Video Article

Heterotopic Cervical Heart Transplantation in Mice

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Abstract

The heterotopic cervical heart transplantation in mice is a valuable tool in transplant and cardiovascular research. The cuff technique greatly simplifies this model by avoiding challenging suture anastomoses of small vessels thereby reducing warm ischemia time. In comparison to abdominal graft implantation the cervical model is less invasive and the implanted graft is easily accessible for further follow-up examinations. Anastomoses are performed by pulling the ascending aorta of the graft over the cuff with the recipient's common carotid artery and by pulling the main pulmonary artery over the cuff with the external jugular vein. Selection of appropriate cuff size and complete mobilization of the vessels are important for successful revascularization. Ischemia-reperfusion (I/R) injury can be minimized by perfusing the graft with a cardioplegic solution and by hypothermia. In this article, we provide technical details for a simplified and improved cuff technique, which should allow surgeons with basic microsurgical skills to perform the procedure with a high success rate.

Video Link

The video component of this article can be found at http://www.jove.com/video/52907/

Introduction

The heart transplantation model is frequently used to investigate I/R-injury, allograft rejection, post-transplant vasculopathy and the efficiency of immunomodulating agents. Mouse models combine many advantages like the known immunological and genetic backgrounds, the availability of many inbred and transgenic strains and relatively low experimental costs.

In 1973, a technique of abdominal heart transplantation in mice was first described by Corry *et al*[1]. In this model grafts are revascularized by end-to-side anastomoses of the ascending aorta to the recipient's abdominal aorta and of the main pulmonary artery to the inferior vena cava. In 1991, a suture technique for cervical heart transplantation in mice was described by Chen *et al*[2]. In this model a retrograde coronary artery perfusion is established by anastomosis of the recipient's carotid artery to the ascending aorta of the graft. The venous blood drains via the coronary sinus into the right atrium, the right ventricle and is ejected through the pulmonary artery into the recipient external jugular vein (**Figure 1**). In comparison to the abdominal graft implantation the cervical model is less invasive and the superficial location of the graft enables a very easy accessibility for additional follow-up examinations (*e.g.* palpation, echocardiography, intravital fluorescence microscopy)[3,4].

Despite technical improvements[5,6], a more widespread use of this model has been limited due to technical difficulties to perform sutured anastomoses of small vessels with a high incidence of anastomosis leakage and thrombosis. In 1991, Matsuura *et al.* introduced the cuff technique in which the vessel wall is everted over a synthetic cylinder, which greatly facilitates the model of cervical heart transplantation[7]. The technique was further adopted for various experimental transplant models including renal[8], pancreatic[9], limb[10], hepatic[11,12], lung[13,14] and vascular transplantation[15]. In comparison to the suture technique the required time for anastomoses and, thus, the warm ischemia time is shorter and there are significantly less vascular complications using the cuff technique[16]. Moreover, it enables surgeons with little training in microsurgery to perform the operation with a high success rate. A disadvantage of the cuff technique is the prerequisite ligation of the common carotid artery, which may alter cerebral perfusion.

In this video paper we present an improved and simplified cuff technique for cervical heart transplantation in mice.

Protocol

All experiments were approved by the German Heart Center Munich and the Government of Bavaria, Germany (Gz. 55.2-1-54-2532.3-17-13). Animals were housed in a specific pathogen free facility and received human care in compliance with the ”Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the ”Guide for the Care and Use of Laboratory Animals” provided by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication no. 86-23, revised 1985).
NOTE: A complete set of sterile microsurgical instruments, including two curved forceps and vessel dilatators, is necessary for the procedure (**Figure 2a**). Perioperative antibiotic prophylaxis is not routinely necessary but may be indicated in immunocompromised animals. Animals were sacrificed using terminal isoflurane inhalation.

