Video Article

Murine Kidney Transplant Technique

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Abstract

The first mouse kidney transplant technique was published in 1973[1] by the Russell laboratory. Although it took some years for other labs to become proficient in and utilize this technique, it is now widely used by many laboratories around the world. A significant refinement to the original technique using the donor aorta to form the arterial anastomosis instead of the renal artery was developed and reported in 1993 by Kalina and Mottram [2] with a further advancement coming from the same laboratory in 1999 [3]. While one can become proficient in this model, a search of the literature reveals that many labs still experience a high proportion of graft loss due to arterial thrombosis. We describe here a technique that was devised in our laboratory that vastly reduces the arterial thrombus reported by others [4,5]. This is achieved by forming a heel-and-toe cuff of the donor infra-renal aorta that facilitates a larger anastomosis and straighter blood flow into the kidney.

Video Link

The video component of this article can be found at https://www.jove.com/video/52848/

Introduction

Since 1973 the kidney transplant model in mice has been a valuable research tool, but technical issues have hampered its widespread use. Over the years several papers have been published detailing improvements/refinements to this procedure. As a model of primarily vascularized solid organ transplantation this procedure is probably second only to the heterotopic heart transplant model which was also devised by the Russell laboratory in 1973 [6]. Both models lend themselves to research into allogeneic rejection responses, the development of delayed graft function and ischemia reperfusion injury.

One of the most common issues to be reported with kidney transplantation is the relatively high incidence of arterial thrombosis [4,5,7] which we also experienced in our laboratory. Therefore we set out to perform a literature review of thrombus formation and possibly find the cause of this technical issue and to also devise a possible solution. The most likely cause of thrombosis is the somewhat tortuous path the blood takes from the recipient aorta, into the donor renal aorta then on to the donor renal artery. This path causes turbulence in the renal artery which can lead to platelet activation and thrombus formation. Based on the recent observations and a search of relevant literature [8-14] we came up with a new technique that has reduced thrombosis to 0%.

The technique described here varies from previously reported techniques in the formation of an arterial heel-and-toe cuff which facilities improved blood flow and significantly reduces thrombus formation. The cuff is formed by dividing the infra-renal aorta across the face of the renal arterial ostium at an angle less than 45^{o} to the longitudinal axis of the aorta (**Figure 1A & 1B**). This results in a cuff approximately 2mm in length. A venous Carrel patch is formed by transecting the renal vein into the IVC thereby increasing the diameter of the cuff. The infra-renal donor abdominal aorta heel-and-toe cuff is end-to-side anastomosed to the recipient abdominal aorta and the donor renal vein/IVC patch is end-to-side anastomosed to the recipient abdominal inferior vena cava (IVC). The ureter is then introduced into and anchored to the bladder as described by Han et al [3].

For this study untreated transplants with warm ischemia times only (*i.e.*, no cold ischemia) are compared. In this case warm ischemia refers to the time from the cessation of blood flow through the donor kidney (step 1.11 below) and reperfusion of the graft in the recipient (step 2.11 below). Cold ischemia refers to the time that the kidney is not perfused and is kept in cold storage until the beginning of the implant procedure.

Protocol

All mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were housed under pathogen free conditions at the University of Colorado Denver, Barbara Davis Center Animal Facility according to NIH Guidelines and with approval of the University of Colorado Denver IACUC.

1. Donor Kidney Harvest

1. Sterilize all instruments, wear sterile gloves throughout the procedure and maintain a sterile field. Perform all surgeries with the use of an operating microscope.
2. Surgically remove mouse donor kidneys from pentobarbital-anesthetized (60mg/kg IP) donors. Ascertain depth of anesthesia by toe-pinch, and observe the respiratory rate.
3. Clip the fur then immobilize the mouse by 4-way restraints. Prepare the skin with povidone-iodine and drape the mouse in a sterile fashion.
4. Make a 2 cm midline vertical abdominal incision and enter the abdominal cavity. Retract the bowel superiorly and externalize it on to the chest. Keep the bowel wrapped in sterile moist gauze throughout the procedure.
5. Identify the great abdominal vessels and mobilize them, identify any lumbar branches and either cauterize or ligate them with 10/0 nylon suture.
6. Immediately distal to the left renal vessels tease the inferior vena cava (IVC) and abdominal aorta (AA) apart by blunt dissection over approximately 2mm. Ligate with 10/0 nylon and divide small arterial and venous branches from the renal vessels.
7. Now separate the renal vein from the renal artery carefully by blunt dissection of these structures. This allows for the accurate creation of a Carrel patch at the proximal end of the renal vein, which will be used to form an end-to-side anastomosis to the recipients IVC.
8. Identify, ligate and divide the left adrenal vessels. This permits access to the suprarenal aorta and a 6-0 silk suture is placed around the aorta in preparation for ligation, but not tied at this time.
9. Mobilize the kidney, vessels and ureter from the surrounding facia. Rotate the kidney to the right and ligate/divide any posterior branches. Then return the kidney to the left.
10. Now direct attention to the ureter. Without disturbing the renal hilum free the ureter from the surrounding facia taking care to preserve the ureteric vessels. Divide the ureter at the level of the ductus deferens. The kidney is now ready for recovering.
11. Slowly inject 300 units of heparin into the distal IVC thereby heparinizing the donor. Tie down the 6-0 silk suture placed around the suprarenal AA and perfuse the left kidney via the distal abdominal aorta with 0.8ml of heparin saline solution (100 U/ml).
12. Once the perfusion has ceased create a venous Carrel patch immediately to eliminate backflow into the kidney. Retract the renal vein toward the kidney revealing the AA and renal artery beneath.
13. Divide the aorta adjacent to the renal artery creating a heel and toe cuff as shown in **Figure 1**. Remove the kidney, vessels and ureter from the donor.
14. Remove the kidney directly to a pre-prepared recipient (0 min cold ischemia time) or stored at 4 ^{o}C in a solution of choice for a pre-determined time until time of implantation. Euthanized the donors by exsanguanation and cervical dislocation. Total time to recover donor kidney is approx. 15-20 min.

