**Editorial comments:**

Changes to be made by the author﴾s﴿ regarding the manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Answer: We did proofread.

1. Please provide an email address for each author.

Answer: E-mail: Xuan Su, xsu@augusta.edu; Yan Shen, yashen@augusta.edu; Yue Jin, yujin@augusta.edu; Meng Jiang, jiangmeng0919@163.com; Yaoliang Tang, yaotang@augusta.edu.

1. Keywords: Please provide at least 6 keywords or phrases.

Answer: Keywords: Exosome, Duchenne muscular dystrophy, Myogenic progenitor cell, sequential ultracentrifugation, Exosome Transplantation, echocardiography, heart function

1. Please add a Summary section before the Abstract to clearly describe the protocol and its applications in complete sentences between 10‐50 words: “Here, we present a protocol to ...”

Answer: We add SUMMARY: Here, we present a protocol to transiently improve cardiac function in Duchenne muscular dystrophy mice by transplanting exosomes derived from normal myogenic progenitor cells.

1. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols ﴾TM﴿, registered symbols ﴾®﴿, and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. You may use the generic term followed by “﴾see table of materials﴿” to draw the readers’ attention to specific commercial names. Examples of commercial sounding language in your manuscript are: Patterson Veterinary, Triton X‐100, VECTASHIELD HardSet, etc.

Answer: We have removed these commercial languages.

1. Please revise the protocol text to avoid the use of any personal pronouns ﴾e.g., "we", "you", "our" etc.﴿.

Answer: We removed the personal pronous.

1. Please revise the protocol to contain only action items that direct the reader to do something ﴾e.g., “Do this,” “Ensure that,” etc.﴿. The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

Answer: We move the discussion about the protocol to the Discussion.

8. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below.

9. 1.4: Please describe how to collect the supernatant and where it is collected from.

Answer: Use a transfer pipette to collect the supernatant from the cell culture dish.

10. 1.8, 1.10: What volume of PBS is used?

Answer: 1.8 Fill up the entire ultra-clear tube.

1.10 100 μL.

11. 1.9: What is centrifuged? The suspension from step 1.8?

Answer: Yes, the suspension from step 1.8.

12. 2.1: Please mention how proper anesthetization is confirmed.

Answer: confirm the depth of anesthesia by toe pinch

13. 2.6: Is PBS served as control? Please specify.

Answer: PBS was used as a control.

14. 2.7: What is used to close the thoracic cavity, pectoralis muscles and skin?

Answer: 6-0 suture.

15. 3.1: Please specify the concentration of isoflurane.

Answer: We set isoflurane at 1-2% to control heart rate at above 400rpm.

16. 4.1: Please describe how to harvest mouse hearts and specify the temperature at which the heart was fixed.

Answer: We add following detail: anesthetize mice (see 2.1) and incise the skin from pelvis to chin, cut the sternum to open the chest. dissect the inferior vena cava, and perfuse the heart with 3ml PBS followed by 3ml 4% paraformaldehyde (room temperature), using a butterfly catheter with 25g needle attached to a 5 ml syringe with the tip positioned at apex of left ventricle.

17. 4.3: Please specify the concentrations of ethanol solutions used and the time for each treatment.

Answer: we add detail below: 100% xylene (3 min); 100% xylene (3 min); 100% ethanol (1 min); 100% ethanol (1 min); 95% ethanol (1 min); 80% ethanol (1 min); H2O (1 min).

18. Please include single‐line spaces between all paragraphs, headings, steps, etc.

Answer: We change to single-line spaces.

19. After you have made all the recommended changes to your protocol ﴾listed above﴿, please highlight 2.75 pages or less of the Protocol ﴾including headings and spacing﴿ that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

Answer: We highlight 1.5 pages of protocol for the video.

20. Please highlight complete sentences ﴾not parts of sentences﴿. Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Please do not highlight any steps describing anesthetization and euthanasia.

Answer: We highlight the steps.

21. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub‐steps where the details are provided must be highlighted.

Answer: We include all relevant details.

22. Discussion: As we are a methods journal, please also discuss critical steps within the protocol, any modifications and troubleshooting of the technique, and any limitations of the technique.

Answer: The discussion of critical steps within the protocol are there.

23. References: Please do not abbreviate journal titles.

Answer: we change the styles of journal titles.

24. Please upload each Figure individually to your Editorial Manager account as a .png, .tiff, .pdf, .svg, .eps, .psd, or .ai file.