Donor Preparation and Graft Harvest

1. Anesthetize the animal by intraperitoneal (i.p.) injection of midazolam (5.0 mg/kg), medetomidine (0.5 mg/kg) and fentanyl (0.05 mg/kg body weight). Place the mouse in a supine position on the operative field and confirm surgical tolerance by the pedal withdrawal reflex.
2. Disinfect the skin three times with chlorhexidine and perform a midline abdominal incision. Retract the intestines with wet gauze to the left to expose the inferior vena cava.
3. Inject 50 IU of heparin diluted in 0.4 ml saline through the inferior vena cava for anticoagulation. Incise the vessel 1 min after heparinization.
4. Open the diaphragm and perform a bilateral thoracotomy to expose the heart. The anterior chest wall is reflected superiorly and held in place with a clamp.
5. Remove the thymus and bluntly dissect the connective tissue between aorta and pulmonary trunk. Incise the right superior vena cava.
6. Puncture the mobilized ascending aorta at the level of the brachiocephalic trunk with a 30 G needle mounted on a 5 ml syringe and slowly perfuse the heart retrogradually without excessive pressure with 3 ml of 4 °C cold cardioplegic solution. Avoid the entry of air into the coronary circulation. Drop ice-cold saline solution onto the graft to further enhance cardiac arrest.
7. Transect the aorta proximally to the origin of the brachiocephalic trunk.
8. Ligate the inferior and both superior venae cavae close to the heart with 8-0 silk and transect them distally to the ligatures.
9. Dissect free the pulmonary trunk as distally as possible. Ligate the pulmonary arteries close to the bifurcation with 8-0 silk and transect them distally.
10. Ligate the pulmonary veins all together with a single 8-0 silk ligature and transect them.
11. Remove the heart from the donor and store it in ice-cold cardioplegic solution until implantation.

2. Recipient Preparation

1. Prepare the cuffs for anastomoses by cutting the polyimide tubing with a No. 11 scalpel or a scissor under the microscope. The cuff consists of a cylindrical body and an extension ($^1/_3$ of the circumference) for the placement of the vascular clamp, each of a length of 1 mm (**Figure 2b**).
2. Anesthetize the animal and confirm surgical tolerance as described above. Inject 0.25 ml warm saline solution subcutaneously (s.c.) for fluid replacement.
3. Remove the hair of the right cervical region using an electric shaver and place the animal in a supine position on a warm pad. Secure the legs with strands of tape while avoiding overstretching the front limbs as this can compromise respiration. Cover the eyes using ophthalmic ointment to prevent dryness. Disinfect the surgical site three times with chlorhexidine.
4. Make a transverse skin incision from the right mandibular angle to the jugular notch.
5. Bluntly mobilize the external jugular vein. Cauterize side branches with a bipolar forceps and transect them.
6. Divide the external jugular vein between ligatures at the level of the confluence with the vein from the submandibular gland.
7. Pull the external jugular vein through the cuff and occlude the vessel proximally by placing a vascular clamp on the extension of the cuff. Remove the ligature and irrigate the vessel lumen with 1:10 heparinized saline.
8. Evert the vessel wall over the cuff and fix it with a preset circumferential 8-0 silk ligature.
9. Remove the superficial part of the sternocleidomastoid muscle.
10. Mobilize the right common carotid artery and transect the vessel between ligatures below the carotid bifurcation.
11. Pull the carotid artery through the cuff and occlude the vessel proximally by placing a Yasargil clamp on the extension of the cuff. Remove the ligature and irrigate the vessel lumen with 1:10 heparinized saline.
12. Gently dilate the arterial lumen with a vessel dilatator, evert the vessel wall over the cuff and fix it with a preset circumferential 8-0 silk ligature.
13. To gain enough space for graft implantation, remove the right lobe of the submandibular gland.
14. Keep the operative field moist until implantation.

3. Graft Implantation

1. Place the graft in the recipient cervical region upside down with the aorta orientated medially and the pulmonary artery laterally.
2. Pull the aorta of the graft over the cuff with the carotid artery and secure the anastomosis with a circumferential 8-0 silk ligature.
3. Incise the pulmonary trunk on its anterior aspect and pull it over the cuff with the external jugular vein. Again secure the anastomosis with a circumferential 8-0 silk ligature.
4. Remove the clamp on the external jugular vein, following by unclamping of the carotid artery. The hearts fills immediately with blood. To enhance reperfusion drop 37 °C warm saline solution onto the graft. Normally the heart develops sinus rhythm within one minute. Remove the cuff extension at the venous site.

