

Video Article

Murine Cervical Heart Transplantation Model Using a Modified Cuff Technique

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Abstract

Mouse models are of special interest in research since a wide variety of monoclonal antibodies and commercially defined inbred and knockout strains are available to perform mechanistic *in vivo* studies. While heart transplantation models using a suture technique were first successfully developed in rats, the translation into an equally widespread used murine equivalent was never achieved due the technical complexity of the microsurgical procedure. In contrast, non-suture cuff techniques, also developed initially in rats, were successfully adapted for use in mice¹⁻³. This technique for revascularization involves two major steps I) everting the recipient vessel over a polyethylene cuff; II) pulling the donor vessel over the formerly everted recipient vessel and holding it in place with a circumferential tie. This ensures a continuity of the endothelial layer, short operating time and very high patency rates⁴.

Using this technique for vascular anastomosis we performed more than 1,000 cervical heart transplants with an overall success rate of 95%. For arterial inflow the common carotid artery and the proximal aortic arch were anastomosed resulting in a retrograde perfusion of the transplanted heart. For venous drainage the pulmonary artery of the graft was anastomosed with the external jugular vein of the recipient⁵.

Herein, we provide additional details of this technique to supplement the video.

Video Link

The video component of this article can be found at <https://www.jove.com/video/50753/>

Introduction

Cardiac transplantation represents the therapy of choice for patients suffering from different end-stage heart diseases. Advances in surgical techniques, more effective prophylaxis of infections, and novel immunosuppressive regimens resulted in markedly improved outcome of organ transplantation⁶. However, long-term graft survival has not improved rigorously over the past years⁷. Chronic rejection, characterized by transplant arteriosclerosis continues to be a major obstacle to long-term graft survival⁸⁻¹¹.

The model of heterotopic heart transplantation in mice provides an important and valid tool for analysis of immunological mechanism during acute as well as chronic rejection¹²⁻¹⁵.

To date the most frequent transplant model is still the abdominal mouse heart transplantation using the suture technique. The ascending aorta of the donor heart is anastomosed to the abdominal aorta and the pulmonary artery is anastomosed to the recipient's inferior vena cava. Much of the microsurgical difficulty of the suture model is based on the small size of the vessels, which are sutured^{16,17}.

In contrast to the suture model the heart is placed in the neck region of the recipient where the external jugular vein is anastomosed to the pulmonary artery and the common carotid artery the aorta of the donor.

The rationale of the development of the cervical heart transplantation model using the cuff technique was to have an animal model, which allows achieving high success rates with basic microsurgical skills that will allow broad application of this model. The major advantages of this method are the significantly less occurring anastomosis related complications like hemorrhages and thrombosis when compared to the suture model¹⁸.

Protocol

Animals are housed in a barrier pathogen free facility. All animals receive human care in compliance with the "Principals of Laboratory Animal Care" formulated by the National Society for Medical Research" and the "Guide for the Care and Use of Laboratory Animals" prepared by the

National Academy of Sciences and published by the National Institutes of Health (NIH Publication no. 86-23, revised 1985). All experiments are approved by the Austrian Ministry of Education, Science and Culture.

1. Recipient Preparation

1. Anesthetize the recipient animal with an i.m. injection of xylazine (5 mg/kg body weight) and ketamine (100 mg/kg body weight).
2. Remove all hair of the lateral cervical region of the animal and scrub the operative field three times using chlorhexidine.
3. Place the mouse in a supine position on the operative field.
4. Next make a skin incision from the jugular incision to the right lower mandible.
5. Subsequently, bluntly mobilize the right external jugular vein (EJV) and divide the branches between ligatures.
6. Then divide the EJV between ligatures making sure to leave enough length to evert the proximal stump over the cuff body.
7. Next remove the right lobe of the submandibular gland.
8. For the venous anastomosis pass the proximal end of the EJV through the polyethylene cuff and fix it at the handle of the cuff with a micro clamp.
9. Remove the ligature at the end of the vessel, evert the lumen over the cuff and fix it with an 8-0 silk loop (**Figure 1a**).
10. Subsequently transect the right sternocleidomastoid muscle with bipolar cautery to gain access to the common carotid artery.
11. Next mobilize the common carotid artery and cut the vessel between the ligatures.
12. Next mobilize the common carotid artery and cut the vessel between the ligatures.
13. Pass the distal end of the vessel through the cuff and fix it with the artery clamp.
14. Remove the ligature at the end of the vessel and distend the lumen using vascular dilators. Next, in analogy to the EJV, evert the artery over the cuff and fix it with an 8-0 silk loop.

