**List of Changes for Jove Submission JoVE52418—Post-Peer Review**

“Rat Heterotopic Abdominal Heart/Single-Lung Transplantation in a Volume-Loaded Configuration”

The following text modifications were made in track-changes mode.

EDITORIAL COMMENTS

1. 3.4 and 3.22 changed. 3.29 left unchanged, as the instruction depends on the investigator’s endpoint.
2. Done.
3. They are original figures, not previously published.

REVIEWER COMMENTS

I would like to sincerely thank each of the reviewers for their feedback and thoughtful critique of our methods manuscript. I hope that you find the following responses satisfactory.

**Reviewer #1**

*Major Concerns*

1. N/A

*Minor Concerns*

1. That’s a great suggestion. At this point we haven’t confirmed antegrade aortic flow by angiography or echocardiography. We would like the focus of this manuscript to be on the methodology itself, while grouping confirmatory findings of antegrade flow (along with other endpoints) into a future ‘results’ oriented manuscript.
2. Because we are not pursuing recovery surgery at this point in time (certainly this will be a future aim), we find it quicker and more practical to perform tracheotomy. The tracheal cannula is also less likely to fall out compared with rodent orotracheal cannulae.
3. Thank you! We have corrected the 2nd typo, and kept the correct spelling of ‘loupes’.
4. I do use 8-0 prolene for both venous and arterial anastomoses. It is more common to use 10-0 for micro-vascular anastomoses (even at our animal facility), but my feeling is that 8-0 does a great job for both. I’ve experimented with 7-0, which is certainly bulkier and less flexible, and would not recommend that. We use a BV 130-5 needle with the 8-0 prolene.
5. At this point in time we haven’t conducted chronic experiments, so I can’t report any experience with thromboembolic complications in that setting. I can report that we have had a thrombotic complication at the site of IVC anastomosis acutely, likely related to under-dosing of heparin. The paper you cited contained a very nice description of the authors’ learning curve and results with a cohort of 90 rats. In terms of learning curves, we are somewhere in the mid-point of our curve, and I don’t feel such an analysis would be appropriate for us with the current manuscript.
6. Good point. This is a recent, high-quality publication in a relevant area. I’ve added the recommended reference to our manuscript!

**Reviewer #2**

*Major Concerns*

1. Thank you for suggesting this. I agree with your points, and have expanded the introduction. I added a brief discussion about the volume-loaded configurations that have been described by other groups. Further, I highlight that our circulation is unique in 2 respects. First, its exact configuration is unique, and despite not being utilized in rodent transplant research, it is likely the most versatile circulation for transplant investigators. Second, our circulation lends itself to direct functional assessment *in vivo*, and we describe how this may be achieved.
2. This is a good point. Since we have not conducted experiments confirming that the transplanted lung tissue is capable of providing “full” systemic oxygenation, we have reworded the sentence in a more general way.
3. I agree, and will work with the JOVE editors to provide updated *in vivo* figures prior to publication.
4. We currently have no chronic time-points. The methodology reported in this manuscript was developed to serve our broader research interests, which in the short-term only involve acute time-points. In the future, it is our interest to fully explore the benefits that this transplant model has to offer, and report our results in a separate manuscript. For now, however, it would be remiss if we didn’t report on our model’s broad range of investigative applications.

*Minor Concerns*

1. Thank you! Corrected.
2. Modified to indicate that a cannula, of at least 16 Gauge size, should be used.
3. I have specified that I use 3.5x loupes, and an operating microscope with 3.4-21.3x magnification.
4. Changed to indicate that we use the right hindlimb for monitoring purposes.
5. Corrected.
6. Modified to reflect our use of a 14 Gauge tracheal cannula.
7. Modified to indicate that it usually requires 10-15cc of NS over 10-20 seconds. The flush is continued until the aortic run-off appears dilute and the heart arrests.
8. Clarified that we use the 16 Gauge cannula attached to the 10mL cardioplegia syringe.
9. Clarified the volume, rate, and frequency of cardioplegia administration.
10. Revised.
11. Modified to indicate that we retract the bowels superiorly and to the left.
12. Modified to indicate that we use either bulldog or Yasargil clamps.
13. Modified sections 3.10 and 3.20 to indicate that we make a longitudinal incision on the anterior aspect of both IVC and abdominal aorta.
14. Thank you for picking this up! We intend to adhere to “heel and toe” terminology. The relevant manuscript sections were modified accordingly.
15. Modified to indicate that the donor IVC clamp should be left in place.
16. Modified to indicate that we are referring to the ascending aorta. I have also specified that the aorta should be free of peri-aortic fat, and adequately separated from the pulmonary artery in order to prepare it for anastomosis.
17. Modified, as indicated above.
18. Modified.

**Reviewer #3**

*Major Concerns*

1. I agree with you. If the JOVE editors have access to an illustrator/art department, I’d be happy to prepare a rough sketch, and guide the illustration of an appropriate figure.

*Minor Concerns*

1. N/A