5. Ensure correct graft position, anastomoses must be tension-free and vessels without torsion. Do not cauterized vessels of the skin since this may impair wound healing. Bleeding usually stops spontaneously. Close the skin with a single 8-0 continuous suture.

4. Postoperative Care

1. Inject 0.25 ml warm saline solution s.c. for fluid replacement.
2. To antagonize narcotics inject naloxone (1.2 mg/kg), flumazenil (0.5 mg/kg) and atipamezol (2.5 mg/kg body weight) s.c. Left the animal on the warm pad until it has regained sufficient consciousness to maintain sternal recumbency. Observe the animal for at least one additional hour to register any abnormal behavior. Do not return an animal that has undergone surgery to the company of other animals until fully recovered.
3. Provide preemptive analgesia with buprenorphine (0.05 mg/kg s.c. three times per day) or an alternative analgesia according to a local institutional protocol for a minimum duration of 72 hr after the procedure.
4. The surgeon and a veterinarian should see animals regularly. In case of weight loss >15%, apathy, swelling or surgical side infection animals are euthanized using terminal isoflurane inhalation.

Representative Results

Our team could successfully perform the described technique after approximately 20 training surgeries. The most challenging part is to evert the vessel wall of the carotid artery over the cuff, because it easily flips back and multiple attempts may lead to tearing of the wall. After the training period, the mean total operative time was 60 ± 8 min. Around 20 min are required for donor harvest, 25 min for preparation of the cervical vessels and 15 minutes for anastomoses and skin closure.

For investigation of myocardial I/R-injury we performed over 200 operations in syngeneic C57BL/6 mice in various experimental settings with a surgical success rate of 92%. Early death was caused by difficulties in performing the anastomoses (n = 3), anastomotic leakage (n = 2) and cardiopulmonary failure (n = 10). One animal was euthanized after recovery due to neurological impairment. Among perioperative survivors, graft survival was >95% at 7 days (**Figure 4**). A number of syngeneic graft recipients have been kept alive for over 3 months. Despite not using antibiotic prophylaxis, we did not observe any infectious complications.

In a first series, 12 syngeneic C57BL/6 mice were transplanted for histopathological examinations (**Figure 3**). Grafts were exposed to 4 hr of cold ischemia by storage on ice at 4 °C before implantation. Transplants were harvested after 7 days of reperfusion and stained in hematoxylin and eosin (H&E) and Sirius red. Nontransplanted animals (n = 6) served as controls. I/R lead to a strong inflammatory reaction with tissue edema, hyperemia, infiltrating leucocytes, microvascular occlusion and an interstitial fibrosis. In this early series one animal died after a repeated injection of ketamine/xylazine. This complication could be further avoided by using a fully antagonizable anesthesia including midazolam, medetomodine and fentanyl.

