Journal of Investigative Surgery

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iivs20

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To cite this article: Yi Yu , Jun Li , Zirong Bi , Chenglin Wu , Shicong Yang , Qifeng Jiang , Ronghai Deng , Qian Fu , Longshan Liu & Changxi Wang (2020): Cuff Anastomosis of Both Renal Artery and Vein to Minimize Thrombosis: A Novel Method of Kidney Transplantation in Mice, Journal of Investigative Surgery, DOI: 10.1080/08941939.2020.1821264

To link to this article: https://doi.org/10.1080/08941939.2020.1821264

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Published online: 23 Sep 2020.

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JOURNAL OF INVESTIGATIVE SURGERY
https://doi.org/10.1080/08941939.2020.1821264

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Cuff Anastomosis of Both Renal Artery and Vein to Minimize Thrombosis: A Novel Method of Kidney Transplantation in Mice

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ABSTRACT

Objectives: Anastomosis of renal artery and renal vein in mouse models of kidney transplantation is technically challenging. Conventional technique using suture may result in vascular thrombosis. We developed a simple cuff method to anastomose both renal artery and vein.

Materials and Methods: Briefly, the left renal artery was occluded at the junction with abdominal aorta using a small vessel clip, transected at the renal hilum, irrigated with heparinized saline, and passed through the lumen of a seamless tubing made of polyimide. The loose end of the artery was everted over the cuff and secured using an 8-0 silk suture. The cuffed artery was inserted into the donor renal artery and secured with an 8-0 suture. Anastomosis of the renal vein was performed similarly. Isograft transplantation was conducted using BALB/c mice as donor and recipient mice ($n = 20$). The total operative time was 77 ± 3 min, and the cold ischemic time of the graft kidney was minimized to 20 min. One animal was excluded due to anatomic variant vessels and another one died at three day after surgery without thrombosis.

Results: Serum creatinine increased insignificantly after transplantation and remained stable over 12 weeks posttransplant. Five recipient mice were sacrificed for histologic examination at 12 weeks after transplantation. No vascular thrombosis was observed at the site of anastomosis. The isografts showed no evidence of acute and chronic lesions such as extinctive ischemic sclerosis and interstitial fibrosis.

Conclusion: In summary, cuff anastomosis can be used to eliminate thrombosis formation in the mouse model of kidney transplantation.

ARTICLE HISTORY
Received 12 July 2020
Revised 20 August 2020
Accepted 3 September 2020

KEYWORDS
Kidney transplantation; cuff anastomosis; animal model; renal artery; isogeneic graft; vascular thrombosis

Introduction

Murine models are important tools for studies of kidney transplantation due to availability of a variety of genetically well-defined strains and a widest range of molecular probes and reagents. Murine kidney transplantation model was originally described by Skoskiewicz et al. in 1973 [1], with subsequent modifications over the last three decades [2]. Anastomosis of renal artery and renal vein between the donor and recipient mice is conventionally conducted using sutures and requires extensive training in microsurgical technique. Cuff technique has been utilized to anastomose small vessels in animal transplantation models of other organs, including the heart [3–5], liver [6, 7], limb [8], and lungs [9]. Chen et al. recently reported a rapid "cuffed" technique for renal vein anastomosis in mouse kidney transplantation [10]. Inspired by Chen's study, we developed a cuff technique to anastomose both renal artery and vein in mice. The following is a summarization of the method and long-term results of isograft transplantation.

Materials and methods

Animals and equipment

The study protocol was approved by the Institutional Animal Use and Care Committee of Sun Yat-sen University (#2019000134). All experiments were conducted in strict accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (USA National Institutes of Health publication No. 80-23, revised 1996) and the institutional policies of Sun Yat-sen University. All efforts were made to minimize animal suffering. Adult BALB/c (H-2d) male mice (8-12 weeks; 22–25 g; Vital River Laboratory Animal