2. Heart Procurement

1. Anesthetize the donor mouse with an i.m. injection of xylazine (5 mg/kg body weight) and ketamine (100 mg/kg body weight). Then remove all hair of the abdominal region of the animal. Scrub the operative field three times using chlorhexidine. Next place the mouse in a supine position on the operative field as previously described.
2. Following a midline abdominal incision, retract the viscera with Q-tips to the left in order to expose the inferior vena cava (IVC).
3. Inject 400 μ l of a 1:4 heparin- sodium- solution into the IVC for heparinization.
4. Perform a thoracotomy and fold the anterior chest wall over cranially to gain access to the heart.
5. Next, remove the thymus and make venting incisions in the left and right superior vena cava.
6. Subsequently, perfuse the heart in a retrograde fashion with 4° Celsius HTK solution by cannulation of the aortic arch with a 27 G syringe at the level of the brachiocephalic branch.
7. Ligate the inferior and superior vena cava with 8-0 silk and divide them distally to the ligatures.
8. Next divide the aortic arch at the level of the previous cannulation and dissect free the pulmonary trunk and divide it as far distally as possible.
9. Following that, tie the pulmonary veins with a bulk ligation and divide them distally to the ligatures.
10. Finally, remove the heart from the donor site and store it in 4° Celsius HTK solution (**Figure 1b**).

3. Implantation

1. Place the heart graft in the recipient neck region in an upside down position (**Figure 1c**).
2. Next pull the pulmonary trunk of the heart over the vein cuff construct of the recipient and fix it with a silk loop (**Figure 1d**).
3. Perform the anastomosis between the aorta of the graft and the everted artery of the recipient animal in the same fashion (**Figure 1e**).
4. Subsequently, remove the venous clamp first, followed by the arterial clamp. The heart is reperfused and starts beating within 1-2 min. During reperfusion moisten the heart with warm (35° Celsius) saline.
5. Finally close the surgical wound with 6-0 continuous sutures (**Figure 1e**).

4. Postoperative Care, Endpoint

1. Give up to 0.3 ml normal saline i.p. postoperatively for fluid replacement.
2. Place the animal under a heat lamp until awaking from anesthesia.
3. Once awake, return the animal to the housing facility where they receive food and water ad libitum.
4. To minimize pain during the first 7 postoperative days administer carprofen (4mg/kg every 12 hr subcutaneously (s.c.)). In addition, give Buprenorphin (0.1 mg/kg) right after the operation every 12 hr for 5 days.
5. Obtain the weight (g) of each recipient animal weekly to assess for proper nutritional intake. If there is evidence of more than 10-15% weight loss compared to weight at surgery-date, apathy, crippling or a very bent back, sacrifice the animal using terminal isoflurane inhalation before reaching the clinical end point. Also sacrifice the animal after the heart has been rejected.

Representative Results

Figure 2 shows representative survival data of cardiac allografts from BALB/c donors after transplantation into fully MHC mismatched C57BL/6 recipients. Hearts are rejected on day 7 ± 1 after transplantation. Finger palpation of the transplanted allograft is a sensitive method to detect the time dependent course of allograft rejection. All syngeneic heart transplants performed in our series survived indefinitely (>150 days).