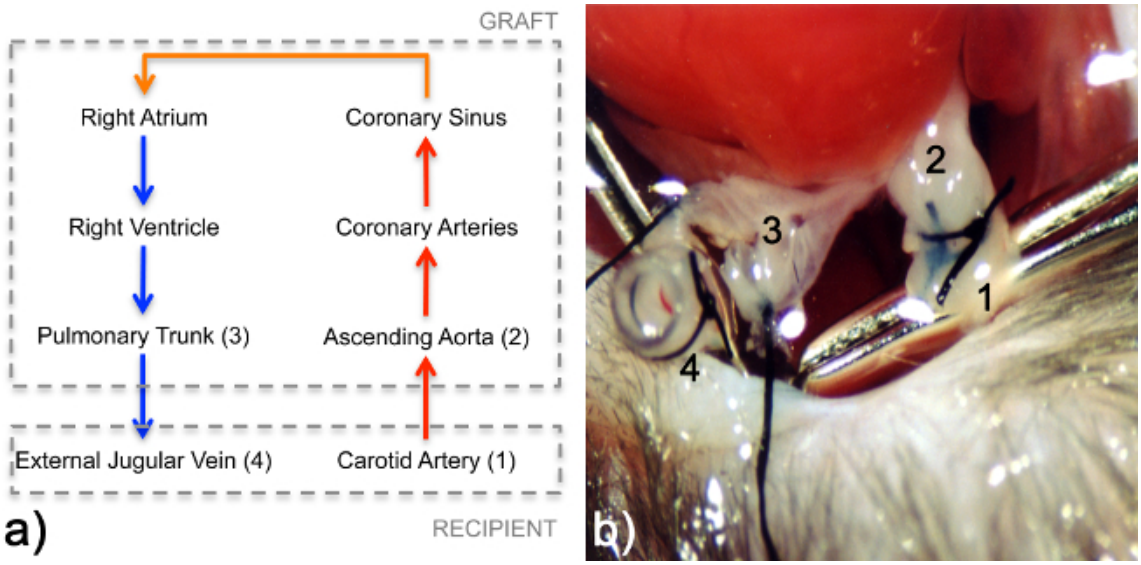

Figure 1: (**a**) Schematic Illustration of Blood Flow in Heterotopic Cervical Heart Transplantation. Blood from the recipient's carotid artery flows retrograde into the ascending aorta and into the coronary arteries to perfuse the cardiac graft. The venous blood drains into the right atrium via the coronary sinus, fills the right ventricle and is ejected through the pulmonary artery into the recipient's external jugular vein. The left ventricle is completely bypassed. (**b**) Intraoperative View of Anastomoses. The ascending aorta (2) is pulled over the cuff with the carotid artery (1) and the pulmonary trunk (3) over the cuff with the external jugular vein (4). In the picture the venous anastomosis had not yet been completed. Please click here to view a larger version of this figure.