Answer: We will upload each figure individually.

25. Please include a title for each figure.

Answer: We add title for each figure.

26. Table of Materials: Please remove trademark ﴾TM﴿ and registered ﴾®﴿ symbols and sort the items in alphabetical order according to the name of material/equipment.

Answer: We remove the trademark and registered symbol, and sorted the items.

Reviewers' comments:

**Reviewer #1:**

This is a well‐written paper reporting.

Answer: We thank the reviewer for his/her positive comments.

**Reviewer #2:**

Manuscript Summary:

The paper describes a technique for isolating exosomes from myogenic progenitor cells and their intramyocardial injection in a mouse model of Duchenne muscular dystrophy. Results were assessed two days after exosome injections and show expression of dystrophin gene expression and improved cardiac function, as assessed by echocardiography.

Major Concerns:

1‐ The technique used for exosome purification is based on a series of untracentrifugations. As such, it does not provide any novel information in that this technique is routinely used for this purpose. Thus, claiming that the paper reports "an improved method for exosome purification" is not really true. Furthermore, in the discussion, the assumption that ultracentrifugation might be the most suitable method is questionable and biased by the inappropriate critiques of the other methods; thus, ultrafiltration is reported to possibly cause structural damage to "large

vesicles", which may not be an issue if the target is to isolate exosomes and no mention is made of tangential filtration which is increasingly recommended.

**Answer:** Thanks to the reviewer’s comments, we do not criticize other methods of purifying exosomes. We also routinely use PEG based protocol to purify exosomes and microvesicles. According to our experience, the advantage of sequential ultracentrifugation is the production of exosome with high purity and cost-efficiency, however, it has disadvantage of the low yield.

2‐ The characterization of exosomes if not fully convincing. Although Figure 1B shows a round‐cup morphology of the isolated particles, the data would have been more meaningful if electron microscopy had been combined with immunostaining against typical markers like CD63 or CD81. Representative Nanoparticle Tracking Analysis spectra should also have been provided to give information on the number and size distribution of the isolated particles.

**Answer**: Thanks for the reviewer’s comments. The detailed characterization of exosome, including nanoparticle tracking analysis, was published (doi: 10.1007/s12265-018-9826-9), we added Western blot data, which confirmed the presence of exosome markers, including CD63 and TSG101 (Fig. 1C).

3‐ In the animal study, no data are given regarding the number of mice in each group, the use or not of randomization and blind assessment of outcomes, the nature of the control medium which was presumably injected. No mention is made either on the limitations of the mdx model for duplicating the patterns seen in Duchenne myopathy.

**Answer**: Thanks for the reviewer’s comments, we had 6 mice in each group, see detail in our published paper (doi: 10.1007/s12265-018-9826-9). We agree with the review that there are limitations of using mdx mice to duplicating the symptoms in Duchenne myopathy. We used DBA/2J-DmdMDX mice, which is a superior Duchenne muscular dystrophy model as it better recapitulates several of the human characteristics of DMD pathology (Jackson laboratory).

4‐ While the rapid internalization of exosomes in the target cells might account for the expression of dystrophin shown in Figure 3A, the functional data are more than questionable in view of the shortness of follow‐up ﴾2 days﴿. Further doubts about the reality of the reported functional improvement come from the fact that neither posttransplantation nor baseline actual echocardiographic values are reported while it would be really surprising that function can improve so rapidly ﴾2 days after treatment﴿. Furthermore, the histological data are limited to an image of dystrophin expression without details about the number of hearts/slides in which this result was observed and without any information on other important histological patterns such as those in relation with inflammation and a possible immune response.

**Answer**: Thanks for reviewer’s comments, we randomly selected 10 fields in each heart section, and assigned the field with dystrophin- positive cardiomyocytes as positive field, and compared the percentage of positive field in each heart section between PBS- and MPC-Exo-treated hearts of MDX mice (n = 18, \*p < 0.05). See our published paper (doi: 10.1007/s12265-018-9826-9). Since we observed recovered dystrophin protein expression in Mdx mouse hearts, we assume this is the mechanisms for improved heart function, however, we cannot exclude other mechanisms, such as anti-inflammation, a recent report demonstrated that mesenchymal Stem Cell-Derived Exosomes Improve the Microenvironment of Infarcted Myocardium Contributing to Angiogenesis and Anti-Inflammation (doi: 10.1159/000438594), moreover, Aminzadeh MA et al recently reported that cardiosphere-derived cells (CDCs) and their exosomes could transiently restore the expression of full length dystrophin in DMD mice (doi: 10.1016/j.stemcr.2018.01.023).