2. Kidney Implant Technique

1. Anesthetize the recipient mouse with pentobarbital (60mg/kg IP initial dose, 25mg/kg IP supplemental dose if required). Ascertain depth of anesthesia by toe-pinch, and observe the respiratory rate. Clip the fur then immobilize the mouse by 4-way restraints and apply ophthalmic ointment to the eyes. Prepare the skin with povidone-iodine and drape the mouse in a sterile fashion.
2. Make a 2 cm midline vertical abdominal incision and enter the abdominal cavity. Retract the bowel superiorly and externalize on to the chest. Keep the bowel wrapped in sterile moist gauze throughout the procedure.
3. At this time perform a right nephrectomy. Ligate the renal artery and vein with 6-0 silk sutures. Excise the kidney distal to the sutures. Ligate the ureter with 6-0 silk and then divide proximal to the kidney.
4. Isolate the abdominal aorta and inferior vena cava (IVC) below the renal vessels. Place 4-0 cotton ties around the aorta and IVC superior then inferior to the anastomosis site. Identify and ligate any lumbar vessels within the field with 10-0 nylon suture.
5. Knot the cotton ties, first the inferior followed by the superior. In this way some blood is retained in the aorta making the aortotomy easier.
6. Form the aortotomy with a 30G needle to enter the lumen of the aorta. Extend the incision with fine micro scissors to a length of approximately 2mm.
7. Make an end to side anastomosis of the donor aortic heel-and-toe cuff to the recipient aorta in the following fashion. Place a 10-0 nylon suture stay stitch in the donor aorta and to the inferior angle of the incision in the recipient aorta and tie.
8. Place a second 10-0 nylon opposite the first in the donor aorta and the superior corner of the incision in the abdominal aorta and tie. Make a running suture line from superior to inferior in the lateral wall of the aorta and tie against the previously placed stay stitch. Then suture the medial wall in a running fashion and tie.
9. Make an end to side anastomosis of the donor renal vein to the recipient IVC in the following fashion. Puncture the IVC with a 30G needle and extend the incision with fine micro scissors. Tie the donor renal vein to the inferior corner of the incision in the IVC with 10-0 nylon. Make a running suture line between the renal vein and the IVC and tie.
   NOTE: It is also vitally important that full thickness passes of the suture needle including the vascular adventitia and the intima are achieved. Eversion of the edges also ensures that there is intima-to-intima contact, which aids in sealing and healing of the anastomoses. While a hemostatic clotting agent can be useful for reducing leaks, we recommend that a surgeon instead rely on good technique.
10. Ensure that the anastomoses are “clean”. That is, that the opposing walls are not caught when placing stitches. This will cause a significant constriction to flow that will result in a failed graft and in extreme cases to hind limb paralysis.
    NOTE: Another vitally important factor is ensuring that the tension of the anastomotic suture lines is also optimal. Too loose and there will be irreversible leaking, too tight and stricture to flow will result. If on the arterial side this will result in poor perfusion of the graft, if on the venous side a congested kidney will result.