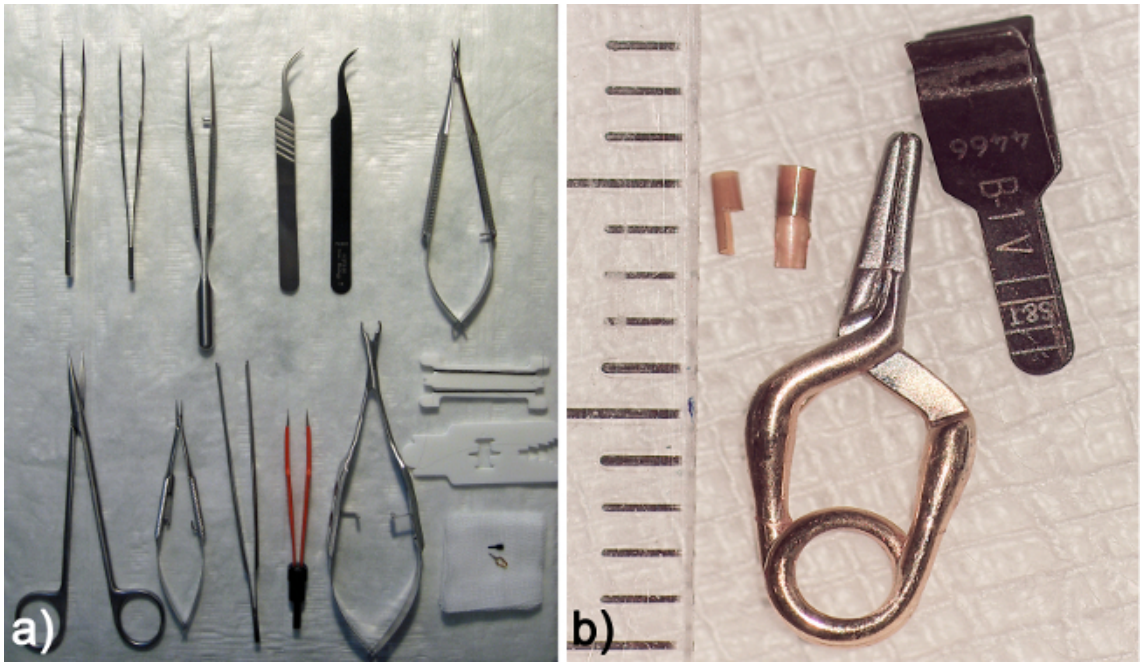

Figure 2: (**a**) A complete set of sterile microsurgical instruments including two curved forceps and two vessel dilatators is required. (**b**) For anastomoses cuffs from 19-24 G with an extension and microvascular clamps are used.

