Video Article

A Modified Method for Heterotopic Mouse Heart Transplantion

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Abstract

Mice are often used as heart transplant donors and recipients in studies of transplant immunology due to the wide range of transgenic mice and reagents available. A difficulty is presented due to the small size of the animal and the considerable technical challenges of the microsurgery involved in heart transplantation. In particular, a high rate of technical failure early after transplantation may result from recipient death and post-operative complications such as hind limb paralysis or a non-beating heart. Here, the complete technique for heterotopic mouse heart transplantation is demonstrated, involving harvesting the donor heart and its subsequent implantation into a recipient mouse. The donor heart is harvested immediately following *in situ* perfusion with cold heparinized saline and transection of the ascending aorta and pulmonary artery. The recipient operation involves preparation of the abdominal aorta and inferior vena cava (IVC), followed by end-to-side anastomosis of the donor aorta with the recipient aorta using a single running 10-0 microsuture and a similar anastomosis of the donor pulmonary artery with the recipient IVC. Following the operation the animal is injected with 0.6 ml normal saline subcutaneously and allowed to recover on a 37 °C heating pad. The results from 227 mouse heart transplants are summarized with a success rate at 48 hr of 86.8%. Of the 13.2% failures within 48 hr, 5 (2.2%) experienced hind limb paralysis, 10 (4.4%) had a non-beating heart due to graft ischemic injury and/or thrombosis, while 15 (6.6%) died within 48 hr.

Video Link

The video component of this article can be found at https://www.jove.com/video/51423/

Introduction

Animal models of organ transplantation can provide valuable information for improving treatment of clinical transplant patients. Mouse models are particularly useful for studying the immune mechanism of organ transplant rejection or acceptance due to the wide range of genetically modified mice and reagents specific for mice that are not available for other animal models[1-3]. A challenge with mouse models of transplantation is the small size of the donors and recipients which requires considerable technical skill to achieve satisfactory outcomes.

A technique was first described for heterotopic transplantation of hearts in rats[4] which was subsequently adapted for mouse heart transplantation by Corry *et al*[5]. This technique involved preparing the recipient prior to the donor operation and no perfusion of the donor heart, both of which are likely to compromise survival of the transplanted heart or the recipient. The procedure has been widely used as originally described to examine the mechanisms of transplant rejection and tolerance[6-8]. Others have adapted the original rat heart transplant procedure of Ono and Lindsey for heart transplants in mice[9,10]. More recently, a technique was published for mouse heart transplantation that overcame some of the problems associated with the method of Corry *et al*[11]. The protocol described here incorporates our modifications, based on the method of Mottram *et al*[12], which include: *in situ* perfusion with cold heparinized saline immediately after thoracotomy and performing the donor operation prior to the recipient operation to minimize recipient operation time. In addition, we use small atraumatic vessel clamps instead of 6-0 silk ties. Although vessel clamps have the disadvantage of taking more space they are easier to control than ties, which should not be too tight or loose and are less straightforward to remove than clamps. Our method uses a single running suture for vessel anastomosis, although in the initial learning stages an alternative is to use stay sutures at the proximal and distal corners to ensure evenness of sutures and thus patency of the anastomosis.

Protocol

Prior to commencement of experiments, obtain approval from the relevant institution's Animal Care Ethics Committee for the planned experiments. Maintain mice in the accordance with the requirements of your institution. The following protocol has been approved by Sydney University and Royal Prince Alfred Hospital committees.

Prior to commencing surgery, all instruments should be cleaned thoroughly and sterilized by soaking in 80% ethanol. Some institutions stipulate autoclaving however this can damage fine microsurgical instruments over the long term. Where possible use sterile disposable equipment.

1. Anesthesia

1. Anesthetize mouse with isoflurane in a sealed container then place it supine on an operating board, quickly connecting anesthetic nose cone. Test adequacy of anesthesia by pinching the hind foot to ensure there is no reflex.
2. Shave the skin with a surgical blade and sterilize with 80% ethanol. In addition, 2% chlorhexidine in 70% isopropyl alcohol can be used for skin sterilization. During induction of anesthesia the isoflurane concentration is 3% but reduce this to 1-1.5% for maintenance. Make minor adjustments to this concentration to maintain regular breathing and heart rate. Maintain temperature of the animal on a heating pad.

