Published in final edited form as:

J Surg Res. 2016 February ; 200(2): 707–713. doi:10.1016/j.jss.2015.08.039.

A simplified cuff technique for abdominal aortic transplantation in mice

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Allograft arteriopathy is still a leading cause of late organ failure (1). Over the last two decades, the abdominal aortic interposition graft model has helped address various mechanisms of graft arteriopathy including, but not limited to, immunologic etiologies as well as effects of specific vascular interventions. The rat aortic allograft model was originally described in the 1990's; however, compared with rat model, the mouse model has advantages of the availability of hundreds of inbred transgenic strains, shorter gestational times, abundant supplies of biological reagents suitable for murine studies, and clear immunologic applications (2, 3). The first heterotopic aortic transplantation model in the mouse was described by Koulack and coworkers in 1995(4). This accepted model and anastomotic technique has been improved by several groups over various iterations (5-10). However, technical difficulties associated with the anastomosis of these small vessels continue to limit its widespread use. A new method permitting rapid aortic transplantation would be of great value in transplantation modeling. To that end, we introduce a simplified and improved procedure utilizing a cuff technique which could be used in orthotopic or heterotopic abdominal aortic transplantation.

Materials and methods

Animals and Equipment

Male Balb/c (H-2^d) and C57BL/6 (B6;H-2^b) mice (8-10 weeks) were obtained from Jackson laboratory (Bar Harbor, ME). All mice weighed 25-30 g and were housed under specific pathogen-free conditions at the Medical University of South Carolina (Charleston, SC) and were used for transplantation studies. In the following series, transplants to male B6 mice were performed under clean conditions using Balb/c donors in the allograft group (Balb/c to B6, n=20) and B6 donors in the isograft group (B6 to B6, n=10). The mice were anesthetized with intraperitoneal injections of ketamine (75 mg/kg, Lloyd Laboratories, Shenandoah, Iowa, USA) and xylazine (16 mg/kg, Vedco, Inc., St. Joseph, MO, USA) diluted in sterile normal saline. An operating microscope with 16×magnification was used

for the surgical procedure. The cuff used for the anastomosis consisted of a 0.6 mm body and a 0.6 mm extension crafted from a 24 gauge polyimide catheter (inner diameter 0.5 mm, wall thickness 0.025 mm, cat. No. 141-0027, Vention Medical, NH, USA). All procedures were approved by the Medical University of South Carolina Committee for Animal Research in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Donor Operation

After satisfactory anesthetic was administered, the donor mouse was placed in supine position and a midline thoraco-abdominal incision was made. 0.4 mL of saline containing 50 U of heparin (SAGENT Pharmaceuticals, IL, USA) was injected into the inferior vena cava as the initial step. For orthotopic transplantation, the bowel was reflected cephalad, the colon was retracted to the animal's left side, and the seminal vesicles and testes were distracted caudally. The segment of aorta between the left renal artery and its bifurcation was separated from the inferior vena cava and harvested (Figure 1a). For heterotopic transplantations, the left lung was reflected to the animal's right side across midline and the diaphragm was incised in order to achieve better exposure. The descending aorta and the intercostal arteries originating from descending aorta were isolated from the surrounding tissues (Figure 1b). All intercostal arteries were cauterized with a low-temperature adjustable fine tip cautery (Bovie Medical Corporation, FL, USA). The distal segment of descending aorta near the diaphragmatic hiatus was harvested and the aortic graft was placed on gauze and flushed free of remaining blood with saline. Once prepared, a 24 gauge cuff was fashioned for insertion into both ends of aortic graft. The cuff extension was handled with fine-tip forceps and the aorta was inserted inside the cuff and everted exposing the endothelial surface (Figure 1c). The cuff was secured around the aorta with 10-0 nylon suture tie (AROSurgical Instruments corporation, Newport Beach, CA). The donor aorta was stored in ice-cold UW solution for further experimentation (Bridge to Life Ltd, SC, USA).

Recipient Operation

The recipient was placed in a supine position, with the tail facing the operator. A midline abdominal incision was performed from the xyphoid to pubic symphysis and a self-retaining retractor was placed to expose the abdominal contents. The intestine, wrapped in moistened sterile gauze, was gently reflected cephalad, the seminal vesicles and testis were covered and retracted caudally. The mesosigmoid was divided and a strip of sterile gauze was allowed to pass under the sigmoid colon, which was used to retract the sigmoid colon to the animal's left side (Figure 2a). 0.2 mL of saline solution containing 25 U of heparin was injected into abdominal cavity. Using 2 super fine tip forceps and cautery, the retroperitoneal tissue surrounding the abdominal aorta and inferior vena cava (IVC) including the reproductive vessels were divided, which allowed for exposure of lumbar arteries and veins between the renal vessels and the iliac bifurcation. The lumbar vessels were cauterized and isolated from abdominal aorta and IVC (Figure 2b), and lumbar arteries to the right were suture ligated below the right lumbar vein (Figure 2c). The infrarenal aorta between the left renal artery proximally and iliac bifurcation distally was dissected free and fully mobilized. The proximal and distal portions of the aorta were clamped by 2 stainless steel micro-serrefines (Fine Science Tools, CA, USA) (Figure 3a and 3b). A small incision was made at the

