4，page 24,25,26,31,37）

⑸ The number of relative angiogenesis gene distributions in the VCAM-1/ TNFα/IL-6 group have been shown in one graph.（R1.5，page 41）

⑹Although "Pro inflammatory stimuli enhance the immunosuppressive functions of adipose mesenchymal stem cells-derived exosomes." also showed the Pro

inflammatory stimuli impacted functions of mesenchymal stem cells-derived exosomes, our present study found that the inflammatory cytokines VCAM-1, TNFα and IL6 impacted the size and morphology of MSCs-exo and the diversity of miRNAs they can produce, **especially their miRNAs impacting angiogenesis**.（R1.6，page 12,41）

Reviewer #2 (Reviewer's code: 02566952): An interesting in vitro study investigating on MSC released exosomes under inflamatory conditions, with special focus on their miRNA content. Introduction Actually MSC exosomes are NOT used clinically in any application as of yet (January 2019) Reference cited in regard to cardiovascular diseases is a review referring to animal models of disease which stresses in itself THE NEED for clinical studies. Material and methods It is good to mention the method of "purifying" cord blood MSCs used here is one of negative magnetic separation (reader should not be obliged to know what Isolex is but the principle of the method needs to be disclosed by the authors) What was the length of MSC exposure to adhesion molecule and inflammatory cytokines? Minor typo in the bioinfo description " functional heatmap" is maybe the correct form Results and discussion Accurate introduction of results and their discussion. I miss, however, an (at least) in vitro validation of bioinfo results (say an in vitro angiogenesis test, perhaps endothelial cells exposed to "normal" MSC-exo compared to those obtained under "inflammatory" like stimulation) The length of "inflammatory" stimulation is important as well (different exposure times should be used maybe in a further study) as this might explain the paradoxical findings reported about pro or anti angiogenetic role of MSC exo The role of intracellular signaling PI3K/AKT is pleiomorphic and not only related to angiogenesis, its major role in cell cycle and cell quiescence could be discussed (and eventually in vitro tested as well)

Response: Thank you for your suggestions.

(1) I am sorry to delete some sentences. A confidentiality agreement had been

signed about our principle of the method. （R2.1，page 5,6）

⑵ The length of MSC exposure to adhesion molecule and inflammatory cytokines was 48 hours. （R2.2，page 6）

⑶According to a Gene Ontology enrichment analysis, miRNAs of exosomes exposed to inflammatory cytokines, compared to controls, had a different regulatory effect on cellular proliferation and differentiation, molecular signal transduction, immunosuppressive functions, angiogenesis and so on. （R2.3，page 12,13）

⑷ **An in vitro angiogenesis test and western blotting test have been added to the paper.**

Our study demonstrated that MSCs-exo maybe induce HUVECs to form capillary-like structures in vitro. The effect of MSCs-exo promoting angiogenesis would be reduced when the stem cells are cultured with TNFα and IL6 stimulation. Besides endothelial cell angiogenesis-related molecular expression, functional characteristics such as PI3K-AKT signaling pathway may be down-regulated with MSCs-exo which are stimulated by IL6. （R2.4，page 8,9,11,13）

Reviewer #3 (Reviewer's code: 02728252): It is an interesting study that characterizes inflammatory factor-induced changes to mesenchymal stem cell exosomes and exosomal microRNA via sequencing analysis. There are major concerns about the manuscript 1. It should be formatted according to the style of the journal. 2. Great language polishing is recommended and I suggest sending the manuscript to a company editing service. 3. Figures are too much for a paper and should be condensed in panels. 4. Clear hypothesis should be added.

Response: Thank you for your suggestions.

⑴ According to the style of the journal, the form has been corrected.

⑵ The manuscript has been edited for English language, grammar, punctuation, and spelling by iCoreMed Technology and Service LLC, an medical and scientific writing company.

⑶ Figures have been condensed.

(4) Clear hypothesis has been added. （R3.4，page 4）

Reviewer #4 (Reviewer's code: 00504335): The authors intended to study effects of three differnt proinflammatory cytokines (VCAM-1, TNF-α and IL-6) on exosome production and miRNA syshesis in human umbilical MSCs. However, VCAM-1 is not proinflammatory cytokine It is a cell membrane molecule CD106 (Cluster of Differentiation 106). The purified VCAM-1 is sold by R and D Systems, but not as a cytokine. VCAM-1 plays a role as adhesive molecule in some interactions among cells or in immunoregulation, but i tis not secreted cytokine. It is ot possible to compare VCAM-1 with cytokines. Results are desctibed on one and half page, but demonstrated in 13 figures and 4 tables. No figure legends are provided. The authors should ask some experienced scientist to help them with preparation of the manuscript. At the present form, the paper is not suitable for the international journal.