2. Donor Operation

1. Make a thoracotomy to expose the heart and vessels by cutting the chest through both sides of the rib cage from the rib edge up to the axilla followed by a transverse cut at the level of the xiphisternum to form a chest wall flap. Lift this flap up beside the head and pin it to the operating board. Tear off pericardium to expose the heart and vessels.
2. Lift the inferior vena cava with forceps in one hand and with the other inject 1 ml cold heparinized saline proximal to heart through the IVC, then put a small artery clamp on the IVC to prevent flow of perfusion solution back through the needle hole.
3. Using gauze and a cotton bud, retract the heart downward to expose the ascending aorta and pulmonary artery. Pass one blade of a pair of microscissors through the channel (transverse sinus) posterior to the bundle of aorta and pulmonary artery and cut the aorta and pulmonary artery together as far distally as possible to ensure sufficient length for anastomosis.
4. Tie and divide the IVC, right superior vena cava (SVC), left SVC and pulmonary veins using 6-0 silk thread. Tie the IVC and right SVC separately then place a single thread posterior to the heart to encircle the left SVC together with pulmonary veins and tie it. Harvest the heart from the donor site by cutting the vessels distal to the ties, then store it in cold sterile saline at 4 °C until transplantation. This results in death of the donor by exsanguination.

3. Recipient Operation

1. Anesthetize the recipient as above (section 1). Carefully shave abdomen to avoid irritation, then make a laparotomy by midline incision from pubis to xiphisternum and retract using paper clips bent to form retractors. Wrap the bowel in warm sterile saline soaked gauze and retract to the upper right of the abdomen.
2. To expose the infra-renal aorta and IVC, free the bundled segments of aorta and IVC from the left renal artery and vein to the iliac bifurcation by dividing them from the lumbar vessels using a cautery device. Take care that the cautery is at the correct temperature and used for sufficient time to divide and seal the vessel ends.
3. Apply small atraumatic vessel clamps to the aorta and IVC segments distally and proximally. Make an incision in the front wall of the aorta by first puncturing a hole with a 30 G needle; then cut an incision vertically with microscissors to match the size of the donor aorta. Flush the aorta lumen with heparinized saline to remove any blood clots.
4. Bring the donor heart to the recipient site covered with gauze soaked in cold saline and place it to the right side of the abdomen. Make sure the donor aorta is positioned next to the incision on the recipient aorta and the donor pulmonary artery positioned next to the recipient IVC.
5. Anastomose the donor aorta end-to-side to the recipient aorta using running 10-0 nylon sutures starting from the proximal corner and suturing along the left side first until reaching the distal corner, at which stage the animal is rotated through 180°. Gently move the donor heart to the left side of the abdomen to expose the right side of the aorta and continue suturing through the right side of the aortic wall from the distal end to the proximal end. Before closing the aortic anastomosis, gently flush the lumen with heparinized saline to remove any clot and air.
6. Anastomose the donor pulmonary artery (PA) end-to-side with the recipient IVC. Make an incision vertically with microscissors in the front wall of the IVC at a site in accordance with the artery anastomosis. Anastomose the donor PA to recipient IVC with running 10-0 nylon sutures starting from the distal end of the left wall within the lumen of the IVC. After reaching the proximal end, continue sutures along the front right side wall up to the distal end to complete the anastomosis. Before closing the anastomosis, gently flush the lumen to remove any clots and air.
7. Before releasing the vessel clamps, place pieces of Gelfoam around the anastomosis sites, and apply gentle pressure with a cotton applicator until hemostasis is achieved. At the time of revascularization, release the distal clamp first, followed by the proximal clamp.
8. After revascularization, apply warm saline at 37 °C to the graft externally to assist its recovery. The graft usually starts fibrillation immediately and reverts spontaneously to sinus rhythm within a few minutes. Inject 0.6 ml warm saline subcutaneously to maintain hydration of the recipient. Inject buprenorphine subcutaneously for analgesia prior to completion of surgery
9. Close the abdominal wound with one 5-0 absorbable running suture for both the layers. Start by completing the inner layer and continue along the skin.