11. Release the distal 4-0 cotton tie re-establishing venous flow. Once hemostasis of the venous anastomosis has been observed gradually loosen the proximal 4-0 cotton tie and observe the arterial anastomosis for hemostasis.
12. Remove the cotton ties from the mouse once both anastomoses are considered secure. Pierce the bladder with a 20 G needle creating two holes.
13. Pass the tips of curved forceps through the holes and pull the donor ureter through the bladder with the forceps with the proximal end of the ureter being anchored to the bladder wall with two 10-0 nylon sutures. Trim the excess length of ureter protruding from the second hole allowing the ureter to retract within the bladder and close the hole with two 10-0 nylon sutures.
14. Return the bowel to the abdomen. Close the abdominal wall in two layers using 5-0 Silk suture in a running fashion.
15. Administer a 1.0 ml bolus of sterile, warm normal saline into the abdomen as fluid resuscitation upon closing, and inject 0.8 ml of normal saline subcutaneously post-operatively. No other supportive measures are required during the surgery.
16. Recover the animal on a warming blanket. Total implant time is approx. 35-45 min. Administer analgesics such as buprenorphine, 0.05 mg/kg, SC, 0.1-0.2 ml at the beginning of the procedure and every 6-12 hr for 72 hr post-op.

3. Contralateral Nephrectomy

1. Depending on the protocol requirements, several days after the implant procedure, perform a contralateral nephrectomy under isoflurane anesthesia (5% inhaled isoflurane for induction, 1.5-2.0% for maintenance).
2. Enter the abdominal cavity and gently retract the bowel to the animal's right. Expose the left kidney and blunt dissect from the surrounding fascia.
3. Ligate the renal artery, vein and ureter with 6-0 silk sutures and then excise the kidney above the sutures and remove the kidney. Total operative time is approx. 10 min.

4. Graft Assessment

1. Measure serum creatinine using the alkaline picrate method (Jaffe reaction).

Representative Results

This surgical technique allows for either simple graft survival/rejection studies, or quite complex experimental protocols. In the figures below we demonstrate the advantages of using this improved arterial anastomosis technique. Using this technique we have significantly reduced the incidence of arterial thrombosis from 35% to 0% thus increasing productivity. We have used this technique for over one year with the same 0% thrombosis result maintained. **Figure 1** describes the method for the formation of the arterial heel-and-toe cuff which is the basis of this new technique. This cuff provides for a longer anastomosis and a straighter blood flow path into the kidney. Both of these points result in significantly lessened turbulence thereby reducing the likelihood of platelet activation and thrombus formation.

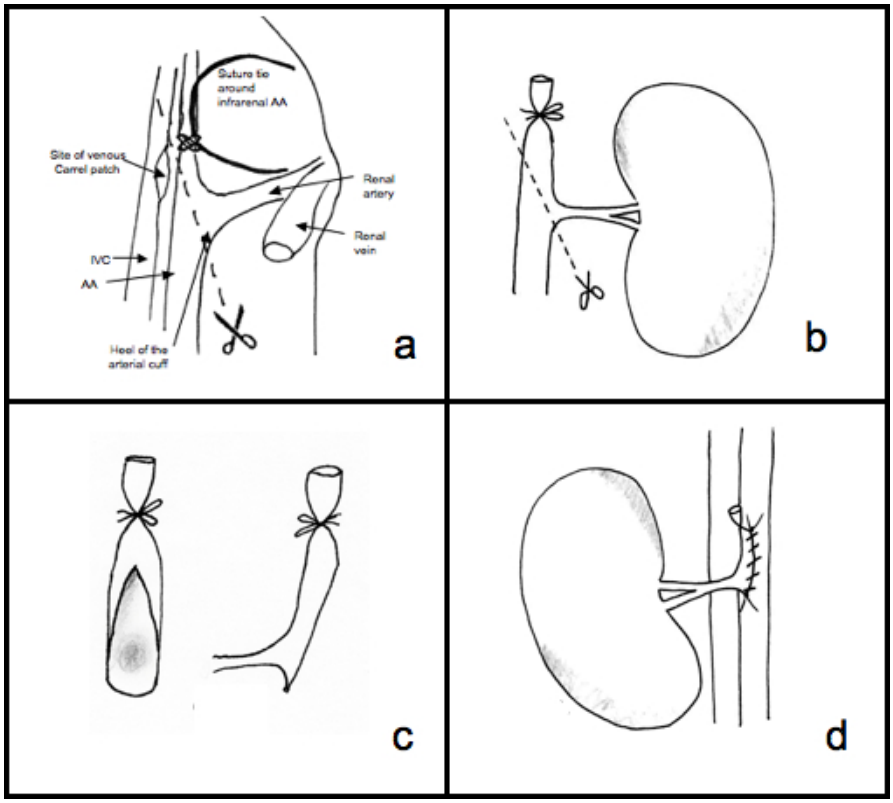

Figure 1. Schematic representation of the preparation of the donor cuff and the resulting arterial anastomosis. (A) A line diagram depicting the formation of the arterial heel-and-toe cuff. The cuff is formed by dividing the abdominal aorta obliquely across the renal arterial ostium at an angle less than 45^{o} to the longitudinal axis of the aorta. **(B)** A simplified diagram showing how the infra-renal aorta is divided to form the cuff. **(C)** The cuff depicting the resulting lumen. **(D)** The completed anastomosis (venous and ureteric structures not shown for clarity) results in a large cross-sectional lumen and a straight blood flow path.