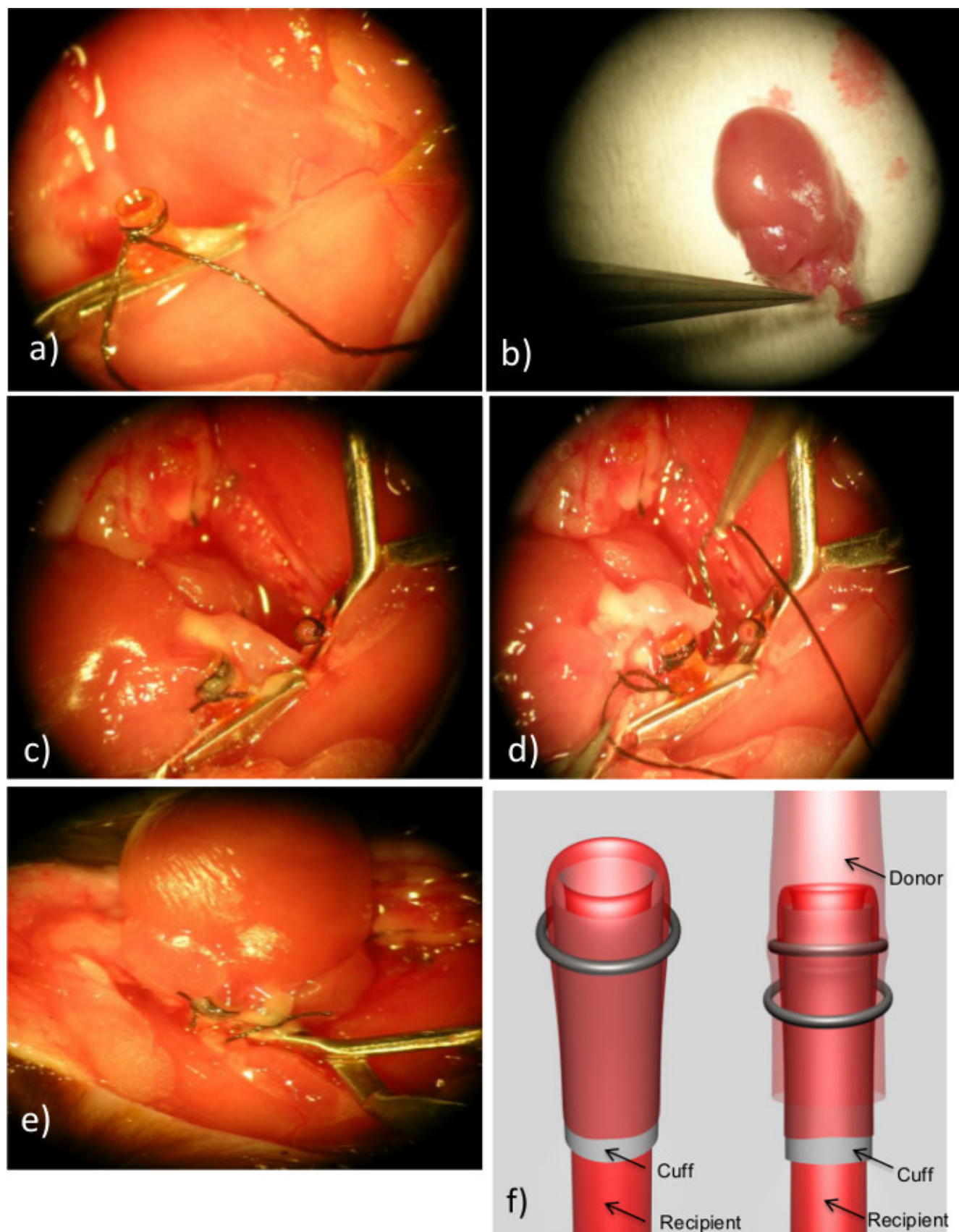


Figure 1: Intraoperative pictures. (a) Operative field during recipient preparation. (b) Heart graft after procurement. (c) The heart graft placed in the recipient neck region in an upside down position. (d+e) The donor site prior to implantation. (f) Schematic diagram illustrating the recipient vessel, the donor vessel and the cuff

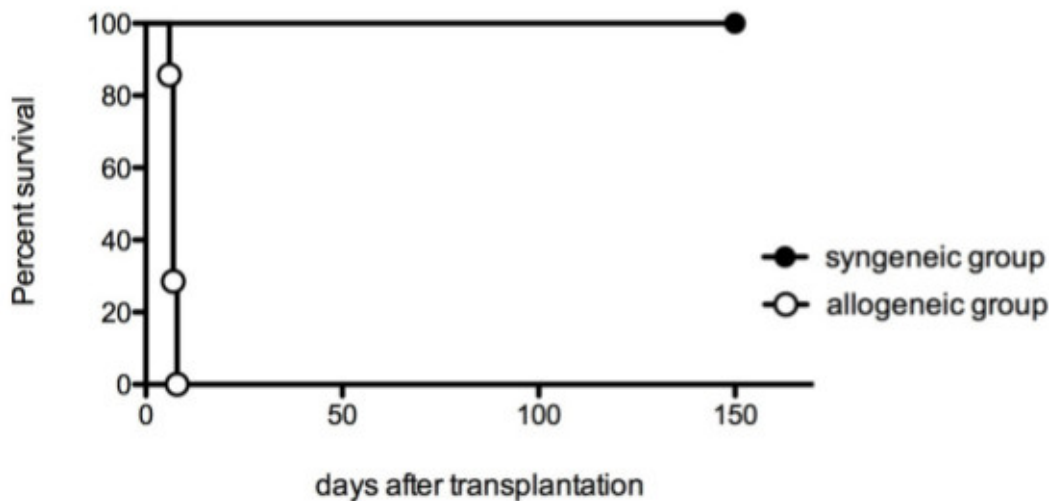


Figure 2: Cardiac allograft survival. Kaplan-Meier plot displaying the survival of cardiac allograft from BALB/c mice after grafting into fully MHC- mismatched BL6 recipients (n = 7; mean survival time = 7 days) as well as a syngeneic control group (n = 7; graft survival >150 days). The day of rejection was defined as the day of cessation of heart beat.