4. Recovery and Graft Monitoring

1. Inject ampicillin for prophylaxis of infection and place the recipient on a heating pad at 37 °C for recovery. Most animals recover rapidly and are usually drinking and often eating within 3 hr. If the mouse shows signs of distress, examine closely to determine cause. If no obvious cause, treat with buprenorphine and monitor closely. Consult a veterinarian if symptoms are severe or persist more than 8 hours and consider euthanasia. If necessary, give continuing 12 hourly injections of buprenorphine until symptoms resolve. If the mouse shows signs of distress after 8 hr, give continuing 12 hourly injections of buprenorphine until they resolve. Consult the veterinarian if symptoms continue for more than 48 hr and euthanize if necessary.
2. Monitor graft heart beat by direct abdominal palpation and record the strength of beat as ++++ for a healthy graft to + for weak beat due to advanced rejection and – as non beating due to complete rejection of the graft. Monitor mice daily for the first 10 days, then 3x per week for the duration of the experiment.

Representative Results

After an initial training period, 227 cases of mouse heterotopic heart transplantation in our group were analyzed. The success rate within the first 24 hr was 90.3% and at 48 hr it was 86.8%. Of the 30 (13.2%) failures within 48 hr, 5 (2.2%) experienced hind limb paralysis and had to be euthanized, 10 (4.4%) had a non-beating heart due to graft ischemia injury and/or thrombosis, while 15 (6.6%) died within 48 hr. Some experimental graft survivals are shown in **Table 1** with a variety of strain combinations. Heart transplants between the same donor and recipient strain were accepted long-term while transplants between non-identical strains were rejected.

The appearance of transplanted hearts is shown in **Figure 1**. The cause of heart transplant failure is generally determined by the time after transplant when the heart stopped beating in combination with the appearance of the heart. If necessary, the cause of cessation of beat should be confirmed by histological analysis of the heart. For example, in a C57BL/6 strain recipient of a DBA/2 donor heart transplant, the heart ceased beating on day 2, which is most unusual as rejection in unsensitized recipients usually takes at least a week. **Figure 1A** shows the heart from this recipient with evidence of thrombosis and infarction of the heart tissue. In the strain combination of C57BL/6 donor to C57BL/6 recipient, the heart was not rejected and survived for >100 days (**Figure 1B**). In this case the heart had reduced slightly in size due to muscle atrophy secondary to its non life sustaining status. In contrast, in the rejecting strain combination of DBA/2 donor to C57BL/6 recipient, the transplanted heart had ceased to beat by day 7 and showed evidence of rejection (**Figure 1C**). It was completely fibrotic and shrunken by day 100 (**Figure 1D**).