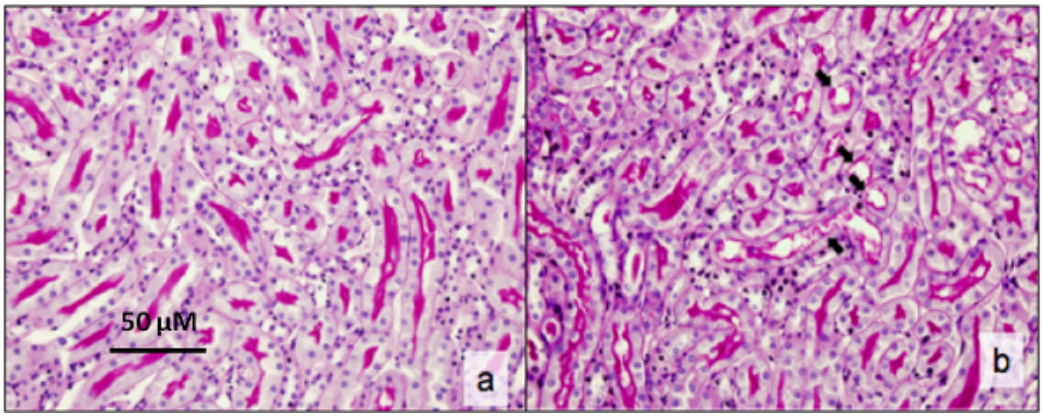

Figure 2. Immunohistochemical analysis. (A) Periodic Acid-Schiff stained section of a control non-transplanted kidney. **(B)** A syngeneic kidney transplant at POD 8 using the Revised technique demonstrates normal proximal tubular cells, which are cuboidal, with a clear cytoplasm and a round light nucleus in the middle of the cell. There is evidence of mild brush border injury (arrows) but the majority of brush borders are regular and well preserved. Magnification: 400x.

	n	Incidence of thrombosis		Warm ischemia time	
Traditional Technique	20	35%	P<0.001	38 +/- 5mins	p=NS
Revised Technique	40	0%		38 +/- 4mins	

Table 1. Comparison of thrombosis incidence and warm ischemia times between the two techniques. All values are expressed as mean ± SD. For single comparisons, normally distributed data are evaluated using unpaired, two-tailed Student t tests, and non-normally distributed data are analyzed by the nonparametric unpaired Mann-Whitney U test. P values of less than 0.05 are considered statistically significant.

Discussion

Mastering this transplant technique is difficult, but once accomplished it is a very powerful research tool. The patient surgeon/researcher will be rewarded by attention to detail and consistency of technique, which is the key to mastering any surgical procedure, even more so in small animal models. The technical difficulties of mastering the mouse kidney transplant are many folds, and it is highly probable that experience in other small animal transplant models must be gained before tackling this procedure.

It is important to ensure that the anastomoses are “clean”; *i.e.,* the opposing vascular walls must not be caught when placing stitches in order to maintain a patent lumen. Otherwise there will be significant constriction to flow that will more than likely result in a failed graft and in extreme cases lead to hind-limb paralysis. It is also vitally important that full thickness passes of the suture needle including the vascular adventitia and the intima are achieved as this results in proper evertion of the edges ensuring that there is intima-to-intima contact which aids in sealing and healing of the anastomoses. While a hemostatic clotting agent can be useful for reducing leaks, we recommend that a surgeon instead rely upon good technique.

Also attention must be paid to ensuring the tension of the anastomotic suture lines is optimal, too loose and there will be irreversible leaking, to tight and reduced flow will result. If on the arterial side this will result in poor perfusion of the graft, if on the venous side a congested kidney will result. Attaining this correct tension is a matter of practice and experience with other small animal microvascular models will be of great assistance. Above all consistency of technique and unwavering attention to detail will yield excellent mouse and graft survival, and kidney transplant function.

The limitations of this technique are governed only by the uses which can be applied by the investigator. As with any microvascular procedure, so long as good technique is observed the results should be reproducible.

The incidence of arterial thrombosis using this technique has been drastically reduced. This results in at least 1/3 of all kidney transplants being converted from potential technical failures to usable data, resulting in reduced costs and increased productivity.

As a fully vascularized, orthotopic transplant model the future applications will include rejection/tolerance studies, the phenomenon of delayed graft function and investigations into the mechanisms of ischemia/reperfusion injury.

Disclosures

The authors have nothing to disclose.

Acknowledgements

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