Journal of Visualized Experiments

Optimization of the cuff technique for murine heart transplantation

--Manuscript Draft--

Article Type:	Invited Methods Article - Author Produced Video
Manuscript Number:	JoVE61103R2
Full Title:	Optimization of the cuff technique for murine heart transplantation
Section/Category:	JoVE Immunology and Infection
Keywords:	Cervical heterotopic heart transplantation. Cuff technique. Inner tube technique. Operation time. Ischemia time. Cooperate
Corresponding Author:	Guoliang Yan Organ Transplantation Institute, Schoole of Medicine, Xiamen University, Xiamen, Fujian, China XiaMen, Fujian CHINA
Corresponding Author's Institution:	Organ Transplantation Institute, Schoole of Medicine, Xiamen University, Xiamen, Fujian, China
Corresponding Author E-Mail:	zhuanyiyan@126.com
Order of Authors:	Guoliang Yan
	Yunhan Ma
	Baiyi Xie
	Helong Dai
	Tianshu Lan
	Shujiao Liu
	Chenxi Wang
	Zhongquan Qi
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$1200)

TITLE:

Optimization of the Cuff Technique for Murine Heart Transplantation

AUTHORS AND AFFILIATIONS:

Yunhan Ma[*1,2], Baiyi Xie[*1,2], Helong Dai[*3,4,5], Chenxi Wang[6], Shujiao Liu[6], Tianshu Lan[7], Shuangyue Xu[1,2], Guoliang Yan[#1,2,6] and Zhongquan Qi[#1,2,8]

[1] Organ Transplantation Institute, School of Medicine, Xiamen University, Xiamen, Fujian, China
[2] Fujian Key Laboratory of Organ and Tissue Regeneration, Xiamen, Fujian, China
[3] Department of Kidney Transplantation, Center of Organ Transplantation, The Second Xiangya Hospital of Central South University, Changsha, Hunan, China
[4] Clinical Research Center for Organ Transplantation in Hunan Province, Changsha, Hunan, China
[5] Clinical Immunology Center, Central South University, Changsha, Hunan, China
[6] School of Medicine, Xiamen University, Xiamen, Fujian, China
[7] Xiamen Medical College, Xiamen, Fujian, China
[8] Medical College, Guangxi University, Nanning, Guangxi, China

[*]These authors contributed equally

[#]Corresponding Authors:
Guoliang Yan at zhuanyiyan@126.com
Zhongquan Qi at zqqi@xmu.edu.cn

Email Addresses of Co-authors:
Yunhan Ma (mayh186@163.com)
Baiyi Xie (milord1108@hotmail.com)
Helong Dai (helong68888@csu.edu.com)

Keywords:

Cervical heterotopic heart transplantation, Cuff technique, Inner tube technique, Operation time, Ischemia time, Cooperate

Summary:

We introduce an inner tube approach to the cuff technique for mouse cervical heterotopic heart transplantation to help evert the vessel over the cuff. We found that cooperation between two experienced surgeons markedly shortens the operation time.

Abstract:

Murine cardiac transplantation has been performed for more than 40 years. With advancements in microsurgery, certain new techniques have been used to improve surgical efficiency. In our lab, we have optimized the cuff technique with two major steps. First, we used the inner tube technique to insert a temporary inner tube into the external jugular vein and carotid artery blood vessel to facilitate eversion of the vessel over the cuff. Second, we

performed complete heterotopic cardiac transplantation through the collaboration of two experienced surgeons. These modifications effectively reduced the operation time to 25 minutes, with a success rate of 95%. In this report, we describe these procedures in detail and provide a supplemental video. We believe that this report on the improved cuff technique will offer practical guidance for murine heterotopic heart transplantation and will enhance the utility of this mouse model for basic research.

Introduction:

The establishment of mouse heterotopic heart transplantation through end-to-end anastomosis within the abdomen in 1973 was a major milestone in basic transplant immunology research[1]. This model provided an important and valid tool for analyzing the mechanisms of ischemia reperfusion injury[2], immunological rejection, and tolerance[3,4]. However, the complex and time-consuming nature of the surgery as well as the potential for infections can result in severe perioperative abdominal adhesions and inflammatory reactions, resulting in a low efficiency for heterotopic heart transplantation model.

The cervical heterotopic heart transplantation technique was first described by Chen in 1991[5]. In this model, the recipient's external jugular vein is anastomosed to the pulmonary artery of the graft and the carotid artery is anastomosed to the ascending aorta. The main advantages of this method are the convenience of monitoring and the reducing of trauma to the recipient. In the same year, Matsuura described an improved technique, in which the end of the external jugular vein and carotid artery were everted over a Teflon cuff and fixed with a circumferential silk ligature[6]. Some researchers also fixed the cuff to the right pulmonary artery in the donor heart before inserting the cuff into the external jugular vein of the recipient[7]. Thus far, the cuff technique has been widely applied in various vascular pedicle transplant models, including those for lung[8], liver[9], and renal[10] transplantation.

To date, there are several difficulties associated with the cuff technique. For example, the carotid artery is difficult to evert over the cuff due to the additional elasticity, resulting in the tissue flipping backwards. Hence, additional practice and a microsurgical dilator may be required to complete this step. In addition, the cervical vessel preparation can take up to 25 minutes.

To resolve these issues, we introduce the inner tube technique, which is based on the cuff technique and includes fixing the cuff on the external jugular vein and carotid artery using an inner tube to helps with the eversion of the vessel wall. In addition, with simple training, the recipient preparation is reduced to 15.5 minutes. This technique reduces the complexity of the operation and does not require additional practice or the use of a vascular dilator. It can be applied in all transplantation immune research, especially for verifying third-party immune tolerance during which the recipient receives two cardiac allografts, one within the abdomen and the other in the neck[11]. We also recommend co-operation between two skilled surgeons to establish this model, with one surgeon preparing the recipient animal and the other harvesting and implanting the donor heart. Such collaboration can shorten the operation time to 25 minutes. Using this optimized procedure, we have established syngeneic, allogeneic[12-19] , and

xenogeneic mouse heart transplantation models[20].

The rationale for the development of the inner tube technique was to reduce the operation time for the establishment of a mouse heart transplant model with a high success rate. Optimization of the cervical heart transplantation model facilitates the acquisition of high success rates in a short period of surgery time compared to the traditional suture and cuff technique[21]. Further, the cooperation model can further reduce the warm ischemic time of the donor heart compared to surgeries performed with a single operator.

Protocol:

Animals (BALB/c, C57BL/6, male, 8-12 weeks) are housed in a specific pathogen-free facility at the Xiamen University Laboratory Animal Center. C57BL/6 is used as recipient and BALB/c is used as donor. All the procedures are performed according to the Institutional Animal Care and Use Committee (IACUC) guidelines.