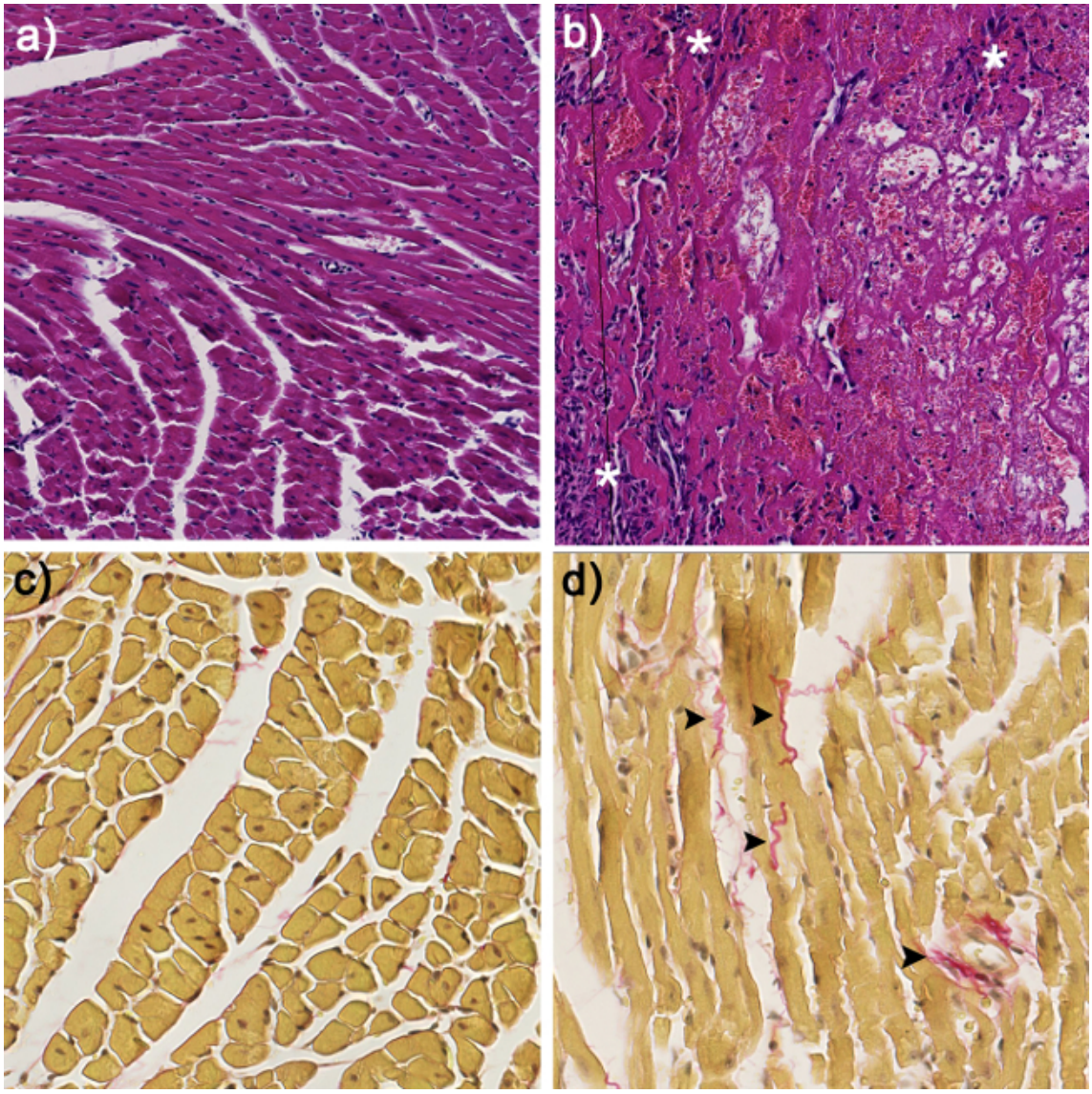

Figure 3: Histological Samples Demonstrating Myocardial I/R-injury in Syngeneic C57BL/6 Mice. H&E staining (20X magnification) of hearts from control animals **(a)** and from animals after 4 hr cold ischemia and 7 days of reperfusion **(b)**. Prolonged I/R resulted in a strong inflammatory reaction with tissue edema, hyperemia, infiltrating leucocytes and microvascular occlusion. In the same experimental setting Sirius red staining (40X magnification) showed marked interstitial collagen deposition after I/R **(d)** compared to the control group **(c)**.