**Reviewer #3:**

Manuscript Summary:

Overall, this manuscript is well written and it will be an useful method for the scientific community to purify the exosomes in different origins. This manuscript can be accepted in the present form.

**Answer:** We thank reviewer for this positive comment.

**Reviewer #4:**

Manuscript Summary:

This article describes the technique of MPC‐Exo purification and transplantation into hearts of Dmdmdx mutant mice.

Major Concerns: None noted.

Minor Concerns:

In the heart image, cam Can the authors show the MPC exosomes are positive for CD9, CD63 and CD812?

**Answer:** Thanks for reviewer’s comments, we did co-stain dystrophin with Tsg101, an exosome marker, in the Mdx hearts treated with MPC-Exo and PBS, however, we did not observe obvious difference of Tsg101 expression in cardiomyocytes with restored dystrophin expression in MPCExo-treated hearts in comparison with cardiomyocytes without dystrophin expression in PBS-treated hearts, see our publication (doi: 10.1007/s12265-018-9826-9).

Is there any quantitative data regarding the cardiac function results?

**Answer:** Thanks for reviewer’s comments, the quantification of cardiac function was shown in our publication (doi: 10.1007/s12265-018-9826-9), Compared with PBS, MPC-Exo administration improved both left ventricular ejection fraction (EF 74.3 ± 2.5% vs 58.2 ± 3.4%, p < 0.05, n = 6) and fractional shortening (FS 42.5 ± 2.4% vs 30.0 ± 2.3%, p < 0.05, n = 6) in MDX mice.

Can the authors comment on the yield of exosomes from the cell culture using the ultracentrifugation method?

**Answer:** According to our experience, compared to PEG purification, the disadvantage of a series of ultracentrifugation is the low yield of exosomes.

**Reviewer #5:**

Manuscript Summary:

In the manuscript titled, "Purification and transplantation of myogenic progenitor cell‐derived exosomes to improve cardiac function in Duchenne Muscular Dystrophic mice", Su X et al., describe a protocol for isolation, purification and transplantation of exosomes derived from myogenic cells in MDX mice. Overall, the protocol and corresponding results are well described. There are some concerns as highlighted that will improve the impact of the paper.

Major Concerns:

1. Authors must include methods and pertaining results to clarify whether the isolated exosome is indeed within the size range of 100‐150. Moreover, include methods for immunoblot analysis of exosome phenotype.

**Answer:** Thanks for reviewer’s comment, we measured the size of isolated exosomes using the Zeta analysis, see Figure 2 in our published paper (doi: 10.1007/s12265-018-9826-9).

2. Please include a picture of PBS only and PBS/exo hearts to clarify whether edema is due to exo/PBS or PBS only Minor Concerns:

**Answer:** Thanks for reviewer’s comments, we added a picture of mouse heart before/after PBS injection (Figure 2A). And no significant difference was observed between PBS and Exo injections, indicating that the edema is a way of judging whether the myocardial injection was successful, regardless of the injected materials.

Section 2. Intramyocardial exosome delivery, sub section 2.6 mentions 50ug in 30ugPBS. Can the authors clarify what they mean? Also, please mention whether the exo/PBS were injected in a single injection site or multiple injections.

**Answer:** Thanks for comments, it is a typo, it has been corrected to “50ug in 30uL PBS”. The exo/PBS were injected in a single injection site.

**Reviewer #6:**

Manuscript Summary:

This article introduces the techniques for exosome isolation from myogenic progenitor cells, purification, and transplantation into hearts of Duchenne muscular dystrophic mice. The title and abstract are appropriate for this methods articles. The steps listed in the procedure are clearly explained and would lead to the described outcome.

Minor Concerns:

a. Please check the formula for calculating LVEF.

**Answer**: We have checked the calculating LVEF.

b. Several typos:

83 1.11﴿ '3μL of exosomal pellet' should be '3μl of exosomal suspension';

**Answer**: Thanks for pointing it out, we made correction.

100 2.6﴿ '50μg in 30μg PBS' should be '50μg in 30μl PBS'.

**Answer**: Thanks for point it out, we made correction of this typo.

c. It would be better if the model and manufacture of Ultracentrifuge and Ultrasound System can be listed.

**Answer**: Thanks for this comment, we add this information to the list.