NOTE: A set of microsurgical instruments, including a micro scissor, micro straight forceps, micro curved forceps and micro needle holders, are necessary for the operation (**Table and Materials, Figure 1B, C, D, E**). One pair of single-use bulldog clamps (**Figure 1F**) is needed. Two cuffs for the external jugular vein and carotid artery are prepared by cutting the customized polyamide tubes with a No. 10 scalpel under a microscope. The diameter of the vein and artery cuff is 0.9 mm and 0.55 mm, respectively. In addition, the diameter of the inner tube for the corresponding vein cuff is 0.6 mm, and these of the inner tube for the corresponding artery cuff is 0.25 mm (**Figure 1G**).

1. Recipient preparation

1.1. Anesthetize the recipient mouse with pentobarbital (60 mg/kg, i.p). Use atraumatic mechanical clippers to remove the hair at the right lateral cervical region.

1.2. Use a sterile cotton tip applicator to wipe the surgical area with iodine antiseptic followed by 70% ethanol.

1.3. Place the mouse in the supine position on the operation platform. Cover the mouse with sterile gauze.

1.4. Use an ophthalmic scissor to make a transverse incision from the lower one-third neck midline to the right shoulder-clavicle joint.

1.5. Isolate the right external jugular vein with micro curved forceps to expose enough length, cut off the branches via electrocoagulation, and ligate the vessel at the distal end using a 6-0 silk suture.

1.6. Clamp the external jugular vein proximally using a bulldog clamp and then transect the vein proximally to the ligature using a micro scissor.

1.7. Wash the vessel lumen with 100 U/mL 0-4 °C heparinized saline to remove any residual blood.

1.8. Pull the external jugular vein through the vein cuff using micro straight forceps; insert the vein inner tube into the lumen as a stent, and evert the vessel wall over the cuff with micro straight forceps (**Figure 2A**).

1.9. Fix the everted vessel endothelium at the proximal end of the cuff using a circumferential 8-0 silk suture (**Figure 2B**).

1.10. Use micro straight forceps to withdraw the vein inner tube from the vein vessel.

1.11. Perform blunt dissection with micro curved forceps to isolate the right carotid artery adjacent to the inner edge of the sternocleidomastoid.

1.12. Clamp the right carotid artery proximally using a bulldog clamp, ligate the carotid artery distally using a 6-0 silk suture, and use a micro scissor to transect the carotid artery proximally to the ligature.

1.13. Wash the carotid artery with 100 U/mL 0-4 °C heparinized saline to remove any residual blood.

1.14. Pass the carotid artery through the artery cuff and insert the artery inner tube into the artery vessel using micro straight forceps (**Figure 2C**).

1.15. Evert the vessel over the cuff using micro straight forceps; fix the everted vessel endothelium using a circumferential 8-0 silk suture (**Figure 2D**).

1.16. Withdraw the artery inner tube from the artery vessel with micro straight forceps.

NOTE: Preserve the submandibular gland of the recipient.

2. Donor preparation

2.1. Anesthetize the donor mouse with pentobarbital (60 mg/kg, i.p). Use atraumatic mechanical clippers to remove the hair at the abdominal region.

2.2. Place the mouse in the supine position on the operation platform. Cover the mouse with sterile gauze.

2.3. Use a sterile cotton tip applicator to wipe the surgical area with iodine antiseptic followed by 70% ethanol.

2.4. Make an abdominal midline incision with an ophthalmic scissor and expose the abdominal cavity.

2.5. Use micro curved forceps to expose the inferior vena cava, and then intravenously inject 200 μL of 100 U/mL 0-4 °C heparinized saline per 20 g of bodyweight through the inferior vena cava.

2.6. Perform thoracotomy with ophthalmic scissors, cut off the ribs through the bilateral midaxillary line incisions, flip the anterior chest wall outwards to expose the thoracic cavity.

2.7. Excise the thymus with micro curved forceps.

2.8. Expose the aorta, and then perfuse 200 μL of 100 U/mL 0-4 °C heparinized saline to the coronary artery through the aortic arch.

NOTE: Avoid perfusing any gas bubbles into the donor heart.

2.9. Use a micro scissor to transect the ascending aorta at the beginning of the aortic arch.

2.10. Transect the pulmonary artery at the beginning of the two main branches with a micro scissor.

2.11. Ligate the superior vena cava and inferior vena cava proximally using a 6-0 silk suture and use a micro scissor to transect vein distally to the ligature.

2.12. Ligate the pulmonary veins together, circumferentially, using a single 6-0 silk suture, and cut off the vein branches distally to the ligature using a micro scissor.

2.13. Remove the heart graft from the surrounding soft tissues; preserve it in 0-4 °C heparinized saline.

3. Heart implantation

3.1. Place the donor heart upside down into the right neck region of the recipient.

3.2. Input the pulmonary artery of the donor heart to a 6-0 silk loop with micro straight forceps.

3.3. Wrap the vessel lumen around the vein cuff, and then tighten the 6-0 silk suture loops around the cuff to band the vessel joint.

3.4. Perform anastomosis of the aorta of the graft and the artery cuff by following the steps described in step 3.2.

3.5. Release the clamped jugular vein followed by the clamped jugular artery. Keep the vessel

joint untwisted and ensure that the blood flow is unobstructed.

NOTE: The sinus rhythm returning to more than 200 times within 1 min is considered normal.

3.6. Moisten the donor heart using warm (37 °C) saline and inspect whether the graft is bleeding. Set the pulsing heart graft into the subcutaneous space, and then suture the incision.

4. Postoperative Care and Graft Assessment

4.1. Record the time to normal sinus rhythm and the preservation of normal sinus rhythm for at least 5 minutes after clamp release to monitor the post-operative graft function.

4.2. Place the recipient alone on a warm blanket until the recipient wakes up from anesthesia. Administer buprenorphine analgesia, 0.05 mg/kg, s.c, at the end of the surgery and every 12 hours for 72 hours post-surgery.

4.3. Record the weight and postoperative recovery status of the recipient daily. In case of >15% weight loss relative to that on the surgery date, hemiplegic paralysis, or infection, euthanize the recipient via terminal isoflurane inhalation[21].

4.4. Monitor graft survival by palpation daily. The surgery is considered to be successful if the murine allograft survives for >72 hours. Grade the graft function, as previously reported[22]: Scale 3 - vigorously pulsate and frequency; Scale 2 - less pulsate; Scale 1- fibrillation and imminent rejection; or Scale - 0, loss of heart beat and complete rejection.