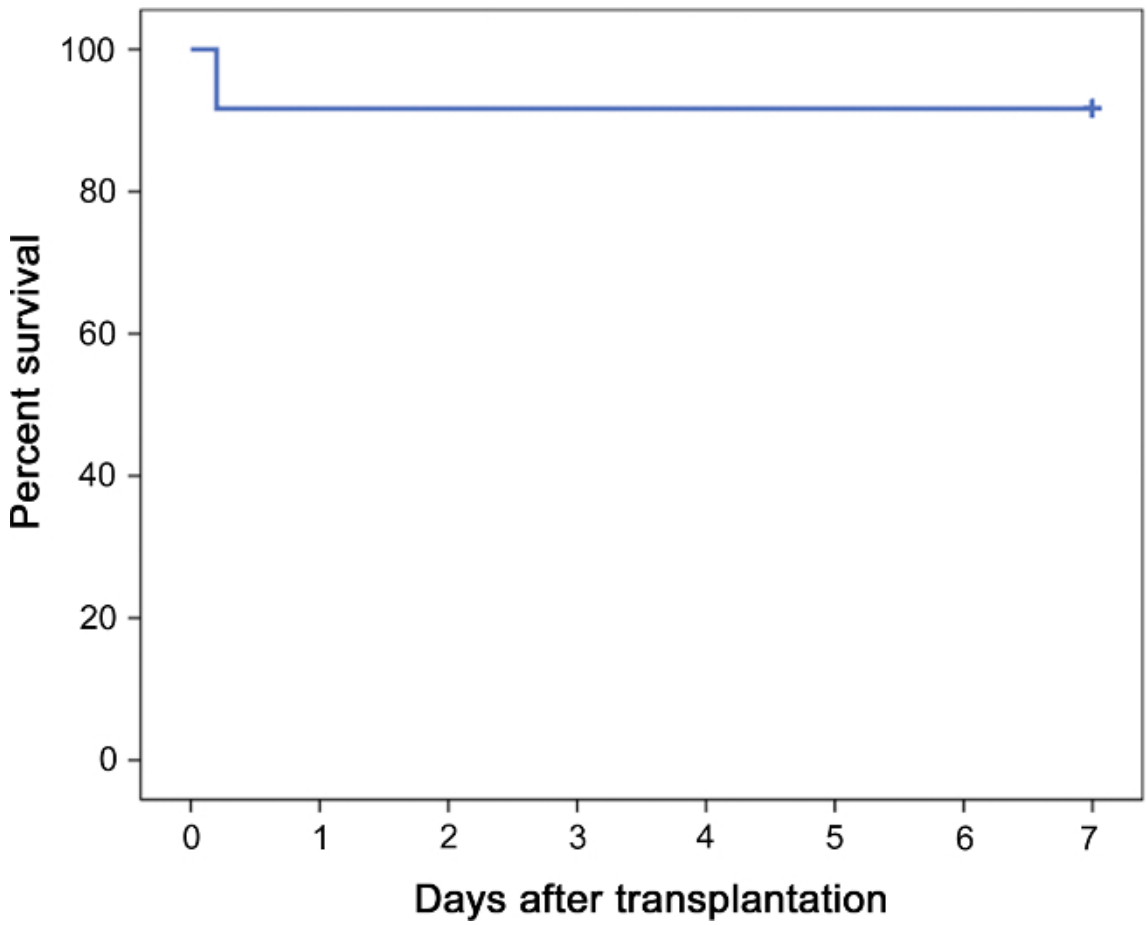

Figure 4: Cardiac Allograft Survival. Kaplan-Meier plot displaying graft survival in syngeneic C57BL/6 mice. Animals ware euthanized on postoperative day 7 for histopathological examination.

Discussion

We have completed over 200 cardiac transplants with a success rate over 90% in various experimental settings. With the technique described here, the complete procedure can be done within 60 min by an experienced surgeon. By preparing the cervical vessels before organ procurement, total graft ischemia time can be limited to 20 min. Time for implantation (warm ischemia time) is significantly shorter using the cuff technique compared to the suture technique[16].

Instead of simply excising the heart as previously described[5,17], we induce cardiac arrest by perfusing the graft with a clinically used cardioplegic solution and by hypothermia. The crystalloid Custodiol HTK solution contains 10 mmol/l potassium and low concentrations of sodium, calcium and magnesium, and effectively arrests the heart. The additives histidine, tryptophan and a-ketoglutarate reduce acidosis, provide ATP and protect cell membranes during ischemia. This resembles the clinical condition of a controlled cardiac arrest during open-heart surgery and makes this model ideal for investigation of myocardial I/R-injury. Moreover, it allows application of pharmacological agents within the cardioplegic solution at the beginning of cold ischemia. Storage of grafts at 0-4 °C is possible for a maximum time interval of 4 hr. A prolonged ischemia time may lead to myocardial hypocontractility complicated by overextension of the right heart chambers and bleeding.

In our experience, a few critical key-points will facilitate successful revascularization. By complete mobilization of the carotid artery and the use of a low profile Yasargil clamp maximum vassel length for anastomosis can be preserved. Vasospasm can be avoided by dropping 1:10 diluted papaverine onto the artery. To avoid neurological impairment the carotid artery is ligated below the bifurcation to preserve collateral circulation to the internal carotid artery. The vessel lumen is carefully dilated with a vessel dilatator with a superfine (0.2 mm) tip while avoiding tearing of the wall. In our experience the placement of holding sutures as previously described[7] is not mandatory. The selection of cuff size is crucial for successful revascularization. The inner cuff diameter for arterial anastomosis should be somewhat larger than the outside diameter of the common carotid artery (250-300 µm) to account for the mismatch with the larger ascending aorta of the graft. For mice weighing 20-25 g use cuffs from 22 to 24 G (inside cuff diameter from 0.510 to 0.643 mm) for the carotid artery and cuffs from 19 to 21 G (inside cuff diameter from 0.724 to 0.912 mm) for the external jugular vein. We prefer polyfilament suture material (silk) as this facilitates knotting. The microvascular clamp is placed on the extension of the cuff. This immobilizes the vessel and facilitates eversion of the wall.

Hypothermia must be carefully avoided since it prolongs recovery and is a major cause of perioperative mortality. Antagonizable neuroleptanalgesia using midazolam, medetomodine and fentanyl provides good surgical tolerance and fastens recovery compared to ketamine/xylazine[18].

Although the cervical heart transplant model is a non-physiologic, non-loaded heart model due to the unique directionality of intracardiac blood flow, it is an indispensable tool in experimental cardiac transplantation. The technical details shown in this video paper should allow researches to establish this model relatively easy in their laboratories.

Disclosures

The authors have nothing to disclose.

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