Discussion

Rejection of vascularized allografts entails a plethora of different steps which can only be insufficiently assessed using *in vitro* models. Phenomena like the sensitization of recipients, antigen processing in secondary lymphoid organs but also the differentiation and proliferation of immune-competent cells are better characterized *in vivo*. Animal models are thereby the ideal tool for translational research¹⁶. Mouse models still represent the gold standard in basic transplant and immunologic research since a broad range of transgenic and gene knockout mice are available, and a large number of immunologic and diagnostic tools have been developed exclusively for mice¹⁸. The murine cervical heart transplantation model offers a unique tool to address questions and perform mechanistic studies related to ischemia reperfusion injury, immunosuppression, acute and chronic rejection and tolerance induction^{1-3, 17, 22}.

The mouse abdominal heart transplantation model using a suture technique was first described by Corry *et al.* in 1973¹⁹. Technical challenges associated with the suture- anastomosis of small vessels, however, limited its widespread use.

In 1991, Matsuura *et al.* introduced the cervical heart transplantation model using a non-suture cuff technique in mice¹⁶. Since its introduction by Zimmerman *et al.*, and Kamada and Calne "the cuff technique" has been used for microvascular anastomoses in several different murine models of organ transplantation²⁰⁻²².

We have developed a modified non-suture cuff technique for revascularization and have completed over 1,000 successful transplants in the past several years, with success rates >95%. Several different strain combinations and treatment regimens were used. Applying the technique described here, the total operation time can be reduced to approximately 45 min. Importantly the total ischemia time can be kept at 20 min with an implantation time of less than 7 min, which in contrast is usually more than 15 min when using the suture technique²³. Compared with the mouse abdominal heart transplant model, cervical heart transplant has several advantages, such as minor postoperative stress and high surgical success rate. The beating of the heart graft can easily be monitored owing to the superficial position. Complications associated with suture anastomosis, including bleeding and thrombosis are significantly less common when using the cuff technique¹⁸.

The following modifications of this technique proposed by our group led to an improved success rate and to a dramatic reduction of the operation time:

- Use a retrograde perfusion through the aortic arch with 3 ml HTK solution.
- Keep the heart moist using HTK solution during the whole harvesting procedure.
- Do not dissect and selective ligate the left superior vena cava.
- Use the micro clamps to occlude the vessels but, importantly, also to fix the cuffs.
- Use vascular dilators with superfine tips to distend the common carotid artery which makes it much easier to evert the vessel over the cuff.
- Do not place holding sutures as original described by Matsuura *et al.*
- For the arterial anastomosis use a cuff with an inside diameter of 0.5 mm and an outside diameter of 0.63 mm, for the venous anastomosis a cuff with an inside diameter of 0.75 mm and an outside diameter of 0.94 mm.
- Use cuffs with a length of 2 mm including a 1 mm handle which serves to its proper fixation to the vessel, using a vascular clamp.

One limitation that this method might have is that it may be difficult to obtain appropriate cuffs. We therefore used commercially available polyethylene cuffs from Rivertech medical (Chattanooga, TN).

Main trouble shooting during the procedure were as follows.

Blood flow disorder: Flush donor heart until the color becomes white in color. Flush without too much pressure as excessive pressure damages the graft.

- Difficulty to evert the vessel over the cuff: Ascertain that the vessel is (a) of sufficient length, (b) free of attached fat and connective tissues and (c) make sure that the vessel was well distended, using vascular dilators.
- Bleeding at anastomotic site: use Q-tips to apply pressure at affected site for approximately 5 min.
- Venous blood flow obstruction: Mainly caused by torsion of the jugular vein during the processes of cuff placement. Remove the cuff handle at the venous site.
- Graft twisting and strangulation: Make sure that the graft is placed properly in the donor side prior to skin closure.

Despite the fact that the heterotopic heart transplantation in mice is technically challenging, it remains the gold standard for any mechanistic *in vivo* studies related to transplant immunology. Our modified approach allows achieving high success rates with basic microsurgical skills that will allow broad application of this model. This video aims to help and guide other researches to establish the model in their laboratories.

Disclosures

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