Representative Results:

Surgical Operation Time

After training, a skilled surgeon can successfully perform the operation within 35 minutes using the inner tube technique, wherein approximately 15.5 minutes are required for recipient preparation, 10.9 minutes are required for donor preparation, and 4.4 minutes are required for donor heart anastomoses. The cold and warm ischemia time (from donor preparation to heart implantation) is reduced to 15.3 minutes compared to the operation using the cuff technique without the inner tube technique and suture technique (**Table 1**)[21].

We designed a cooperation model to further improve the efficiency of the operation. As shown in the schematic (**Figure 3**), one surgeon begins performing recipient preparation first, followed by initiation of the donor preparation by a second surgeon after 4-5 minutes. After 15-16 minutes, the first surgeon should have finished the recipient preparation, at which point the second surgeon having finished harvesting the donor heart, should begin anastomosing the donor heart within the recipient. This cooperation model requires each surgeon to be trained in only a single part of the cuff technique and further shortens the total operation time to approximately 25 minutes. An analysis of >600 heterotopic murine transplantations performed via cooperation between two surgeons over the past two years at the Organ Transplantation Institute of Xiamen University indicates a success rate for cardiac transplantation using this

technique of up to 95%.

Survival of Major Histocompatibility Complex Cardiac Mismatched and Matched Cardiac Grafts

Major Histocompatibility Complex (MHC), designated "H-2", has been used to determine genetic disparities and similarities. Donor-mismatched MHC antigens can trigger graft rejection by interacting directly with the recipient T cells or indirectly as donor MHC-derived peptides expressed on recipient MHC molecules[23]. A fully MHC mismatched BALB/c (H-2^d) allograft heart can be rejected, with a median survival time of 7.5 days after transplantation into C57BL/6 (H-2^b) recipient mice (**Figure 4A**). In our studies, syngeneic heart transplants survived more than 100 days, except in one rare case due to a 15% weight loss compared to normal weight before the operation.

Heart allografts can be harvested for histopathological examination at the time of rejection. **Figure 4B** shows the appearance of marked cell-mediated rejection characteristics, such as inflammatory cell infiltration, tissue edema, and microvascular occlusion. Syngeneic grafts are near normal with no evidence of myocyte necrosis or inflammatory cell infiltration.

Effect of Inner Tube on Vascular Endothelium

To evaluate the damage on the vascular endothelium after inserting the inner tube into the lumen, 100 days after syngeneic heart transplant, the vascular endothelium of anastomosis site can be collected and stained by immunofluorescent. In this analysis, no obvious narrowing of vascular wall, thrombosis, or thickening of the intima were observed (**Figure 4C**). Electron microscopic imaging revealed that a smooth endothelium and a regular longitudinal crest formation, with the endothelial cells arranged neatly and closely, with no obvious sediments on the surface (**Figure 4D**).

Figure and Table Legends:

Figure 1. A set of Sterile Surgical Instruments: (**A**) Fine forceps and ophthalmic scissor; (**B**) Micro curved forceps; (**C**) Micro straight forceps; (**D**) Micro needle holders; (**E**) Micro scissors; (**F**) Bulldog clamps; (**G**) An artery inner tube (red dotted arrow) and cuff (red solid arrow), along with a vein inner tube (black dotted arrow) and cuff (black solid arrow).

Figure 2. Recipient Preparation. (**A**) Insert the vein inner tube into the external jugular vein vessel; (**B**) Evert the vein vessel over the cuff and fix it using a circumferential 8-0 silk suture; (**C**) Insert the artery inner tube into the lumen of the artery vessel; (**D**) Evert the vein vessel over the cuff and fix it using a circumferential 8-0 silk suture.

Figure 3. Operation Time of Each Step in Heterotopic Murine Transplantation.

Figure 4. (**A**) Cardiac graft Survival Time. Kaplan-Meier plot displaying the survival of heart allografts (BALB/c) and syngeneic grafts (C57BL/6) from donor mice transplanted into C57BL/6 recipient mice (n=12 mice/group); (**B**) Microscopic Examination of C57BL/6 (left) Isograft and

Wild Type BALB/c Allograft (right) on Day 7 after Transplantation (Scale bars, 50 um; magnification ×400); (**C**) Immunofluorescence (Scale bars, 50 μm; magnification ×400) (**D**) Electron Microscopy Scanning of Vascular Endothelium in Transplant (left) and Naive (right) Recipient.

Table 1. Comparison of Time of Different Phases in Different Mouse Heart Transplantation Techniques

Discussion:

Mouse heart transplants models are important tools for transplant immunology research, as tools and materials for evaluating the immune mechanisms of this model and a large number of gene-modified mice are available. However, microsurgical technical challenges, such as vessels suture and eversion, have limited its widespread use. In the present study, we have investigated certain core technical challenges of murine heart transplantation and have obtained good outcomes. A critical step of the protocol the insertion of an inner tube into the lumen as a stent to evert the vessel wall over the cuff. This optimization step resolves the technical challenge of everting the artery vessel due to the need for an extensive stretch. Individuals with no microsurgical skill can begin performing the technique after two months training, which will also aid in the broad application of this model.

In our experience, an appropriate inner tube will enhance transplantation outcomes. The outside diameter of the inner tube should be slightly smaller than the inside diameter of the recipient blood vessel. Moreover, blunt polypropylene tubes or cylinders with a slippery surface should be used as the temporary inner cores to avoid damaging the vascular endothelium. In our hands, no thrombosis occurred in the 5% of models that failed, even though thrombus is a major factor for failure after anastomosis using the suture technique. Using these mature models, our labs have published several basic research articles that have been recognized by peer reviewers[14-19].

Surgeries performed within 35 minutes were not significantly different compared to the traditional cuff technique, but the cold and warm ischemia time were significantly lower than other techniques (**Table 1**). Using the cooperation model further reduces the average operation time to 23-25 minutes, which is reflected in the anesthesia time of the recipient mouse and in the donor heart implantation time. Another advantage of the cuff technique that it limits the warm ischemic time (**Table 1**). As no ice bag is used to protect the heart graft from the warm body temperature of the mouse recipient, the warm ischemic time is equivalent to the anastomosis time. The optimized cuff technique involves the preparation of both cuffs on the recipient to simplify the anastomosis procedure and accordingly shortens the anastomosis time. Therefore, the cuff technique limits the warm ischemic time to only 4.4 minutes on average.