proximal and distal ends of the aorta with vannas spring scissors (Fine Science Tools, CA, USA) and the arterial lumen was flushed with heparinized saline through the incisions. A suture was used to retract the lateral wall of the incised artery to the animal's left side (Figure 3c). The opposite arterial sidewall was grasped with super fine tip forceps, and the cuffed end of donor aorta was inserted into recipient's proximal arterial lumen (Figure 4a), and fixed by a circular ligature of 10-0 silk suture (Figure 4b). The distal side of the cuffed end was inserted in a similar fashion (Figure 4c). The curved donor aorta was lifted gently and the recipient's native aorta was resected (Figure 5a). The entire abdominal aorta including the donor aorta was then straightened in line (Figure 5b). The distal and proximal clamps were released in sequence, and pulsatile flow in the donor arterial segment was clearly visible (Figure 5c). The abdominal contents were returned to the abdominal cavity, and the incision was closed with a running 4-0 silk suture.

Postoperative Care

After the operation, 1 ml of sterile saline was administered to the recipient animal by subcutaneous injection for fluid resuscitation. The recipients were separated in a single cage, placed on a heating pad postoperatively for 24 hours and given regular food and water ad libitum. No antibiotics or immunosuppression were administered.

Histopathological examination and statistical analyses

Technical success was defined as survival without paralysis during the first 7 days after transplantation. Under anesthesia, the abdomen was exposed and after visual examination the segment of the grafted aorta between proximal and distal sutures was harvested. The graft was subsequently embedded in paraffin. Four micrometer aortic sections were stained with Hematoxylin and Eosin (HE) and Verhoeff-van Gieson's staining kit (EvG, HT25A, SIGMA, St. Louis, MO, USA). Morphometric analysis of transplant arteriosclerosis on VVG stained sections were measured as described (11). Briefly, percentage intimal expansion (IE) was calculated using the following formula: % intimal expansion = $((A_I - A_L)/A_I) \times 100$, where A_I is the area with the internal elastic lamina and A_L is the luminal area. Statistical evaluations were performed using Graphpad Prism software. Statistical significance was determined using Mann-Whitney U test. $P < 0.05$ was considered statistically significant.

Results

Graft Outcomes

Thirty aortic grafts were performed with this cuff technique for anastomosis in 10 isogenic and 20 allogeneic combinations. Implantation of grafts could be completed in an average of 23 minutes (range, 18-27 minutes). There was no technical failure in all 60 anastomoses. Two animals died of unrelated causes (no paralysis, bleeding or thrombosis) during the follow-up period, 1 of dehydration at day 4, the other of sepsis at day 7. The overall survival rate was 93.3%. None of the grafts were occluded at examination on the day the mice were killed.

Histopathology

At day 28 after implantation, all isografts and allografts were harvested for histologic examination respectively. On gross inspection, all grafts were patent. For isografts, no evidence of rejection was found (Figure 6a). H&E staining of allografts showed diffuse infiltration and typical aspects of chronic rejection (Figure 6b). The intima of allografts was thickened (Figure 6c and 6d) and the percentage of intimal expansion was higher than isografts (Figure 7, $P<0.01$).

Discussion

The aortic allograft model in rodents has been used to study chronic rejection and has given useful information in the development of graft arteriosclerosis (1, 3, 4, 12, 13). Compared with the rat model, mouse models offer many advantages including ample progeny, wide availability of identical genetic background strains, and lower experimental cost. Although aortic graft transplantation models in mice have been applied to study transplantation immunity by several groups (1, 12, 13), technical problems associated with the anastomosis of mouse aortas have stymied their global acceptance. A routine technique related to aortic implantation in mice is an end to end suture anastomosis (4, 7, 8). In fact, our own studies have previously successfully applied the end to end anastomotic technique to a humanized aortic interposition graft model utilizing human arterial segments into immunodeficient mice as a preclinical in vivo assay (1, 14). However, this accepted standard technique results in a higher rate of thrombosis (4) (15% to 20%), perhaps because of endothelial injury during the anastomosis and long anastomotic times (30-60 min) (4, 6). Since 1995, several modified techniques have been developed in anastomosis of small vessels, which include end to side suture anastomosis(6, 7), a temporary assisting suspension suture technique(8), the sleeve technique(10), and an “underwater” anastomosis by intermittent irrigation(15). However, the technical difficulty of this anastomosis still exists, which requires experienced microsurgical skills and long-term training.