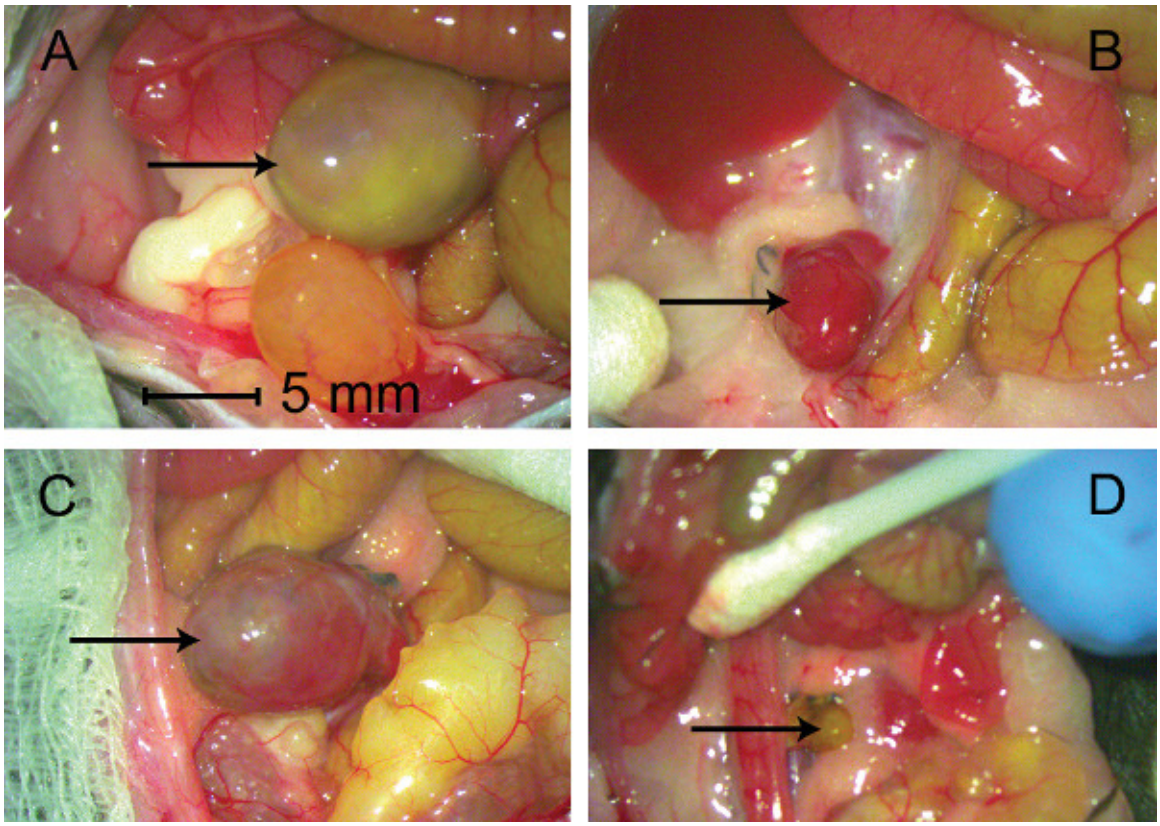

Figure 1. Appearance of thrombosed, rejecting and non-rejecting transplanted hearts. (A) DBA/2 heart transplanted to C57BL/6 recipient showing thrombosed and infarcted heart 7 days after transplant. **(B**) C57BL/6 heart transplanted to C57BL/6 recipient showing a well perfused and beating heart 100 days after transplantation. **(C)** DBA/2 heart transplanted to C57BL/6 recipient showing rejected heart 7 days after transplantation. **(D)** DBA/2 heart transplanted to C57BL/6 recipient showing rejected heart 100 days after transplantation. Arrows indicate the transplanted heart.

Combinations	Survivals (days)	n	MST (days)
B10.BR→B10.BR	>200 x7	7	>200
C57BL/6→C57BL/6	40, >200 x2	3	>200
F1→B10.BR	8 x2, 9, 12	4	8.5
178.3→B10.BR	7 x2, 8 x2, 9, 10, 12, 13	8	8.5
C57BL/6→B10.BR	8 x4, 9 x2, 10 x5, 11, 12, 14 x2, 15	16	10
BALB/c→C57BL/6	7 x9	9	7
DBA/2→C57BL/6	6, 7 x10	11	7

Table 1. Heart graft survival in different donor/recipient combinations.

Discussion

Mouse heart transplantation is a challenging microsurgical method that requires considerable surgical skill to master. The most challenging aspect is the small diameter of the vessels. In addition, it is necessary to limit the recipient operation time and bleeding. The technique for mouse heart transplantation was first described by Corry *et al*. in 1973 and subsequently by Mottram *et al*[12]. Our modifications include the following points. Firstly, immediate perfusion of the donor heart with cold heparinized saline following thoracotomy and transection of aorta and pulmonary artery bundle soon after perfusion prevents warm ischemia during harvesting. Secondly, starting the recipient preparation soon after completion of donor harvesting, instead of preparing the recipient before the donor, reduces the abdominal exposure time in the recipient with consequent improvement in survival. Finally, use of small atraumatic vessel clamps to block blood flow, instead of ties for the recipient aorta/IVC bundle, usually results in less damage to the vessels and can reduce the incidence of hind limb paralysis.