However, there are certain key steps to note with the new technique discussed. Be sure to preserve the submandibular gland of the recipient mouse in cervical heterotopic heart transplantation[30], as the submandibular gland can be used to fix the heart graft and avoid

twisting of the vessels. Avoid damaging the vagus nerve when isolating the external jugular vein and carotid artery, as injury can lead to neck hemiplegia in the recipient. The pressure of the bulldog clamps should be maintained at 20-25 grams to avoid clip injury or vessel leakage. Wash the lumen of the vessels and the cuffs with 0-4 °C heparinized saline to remove any residual blood and gas bubbles; this prevents embolism in grafts after reperfusion. Use a 1 mL syringe for perfusing the donor with 0-4 °C heparinized saline and increase the speed to 50 μL per second to maintain an appropriate pressure.During anastomosis, do not band the circumferential 8-0 sutures (used to fix the everted vascular endothelium to the cuffs) into the lumen of the graft arteries.

Although there are limitations to the cooperation model, which include the need for microsurgical techniques, the availability of two simultaneous microscopes, and double the number of skilled surgeons, it has nevertheless shown to be a successful approach to performing vascularized organ transplantation. Its broader application may further contribute to developing novel immunosuppressive protocols and study of the mechanisms of acute and chronic rejection in the transplant area.

Acknowledgements:
This work was supported by the Fujian Provincial Health Education Joint Research Project (WKJ2016-2-20), the National Natural Science Foundation of China (81771271 and 81800664), the National Key R&D Program of China (2018YFA0108304) and the Education and Scientific Research Project for Young and Middle-aged Teachers in Fujian Province (JAT170714), Natural Science Foundation of Hunan Province of China (2019JJ50842) and Huxiang Young Talents of Hunan Province (2019RS2013).

Disclosures:
The authors have nothing to disclose.

REFERENCES:
1 Corry, R. J., Winn, H. J., Russell, P. S. Heart transplantation in congenic strains of mice. *Transplantation Proceedings.* **5** (1), 733-735 (1973).
2 Que, W. et al. Prolonged cold ischemia time in mouse heart transplantation using supercooling preservation. *Transplantation.* (2019).
3 Wang, C. Y. et al. Suppression of murine cardiac allograft arteriopathy by long-term blockade of CD40-CD154 interactions. *Circulation.* **105** (13), 1609-1614 (2002).
4 Hasegawa, T., Visovatti, S. H., Hyman, M. C., Hayasaki, T., Pinsky, D. J. Heterotopic vascularized murine cardiac transplantation to study graft arteriopathy. *Nature Protocols.* **2** (3), 471-480 (2007).
5 Chen, Z. H. A technique of cervical heterotopic heart transplantation in mice. *Transplantation.* **52** (6), 1099-1101 (1991).
6 Matsuura, A., Abe, T., Yasuura, K. Simplified mouse cervical heart transplantation using a cuff technique. *Transplantation.* **51** (4), 896-898 (1991).
7 Wang, Q., Liu, Y., Li, X. K. Simplified technique for heterotopic vascularized cervical heart transplantation in mice. *Microsurgery.* **25** (1), 76-79 (2005).

8 Li, W. et al. Surgical technique for lung retransplantation in the mouse. *Journal of Thoracic Disease.* **5** (3), 321-325 (2013).
9 Kamada, N., Calne, R. Y. A surgical experience with five hundred thirty liver transplants in the rat. *Surgery.* **93** (1 Pt 1), 64-69 (1983).
10 Chen, H., Zhang, Y., Zheng, D., Praseedom, R. K., Dong, J. Orthotopic kidney transplantation in mice: technique using cuff for renal vein anastomosis. *PLoS One.* **8** (10), e77278 (2013).
11 Miller, M. L. et al. Spontaneous restoration of transplantation tolerance after acute rejection. *Nature Communications.* **6**, 7566 (2015).
12 Lin, Y. et al. Overexpression of Jagged-1 combined with blockade of CD40 pathway prolongs allograft survival. *Immunology and Cell Biology.* **93** (2), 213-217 (2015).
13 Xie, B. et al. Combined costimulation blockade inhibits accelerated rejection mediated by alloantigen-primed memory T cells in mice. *Immunological Investigations.* **38** (7), 639-651 (2009).
14 Shao, W. et al. Combination of monoclonal antibodies with DST inhibits accelerated rejection mediated by memory T cells to induce long-lived heart allograft acceptance in mice. *Immunology Letters.* **138** (2), 122-128 (2011).
15 Dai, H. et al. Blockade of CD27/CD70 pathway to reduce the generation of memory T cells and markedly prolong the survival of heart allografts in presensitized mice. *Transplant Immunology.* **24** (4), 195-202 (2011).
16 Yan, G. et al. Inhibition of accelerated rejection mediated by alloreactive CD4(+) memory T cells and prolonged allograft survival by arsenic trioxide. *Immunological Investigations.* **42** (5), 438-454 (2013).
17 Yan, G. et al. Inhibiting accelerated rejection mediated by alloreactive CD4(+) memory T cells and prolonging allograft survival by 1alpha,25-dihydroxyvitamin D(3) in nude mice. *Immunology Letters.* **149** (1-2), 54-61 (2013).
18 Lin, Y. et al. Arsenic trioxide is a novel agent for combination therapy to prolong heart allograft survival in allo-primed T cells transferred mice. *Transplant Immunology.* **25** (4), 194-201 (2011).
19 Shao, W. et al. CD44/CD70 blockade and anti-CD154/LFA-1 treatment synergistically suppress accelerated rejection and prolong cardiac allograft survival in mice. *Scandinavian Journal of Immunology.* **74** (5), 430-437 (2011).
20 Li, Y. et al. A highly reproducible cervical cuff technique for rat-to-mouse heterotopic heart xenotransplantation. *Xenotransplantation.* (2017).
21 Oberhuber, R. et al. Murine cervical heart transplantation model using a modified cuff technique. *Journal of Visualized Experiments.* (92), e50753 (2014).
22 Blanchard, J. M., Pollak, R. Techniques for perfusion and storage of heterotopic heart transplants in mice. *Microsurgery.* **6** (3), 169-174 (1985).
23 Felix, N. J. et al. H2-DMalpha(-/-) mice show the importance of major histocompatibility complex-bound peptide in cardiac allograft rejection. *Journal of Experimental Medicine.* **192** (1), 31-40 (2000).
24 Tomita, Y. et al. Improved technique of heterotopic cervical heart transplantation in mice. *Transplantation.* **64** (11), 1598-1601 (1997).
25 Niimi, M. The technique for heterotopic cardiac transplantation in mice: experience of

3000 operations by one surgeon. *Journal of Heart and Lung Transplantation.* **20** (10), 1123-1128 (2001).

26 Wang, K., Zhang, N., Li, H. Improved technique of mouse heterotopic heart graft retransplantation. *Microsurgery.* **26** (3), 200-202 (2006).