The cuff technique has been applied to anastomose small vessels in many in vivo mouse models, such as the heterotopic heart transplantation (16-18), liver transplantation (19, 20), limb transplantation (21), and even in lung transplantation (22). This technique has proven to be convenient without requirement of intensive training for microsurgical vascular anastomosis. To our knowledge, there has been only one limited report describing aortic transplantation utilizing cuff technique by Yukihiro et al. until now (9). In this report, the author chose the cervical area as an implantation site; however, as illustrated by the author, the length of the mouse common carotid artery remains a limitation to the cervical cuff technique. In fact, we also encountered similar problems when performing cervical heart or limb transplantation in our own laboratory. The novel model described here utilizes the abdominal aorta between left renal artery and the bifurcation which offers enough length for comfortable anastomosis. In fact, Yukihiro et al. also attempted to transplant into the infrarenal aorta with cuff technique; but was unsuccessful due to complications such as paraplegia or ischemia of the lower extremities after transplantation. In our opinion, aortic graft occlusion was encountered in Yukihiro's reports secondary to an inappropriate cuff size. The cuff used in their report was a thick-walled Teflon tube (internal diameter 0.4 mm,

wall thickness 0.2 mm). However, the polyimide catheter reported here was a thin-walled tube (inner diameter 0.5 mm, wall thickness 0.025 mm). In our experience and others, this could be an explanation as to why we did not experience thrombus formation due to the cuff technique in our study (5, 18). Another reason for our success and low thrombotic rate could also be explained by less damage to the aortic endothelial layer during the anastomosis process and no exposed suture to blood flow intraluminally after anastomosis and reperfusion.

Compared with conventional suture techniques, we could rapidly finish the entire recipient operation within 30 minutes with 10-15 minutes of cross-clamp aortic occlusion time. In our experience, this is approximately 50% less time than previously necessary to perform the suture technique, which contributes to higher survival rates and faster recovery time. As reported by the literature, researchers often apply infrarenal aortic occlusion for 30-60 minutes to mimic spinal cord injury in rabbits and rats (23-25). Although this model of injury can also occur with thoracic aortic occlusion in mice (26), we found that infrarenal aortic occlusion longer than 30 minutes, resulted in an increasing hindlimb paralysis rates after the operation because of more spinal cord injury and longer ischemic times of hindlimbs when we performed abdominal heart transplantation or aortic interposition grafts using the conventional end to end suture technique. The most difficult part of our novel model is separation of the infrarenal aorta from the IVC. In our experience, we first recommend using cautery to coagulate the lateral lumbar vessels. We then recommend dissecting periaortic tissues over the lateral side of the aorta, and then suture ligating the lumbar arteries on the medial side of the IVC, which makes it unnecessary to isolate the lumbar artery from IVC. If the lateral side of aorta is clear of tissue, it is much easier to identify the space between the aorta and the IVC after reflecting the aorta laterally (Figure 3a). Another modification that we employed was that we refrained from excising the recipient aorta until the anastomosis was complete, which also made the cuff technique much easier. Each cuff anastomotic step took only 3-5 minutes. If the abdominal aorta was separated from the IVC, grafted aorta could be straightened and lengthened from curved shape immediately after excising the native abdominal aorta. The step of straightening and lengthening is of extreme importance because increased turbulent flow caused by the curved aorta will contribute to thrombosis, which also has been confirmed by aortic arch transplantation(6) and end to side anastomotic studies(7). In conclusion, we have developed an innovative, stable, and simple aortic transplantation model in mice, which is useful for vascular research in transplantation and beyond.

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Figure 1.
Donor operation (a) orthotopic graft. (b) heterotopic graft. (c) cuffed graft.

Figure 2.
Recipient operation (a) operation area exposure. (b) lateral branches cauterized. (c) suture ligation of medial lumbar artery.

Figure 3.
Recipient operation (a) the space between aorta and IVC. (b) mobilization of infrarenal aorta. (c) the proximal end for the cuffed graft.

Figure 4. Recipient operation (a) proximal anastomosis. (b) fixed by 10-0 ligature. (c) the distal end for cuffed graft.

Figure 5.
Recipient operation (a) excision of recipient aorta. (b) straight-stretched graft. (c) after releasing the occlusion clamps.

Figure 6.
Light microscopy of aortas (representative staining, 28 days after implantation). (a) isografts and (b) allografts (H&E). (c) isografts and (d) allografts (EvG).

Figure 7.
Comparison of intimal expansion percentage between 2 groups (28 days after implantation).*, $P < 0.01$