In addition, the following points are important for success. The optimal size of donors and recipients is around 23 to 26 g and optimum age is 10 to 12 weeks. Older animals do not have larger vessels and their increased fat makes exposure of the aorta and IVC more difficult. Dehydration and hypothermia of the recipient during surgery must be avoided. Recipient bowel needs to be well protected from dehydration using warm saline soaked gauze and the recipient can be maintained on a heating mat at 37 °C. The gauze needs to be kept moist by regular application of fresh, warm saline during the operation. To minimize blood clots and air emboli that can lead to hind limb paralysis, the vessel anastomosis sites need to be well flushed before closing. Bowel should be carefully replaced at completion of transplantation to prevent any twisting of the mesentery. Subcutaneous injection of 0.6 ml warm saline assists animal recovery whereas a larger volume of intravenous fluid soon after surgery may cause hypertension and bleeding of anastomoses. Limiting the time that the recipient aorta and IVC are cross clamped to less than 30 min will improve the success rate.

Disclosures

The authors have nothing to disclose.

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References

1. Aramaki, O. *et al.* Interleukin-10 but not transforming growth factor-beta is essential for generation and suppressor function of regulatory cells induced by intratracheal delivery of alloantigen. *Transplantation.* **79**, 568-576 (2005).
2. Chen, R. H., Bushell, A., Fuggle, S. V., Wood, K. J., & Morris, P. J. Expression of granzyme A and perforin in mouse heart transplants immunosuppressed with donor-specific transfusion and anti-CD4 monoclonal antibody. *Transplantation.* **61**, 625-629 (1996).
3. Poulin, L. F. *et al.* Interleukin-9 promotes eosinophilic rejection of mouse heart allografts. *Transplantation.* **76**, 572-577, doi:Doi 10.1097/01.Tp.0000071201.32424.D2 (2003).
4. Ono, K., & Lindsey, E. S. Improved technique of heart transplantation in rats. *J. Thorac. Cardiovasc. Surg.* **57**, 225-229 (1969).
5. Corry, R. J., Winn, H. J., & Russell, P. S. Primarily vascularized allografts of heart in mice. *Transplantation.* **16**, 343-350 (1973).
6. Larsen, C. P. *et al.* CD40-gp39 interactions play a critical role during allograft rejection. Suppression of allograft rejection by blockade of the CD40-gp39 pathway. *Transplantation.* **61**, 4-9 (1996).
7. Saitovitch, D., Bushell, A., Mabbs, D. W., Morris, P. J., & Wood, K. J. Kinetics of induction of transplantation tolerance with a nondepleting anti-Cd4 monoclonal antibody and donor-specific transfusion before transplantation. A critical period of time is required for development of immunological unresponsiveness. *Transplantation.* **61**, 1642-1647 (1996).
8. Callaghan, C. J. *et al.* Regulation of allograft survival by inhibitory FcgammaRIIb signaling. *J. Immunol.* **189**, 5694-5702, doi:10.4049/jimmunol.1202084 (2012).
9. Qian, S. *et al.* Impact of donor MHC Class I or Class II antigen deficiency on first- and second-set rejection of mouse heart or liver allografts. *Immunology.* **88**, 124-129 (1996).
10. Wang, C. *et al.* Spontaneous acceptance of mouse kidney allografts is associated with increased Foxp3 expression and differences in the B and T cell compartments. *Transpl. Immunol.* **24**, 149-156, doi:S0966-3274(10)00159-0 [pii]10.1016/j.trim.2010.12.004 (2011).
11. Liu, F., & Kang, S. M. Heterotopic Heart Transplantation in Mice. *J. Vis. Exp.* (6), e238, doi:10.3791/238 (2007).
12. Mottram, P. L. *et al.* Electrocardiographic monitoring of cardiac transplants in mice. *Cardiovasc. Res.* **22**, 315-321, doi:10.1093/cvr/22.5.315 (1988).