27 Plenter, R. J., Grazia, T. J. Murine heterotopic heart transplant technique. *Journal of Visualized Experiments.* (89) (2014).

28 Ratschiller, T. et al. Heterotopic Cervical Heart Transplantation in Mice. *Journal of Visualized Experiments.* (102), e52907 (2015).

29 Zhou, Y., Gu, X., Xiang, J., Qian, S., Chen, Z. A comparative study on suture versus cuff anastomosis in mouse cervical cardiac transplant. *Experimental and Clinical Transplantation.* **8** (3), 245-249 (2010).

30 Fukunaga, N., Bissoondath, V., Rao, V. Submandibular Gland-preserving Technique for Heterotopic Cervical Heart Transplantation in Mice. *Transplantation.* **102** (11), e464-e465 (2018).

Figure 1

Click here to access/download;Figure;Figure 1.pdf

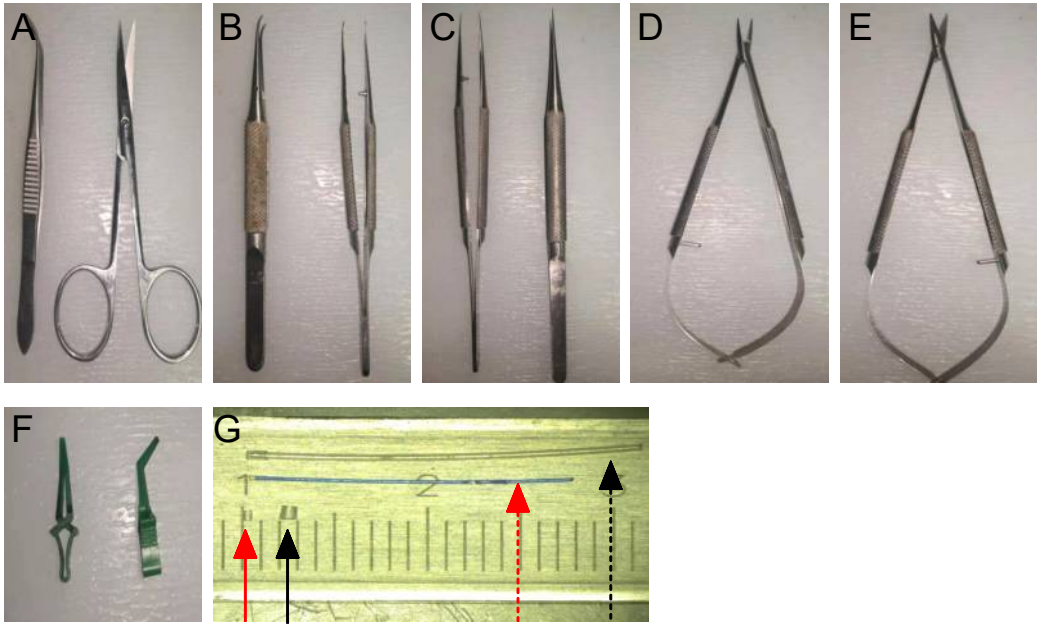


Figure 2

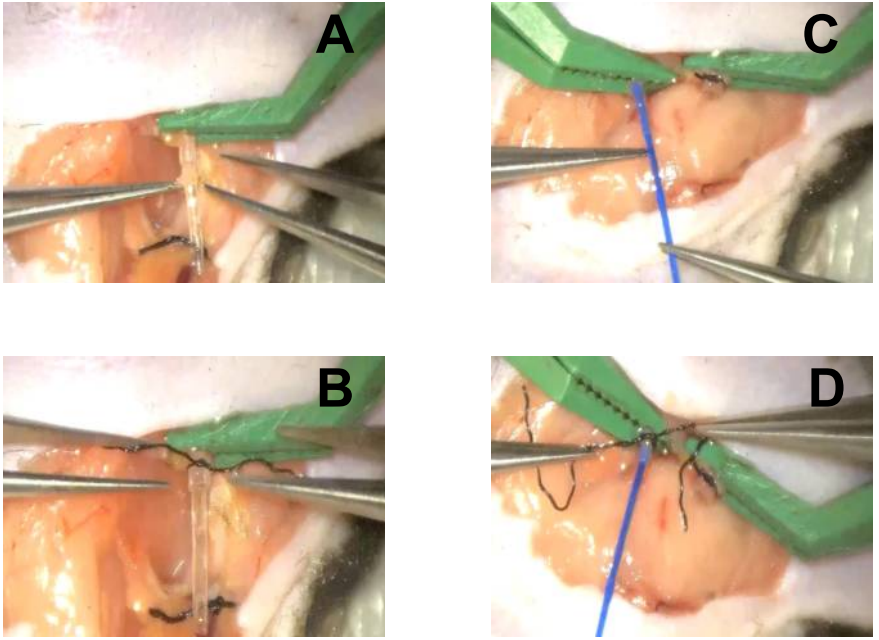


Figure 3

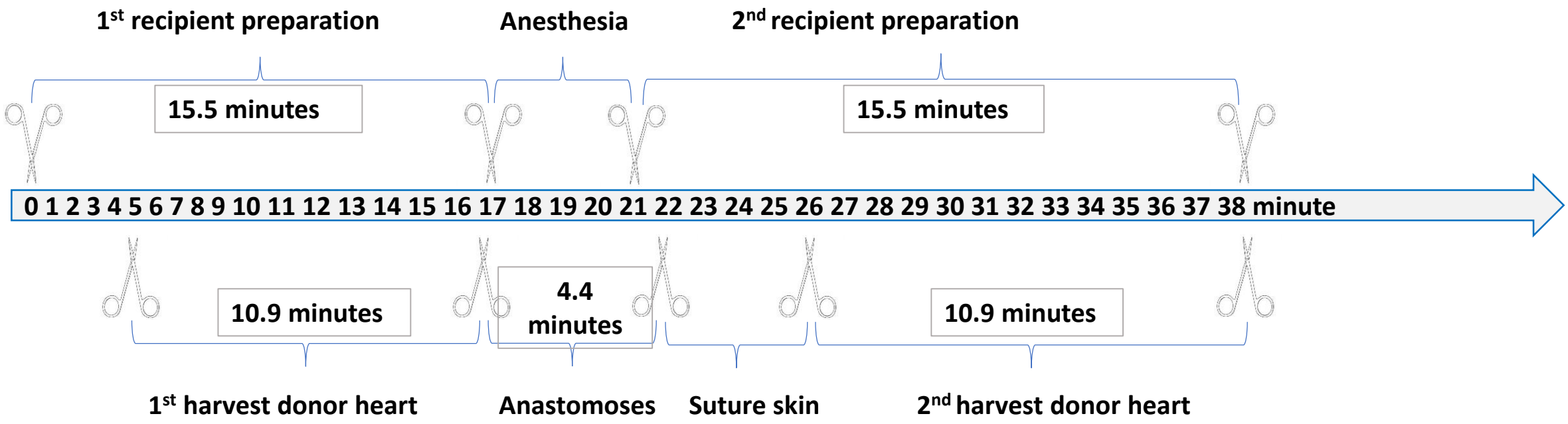
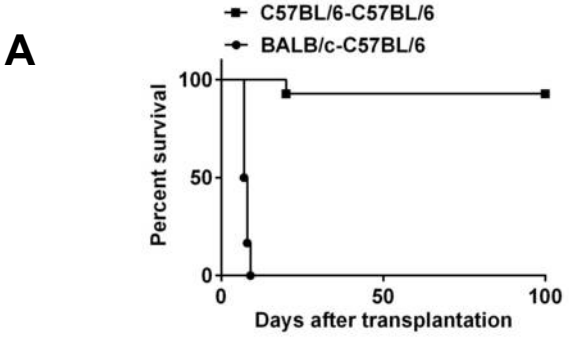
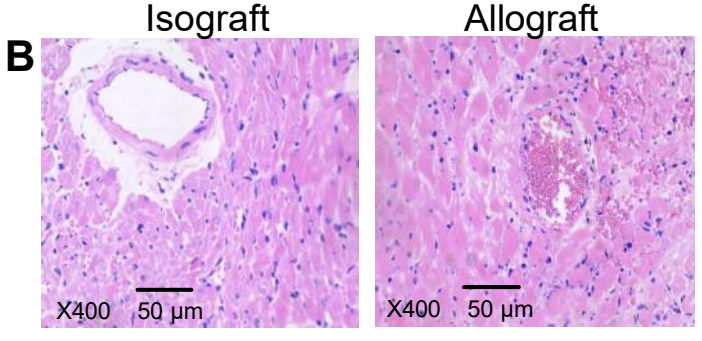
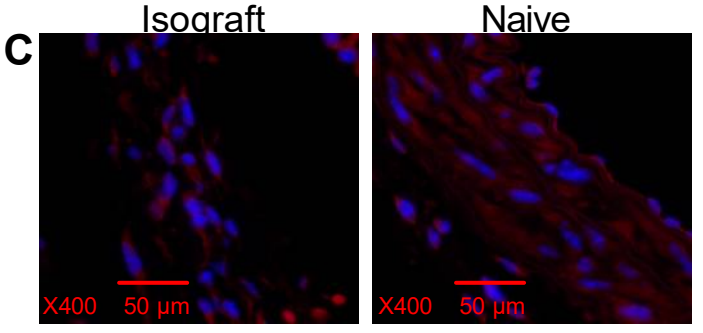


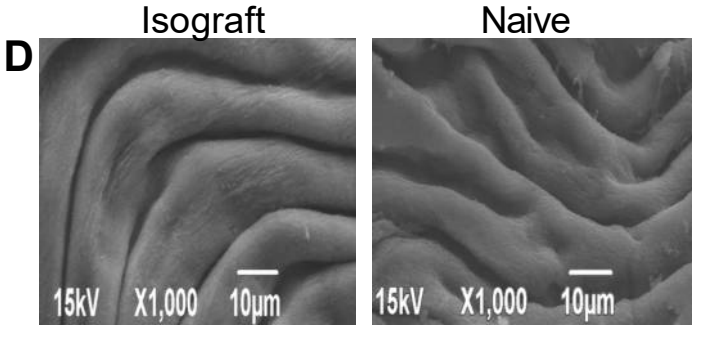
Figure 4

Click here to access/download;Figure;Figure 4.pdf









Table

Table 1. Comparison of Time of Different Phases in Different Mouse Heart Transpla

	Anastomosis technique	Recipient preparation time	Donor preparation time	Heart implantation time
[5] Cervical Heterotopic	Suture	N	N	N
[6] Cervical Heterotopic	Cuff	45 min	15 min	10 min
[24] Cervical heterotopic	Cuff	15 min	20 min	15 min
[25] Abdomen Heterotopic	Suture	N	N	N
[7] Cervical Heterotopic	Cuff	N	20 min	N
[26] Cervical Heterotopic	Cuff	N	20 min	20 min
[4] Abdomen Heterotopic	Suture	60–70 min	6–7 min	N