Response: Thank you for your suggestions.

"…pro-inflammatory molecules ICAM-1 (3 – 6 times) and VCAM-1 (5 – 7 times)."(Subhadeep Chakrabarti, Sandra T. Davidge. Estradiol Modulates Tumor Necrosis Factor-Induced Endothelial Inflammation: Role of Tumor Necrosis Factor Receptor 2. J Vasc Res 2013;50:21-34)

According to the paper above, we thought Vcam-1 could be a generalized inflammatory cytokine.

The figure legends have been provided.

Reviewer #5 (Reviewer's code:03370303): In this study, authors describe the differential profiles of MSC exosomes when MSCs were treated by VCAM-1, TNF- and IL-6 to understand how the characters of MSC exosomes would change under inflammatory situations such as cancer progression. Although the results shown in this manuscript are rather descriptive than determining/identifying/proving specific findings, it is worth reporting since it contains a large mass of valuable information

with detailed analyses. However, there are several concerns in the manuscript. Before publication in World Journal of Stem Cells, they should be properly addressed. Major concerns: 1) Raw data for the integrated miRNA expression should be registered in public databases and their accession numbers should be written in the main text so that readers can share them. 2) In the second section of “Results” in page 7, the concentration of MSCs-exo for the IL-6 group was missing. Please describe the finding. Minor concerns: 1) There are several unusual (or even peculiar) usages of words, such as “precipitate” in page 2 and page 3, although it is properly used in page 5. The word “precipitate” in page 2 and page 3 should be replaced by a common word, for example, “up-regulate”, “augment”, “enhance”, “boost” and so on. 2) There is inconsistency in the usage of the abbreviation including “MSC-exo”, “TNF- ” and IL6. After showing the abbreviated form in its first use, the abbreviated form should be used throughout the manuscript. 3) In page 3, the word “diseasesl” should be corrected as “diseases”. 4）In page 7, the sentence “Correlation analyses showed that miRNA in the IL-6 group（0.583）and TNFα group（0.697）were different than the control group.” is peculiar. It should be replaced by, for example, “Correlation analyses showed that miRNA expression profile in IL-6 group（0.583）and that in TNFα group（0.697）were more different from that of the control group than that in VCAM1 group (0.985).” 5) In page 7, the sentence “Hierarchical Clustering indicated that the IL-6 group compared with the control group downregulated most kinds of expressed genes (Figure 7).” is peculiar. It should be replaced by, for example, “Hierarchical Clustering indicated that the expression levels of the majority of miRNAs in IL-6 group were downregulated compared with the control group (Figure 7).” 6) In page 8, line 4, the word “between” should be corrected as “among”. The word “between” can only be used when comparing the two things.

Response: Thank you for your suggestions.

Major concerns:(1) MiRNA accession numbers have been written in the main text.

(2) The concentration of MSCs-exo (3.01×10^{8}/ml) in the IL-6 group has been

presented. （R5-2，page 10）

Minor concerns:⑴“precipitate” in original page 2 has been replaced by influence. （R5.2，page 3）

“precipitate” in original page 3 has been replaced by enhance. （R5.2，page 5）

⑵ The inconsistency in the usage of the abbreviation has been corrected.

⑶Original page 3 "diseasesl" has been corrected as "diseases". （R5.3，page 5）

⑷In original page 7, the sentence “Correlation analyses showed that miRNA in the IL-6 group（0.583）and TNFα group（0.697）were different than the control group.” has been replaced by “Correlation analyses showed that miRNA expression profile in IL-6 group（0.583）and that in TNFα group（0.697）were more different from that of the control group than that in VCAM1 group (0.985).” （R5.4，page 10）

⑸ In original page 7, the sentence “Hierarchical Clustering indicated that the IL-6 group compared with the control group downregulated most kinds of expressed genes (Figure 7).” has been replaced by “Hierarchical Clustering indicated that the expression levels of the majority of miRNAs in IL-6 group were downregulated compared with the control group (Figure 7).” （R5.5，page 10）

⑹ In original page 8, original line 4, the word “between” has been corrected as “among”. （R5.6，page 10）