[21] Cervical Heterotopic	Cuff	N	N	7 min
[27] Abdomen Heterotopic	Suture	N	10-15 min	N
[28] Cervical Heterotopic	Cuff	25 min	20 min	15 min
[29] Cervical Heterotopic	Cuff	31.9 min	21.1 min	5.1 min
[29]Cervical Heterotopic	Suture	25.2 min	20.5 min	30.8 min
Cervical Heterotopic in Our Protocol	Cuff	15.5 min	10.9 min	4.4 min

ntation Techniques	
Cold and warm ischemia time	Total operation time
< 45 min	N
N	N
25-40 min	< 60 min
N	35 min
30 min	35 min
< 35 min	N
N	75 min
20 min	45 min
N	45-60 min
20 min	60 ± 8 min
28.5 min	57.8±3.9 min
51.3 min	83.9±2.9 min
15.3 min	35 min (Single Operation) 23 min (Cooperation)

Name of Material/ Equipment	Company
Artery cuff	Self-made
Artery inner tube	Self-made
Micro curved forceps	Shanghai Medical Instruments (Group) Ltd., Corp. Surgical Instruments Factory
Micro needle holders	Shanghai Medical Instruments (Group) Ltd., Corp. Surgical Instruments Factory
Micro scissors	Shanghai Medical Instruments (Group) Ltd., Corp. Surgical Instruments Factory
Micro straight forceps	Shanghai Medical Instruments (Group) Ltd., Corp. Surgical Instruments Factory
Scanlan Vascu-Statt Bulldog Clamps	Scanlan International Inc
Vein cuff	Self-made
Vein inner tube	Self-made

Catalog Number	Comments/Description
	Polyamide tube. diameter: 0.55 mm,length: 1.0 mm
	Polyamide tube. Diameter: 0.28mm
WA3050	1/8 arc, 0.3-mm tip without a hook
WA2050	0.2-mm tip
WA1050	Straight, blade length: 10 mm
WA3060	0.15-mm tip without a hook
1001-531	Clamping pressure 20–25 grams
	Polyamide tube. diameter: 0.9 mm,length: 1.2 mm
	Polyamide tube. Diameter: 0.6 mm

Dear Vineeta Bajaj, Ph.D.

Enclosed is our manuscript entitled "**Optimization of the cuff technique for murine heart transplantation**", authored by Yunhan Ma, Baiyi Xie, Helong Dai, Chenxi Wang, Shujiao Liu, Tianshu Lan, Shuangyue Xu, Guoliang Yan, Zhongquan Qi. The submission ID of our manuscript is JoVE61103R1. We would like to send the revision of this work to you with the expectation to have this submission considered for publication in **JOVE**.

We had looked through carefully and revised our manuscript according to you and the reviewers. Some essential data and experiments were also added according to reviewers' suggestions. Furthermore, our revised manuscript was edited by a native English speaker. During the revision of this manuscript, we had tried our very best to address each of the comments raised by reviewers as much as we can. We thank the editor and reviewers for their very constructive comments and suggestions, which are extremely helpful and thus have substantially improved the quality of our manuscript. Please see below for our detailed responses to the reviewers' comments.

Thanks again for your consideration of our revised manuscript!

To Editorial and production comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

2. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep and note in the protocol section. Please use Calibri 12 points.

Answer: We have revised this manuscript in the new version.

3. Please ensure that the long Abstract is within 150-300 word limit and clearly states the goal of the protocol.

Answer: The Abstract in the new version contains 154 words and clearly state the goal of the protocol.

4. Please revise the Introduction to include all of the following:

a) A clear statement of the overall goal of this method

b) The rationale behind the development and/or use of this technique

c) The advantages over alternative techniques with applicable references to previous studies

d) A description of the context of the technique in the wider body of literature

e) Information to help readers to determine whether the method is appropriate for their application

Answer: Thanks to the reviewer for your comments!

a) The overall goal of this method is "This model provided an important and valid tool for analyzing the mechanisms of ischemia reperfusion injury, immunological rejection, and tolerance" which is added in the new version between lines 59 to 60.

b) The rationale behind the development and/or use of this technique is " The rationale for the development of the inner tube technique was to reduce the operation time for the establishment of a mouse heart transplant model with a high success rate." which is added in the new version between lines 91 to 93.

c) The advantages over alternative techniques is "Optimization of the cervical heart transplantation model facilitates the acquisition of high success rates in a short period of surgery time compared to the traditional suture and cuff technique21. Further, the cooperation model can further reduce the warm ischemic time of the donor heart compared to surgeries performed with a single operator." which is added in the new version between lines 93 to 96.

d) The description of the context of the technique is added in the new version between lines 64 to 73.

e) Information in the new version "It can be applied in all transplantation immune research, especially for verifying third-party immune tolerance during which the recipient receives two cardiac allografts, one within the abdomen and the other in the

neck” is added between lines 83 to 86 to help readers to determine whether the method is appropriate for their application.

5. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (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.”

Answer: We have revised the protocol section in the imperative tense.

6. The Protocol should contain only action items that direct the reader to do something.

Answer: We have revised the protocol section in the new version.

7. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please ensure that individual steps of the protocol should only contain 2-3 actions per step.

Answer: We have revised the protocol section in the new version, and make sure that individual steps of the protocol only contain 2-3 actions per step.

8. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Please include details of all action throughout.

Answer: We have revised the protocol section in the new version, which includes details of all action throughout.

9. Lines 91-98: Please do not include materials in the protocol section

Answer: We have deleted that materials part in Protocol section.

10. Please include strain, age, sex of the animal used both for the donor and recipient.

Answer: The strain, age and sex of the animal used both for the donor and recipient is added in the new version between lines 108 to 110.

11. Lines 176-182: Please include a table for this data as well. Please include comparison with other techniques.

Answer: The table named "Comparison of time of different phases in different mouse heart transplantation techniques", is added in the new version.

12. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

Answer: All figures are original in our manuscript.

13. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations, please use paragraph style only.

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

Answer:

a) Critical steps within the protocol "A critical step of our protocol the insertion of an inner tube into the lumen as a stent to evert the vessel wall over the cuff. This

optimization step resolves the technical challenge of everting the artery vessel due to the need for an extensive stretch.” is added in the new version between lines 326 to 328.

b) Any modifications and troubleshooting of the technique are added in the new version between lines 332 to 335.

c) Any limitations of the technique are added in the new version between lines 365 to 367.

d) The significance with respect to existing methods are added in the new version between lines 340 to 350.

e) Any future applications of the technique are added in the new version between lines 367 to 370.

14. For images with microscope please include a scale bar and define it in the figure legend.

Answer: We have revised the figure and figure legend in the new version.

15. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please sort the materials table in alphabetical order.

Answer: We have revised the materials table in the new version.

Video:

1. The video cannot exceed 15 minutes in total length. Your protocol is 19 seconds over the limit. Please shorten this video.

Answer: We have shortened this video in the new version.

2. The video is sped up for surgery footage, this is not allowed. Please shorten individual shots of surgery and edit the video to fit the 15 min length without speeding the footage.

Answer: We have shortened and edited the video in the new version.

3. Introduction narration in the video is off by few seconds.

Answer: We have edited the Introduction narration in the new version.

4. Please increase the homogeneity between the written protocol and the narration in the video. It would be best if the narration is a word for word from the written protocol text.

Answer: We have edited the Protocol section in the new version, and make sure the homogeneity between the written protocol and the narration in the video.

5. Surgical drape should cover the entire hair region to maintain sterility.

Answer: We have edited the video in the new version, and make sure sterility operation.

6. 3:48 the clamp is touching the hair beneath.

Answer: We have edited the video in the new version, and make sure sterility operation.

7. Please ensure that the figures are shown in the same way as these are in the text.

Answer: We have edited the video in the new version, and make sure the figures are shown in the same way as these are in the text.

8. Please include a title card in the end as well.

Answer: We have added a title card in the end.

Production notes.

1. Title card:

a. Please insert a space between "Guoliang Yan" and "Zhongquan Qi".

b. "1 Organ Transplantation institute": "I" in "institute" should be capitalized.

Answer: We have revised the Title card in the new version, as shown in “Guoliang Yan Zhongquan Qi”, and “Organ Transplantation Institute”

2. Ethics Card

a. @01:27 Animal Care and Use Committee and Ethics Committee notice card: Eliminate hypenization here (ensure that words are not broken up between lines, as this make it more difficult for the audience to read the notice.)

Answer: We have revised the Ethics card in the new version.

3. Introduction

a. @00:27 There is an audio drop-out here. If it's not recoverable, re-recording the introduction in a different (and quieter) location may be advised. There seems to be some background noise here that can be heard as well throughout the author's introduction

b. @00:53 There is a "pop" here in the audio, it's not very disruptive but if it can be fixed, that would be desirable.

c. Uses stock music loop. Please reduce the music volume by 25 to 50%.

Answer:

We have re-recorded the audio and reduced the music volume.

4. Conclusion info Card:

a. @15:12 There is a typo in the Conclusion text card. Item 1 "tZechnique" should be "technique".

b. @15:15 Use a fade out on the Conclusion text card. As-is, it hard cuts out (disappears over one frame of video) as the credits are fading in. Use a fade on both ends of this edit.

Answer: We have revised the Conclusion info Card, as shown in “technique”, and used a fade out on the Conclusion text card.

5. Editing Style & Pacing:

a. @00:30 Avoid "jump cuts", which is when you make a "hard" or straight edit between two shots that are very similar, resulting in a "jump" in the video. Use a

quick dissolve instead for these instances.

b. @02:45-03:40 "Fast fo(r)ward" Generally, we don't allow sped up procedures. To express the movement of time, try showing only the key steps of the dissection/surgery by fading from key step to key step.

Answer: We revised the editing style in the new version and avoided “jump cuts” and didn’t speed up procedures.

6. Narration performance

a. @14:58 Narration says "used in our research survived infinitely"- "infinitely" is an awkward choice of word for what you're trying to convey. The word "infinitely" implies that the mouse is immortal and cannot die (which would be quite the scientific discovery!). For a mouse that exhbits normal survival probability, you could say "used in our research survived typically" or "used in our survived normally".

Answer: We revised the Narration in the new version, as recorded in “used in our research survival more than 100 days, except in one rare case due to a 15% weight loss compared to normal weight before the operation ".

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The paper describes a novel technique to reduce the operation time in performing a cervical heterotopic heart transplant in mice. The aim of the manuscript was to facilitate vessel eversion when using the cuff technique for the anastomosis of blood vessels. The authors present a new technique in which an inner tube is inserted into the recipient's vessel to act like a stent to facilitate vessel eversion over the cuff. The authors conclude that the inner tube technique shortens the duration of ischemia and the operation time. Also, they conclude that the inner tube technique was successful in allogenic and syngeneic heart transplant mouse models for long-term studies. The manuscript is well written and only needs a few

minor edits.

Major Concerns:

None

Minor Concerns:

Lines 56 and 57 describe various studies, but no references are provided.

Answer: We have added references in the new version.

Line 162, it reads: "Set the pulsing... "subcutaneous room", perhaps the authors mean the "subcutaneous space or pocket"

Answer: We have revised the sentence in the new version as shown in Line 227.

Lines 170 -172, it would be more appropriate for the authors to provide the original wording of the grading scale obtained from Reference 19: "Scale 3-optimal function, with a vigorously beating allograft; Scale 2-poorer function, with contractions less prominent than Scale 3; Scale 1-imminent rejection: fibrillation is more prominent than contraction; Scale 0- rejection is complete".

Answer: We have revised the sentences in the new version as shown in Line 243 to 245. Scale 3 - vigorously pulsate and frequency; Scale 2 - less pulsate; Scale 1-fibrillation and imminent rejection; or Scale - 0, loss of heart beat and complete rejection.

On line 200, the authors appear to suggest that all syngeneic isografts survived indefinitely. However, the Kaplan-Meier graph on Figure 5 shows that at least one animal in the isograft group died at day 20. This sentence needs to be clarified.

Answer: In our studies, syngeneic heart transplants survived more than 100 days, except in one rare case due to a 15% weight loss compared to normal weight before the operation.

Lines 208 and 209, in panel H of Figure 1 two tubes are shown. The legend for panel H indicates that an inner tube and cuff for the artery and an inner tube and cuff for the vein are shown. This description suggests that there should be 4 tubes, but only two are shown. Perhaps the authors should include all 4 tubes in the image with their respective dimensions (lines 95-98) identified either in the legend or the image.

Answer: We have taken the figure again in the new version.

Reviewer #2:

Manuscript Summary:

Novel interesting technique. 2 surgeons collaborating to decrease operative time to 23-25min.

Major Concerns:

1. More data is needed to show that introducing the inner tube does not introduce endothelial injury or that the endothelial injury that may happen does not have significant sequela. H&E of the vessel wall at the anastomosis site (both arterial and vein vessel wall) and the heart allograft at 6hrs, 24hrs and 1 month. Figure 5 does not show an open lumen. Immunohistochemistry with endothelial markers to show endothelial marker expression over time is needed.

Answer: To evaluate the damage on the vascular endothelium after inserting the inner tube into the lumen, 100 days after syngeneic heart transplant, the vascular endothelium of anastomosis site can be collected and stained by immunofluorescent. In this analysis, no obvious narrowing of vascular wall, thrombosis, or thickening of the intima were observed. Electron microscopic imaging revealed that a smooth endothelium and a regular longitudinal crest formation, with the endothelial cells arranged neatly and closely, with no obvious sediments on the surface.

These results have been added in the new version as shown in Line 284 to 290.

2. The time to normal sinus rhythm and the preservation of normal sinus rhythm for at least 5 minutes after clamp release needs to recorded.

Answer: We have added the records in the new version as shown in Line 224, Line 231 to 232.

1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Optimization of the cuff technique for murine heart transplantation

Author(s): Yunhan Ma, Baiyi Xie, Helong Dai, Guoliang Yan, Zhongquan Qi

Item 1: The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via:

☐ Standard Access ☑ Open Access

Item 2: Please select one of the following items:

☑ The Author is **NOT** a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of The Journal of Visualized Experiments; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and(c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. **Grant of Rights in Video – Standard Access.** This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. **Grant of Rights in Video – Open Access.** This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this **Section 6** is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. **Government Employees.** If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. **Protection of the Work.** The Author(s) authorize JoVE to take steps in the Author(s) name and on their behalf if JoVE believes some third party could be infringing or might infringe the copyright of either the Author's Article and/or Video.

9. **Likeness, Privacy, Personality.** The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

10. **Author Warranties.** The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

11. **JoVE Discretion.** If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

12. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

13. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication of the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

14. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement is required per submission.

CORRESPONDING AUTHOR

Name:	Guoliang Yan, Zhongquan Qi
Department:	
Institution:	Organ Transplantation institute, School of Medicine, Xiamen University, Xiamen, Fujian, China Medicinal College, Guangxi University, Nanning, Guangxi, China
Title:	Optimization of the cuff technique for murine heart transplantation
Signature:	阎国良 钟志权
Date:	2019.7.31

Please submit a **signed** and **dated** copy of this license by one of the following three methods:

1. Upload an electronic version on the JoVE submission site
2. Fax the document to +1.866.381.2236
3. Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02140