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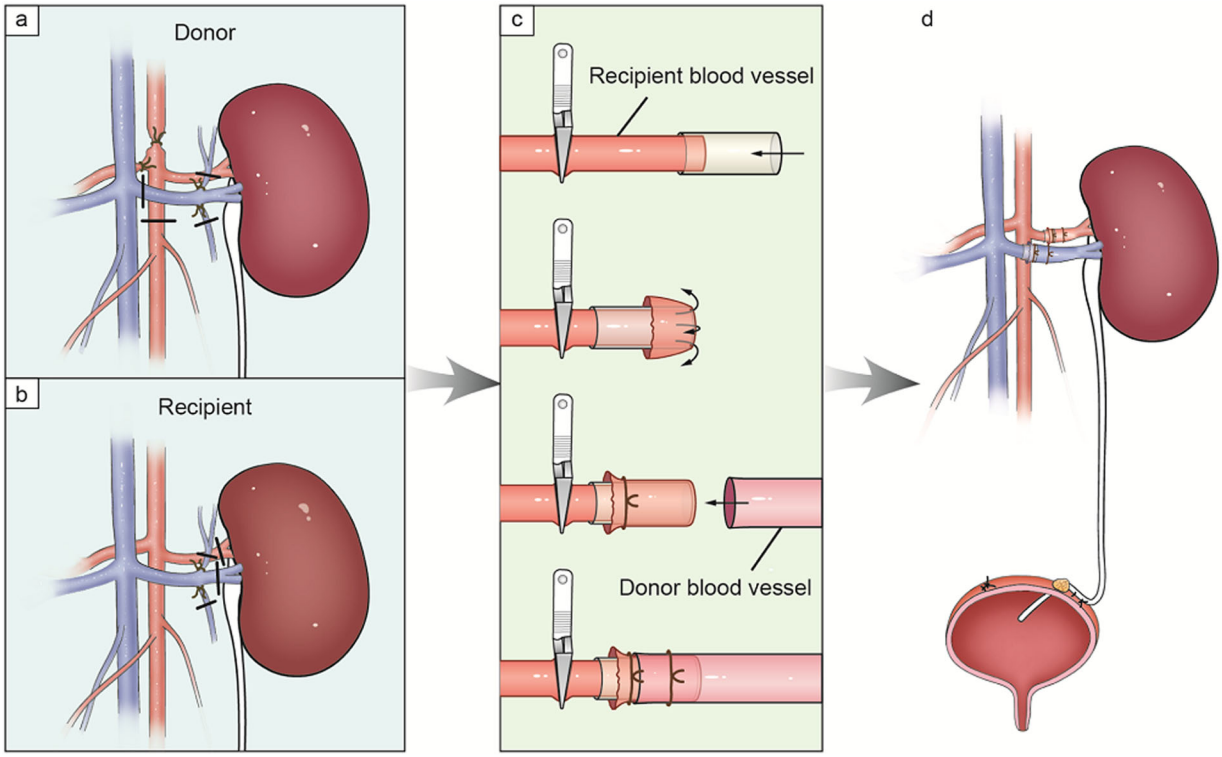

Figure 1. Diagrammatic drawing of mouse kidney transplantation procedures using the cuff technique. (a) donor vessels operation. Straight short lines indicate vessel incision sites and silk lines indicate site of ligature and transection. (b) The recipient vessels operation. (c) The cuff technique. Arrows represent direction of the cuff. (d) Ureterovesical anastomosis is done by transposing the ureter into the bladder.

Technology, Beijing, China) were housed in a specific pathogen free facility. Isograft transplantation was conducted using BALB/c mice as both donor and recipient. The artery cuff used for the anastomosis was made with a seamless capillary (inner diameter: 0.3 mm; thickness: 0.08 mm; Shanghai Qijie Macromolecule Material Co.; https://www.pvc123.com/b-taichang439/) and was clipped to approximately 1 mm in length directly. The vein cuff was made with the inner tube of a 24G intravenous catheter.

Surgical technique

The left kidney, together with the renal artery and vein, was removed from the donor mice under anesthesia with intraperitoneal pentobarbital, as previously described by Tse et al. (Figure 1a) [11], perfused with heparinized Ringer's solution (25 U/mL) and stored at 4 °C prior to transplantation into recipients. Efforts were made to avoid electrocoagulation when ligating the donor kidney vessels.

A mid-line abdominal incision was made to recipient mice under pentobarbital anesthesia. The left kidney was then harvested with enough length of renal artery and renal vein for cuff anastomosis (Figure 1b). The left renal artery was occluded at the junction with abdominal aorta using a small vessel clip, transected at the renal hilum and irrigated with heparinized saline (100 U/mL). Then the loose end of artery was passed through the lumen of a seamless tubing artery cuff made of polyimide (inner diameter: 0.3 mm; length: 1 mm) (Figure 2a), everted over the cuff and secured using an 8-0 silk suture (Figures 1c and 2b). The cuff was soaked in heparinized Ringer's solution, and a small amount of 125-IU/mL heparinized Ringer'solution was applied onto the cannula before use.

The cuffed renal artery in the recipient mice was aligned with and inserted into the donor renal artery and secured with an 8-0 suture. Anastomosis of the renal vein was performed in the same manner, except using the tubing in a 24 G intravenous indwelling needle as the cuff. After the clamps on the vein and artery were released, pulsation in the renal artery could be observed momentarily (Figure 2c). After uretero-bladder anastomosis as described by Han et al. [12], urine discharge typically occurred within 2–4 minutes (Figures 1d and 2d). The right kidney was removed immediately. After abdominal closure, animals were kept warm until they had fully recovered from anesthesia.

Renal function and histopathological examination

Renal function of recipients was monitored for 12 weeks. Autopsy was performed to observe anastomosis thrombosis if the recipient mice died within 4 days. Data were presented as mean ± the standard deviation (SD). Differences were considered as significant at $p < 0.05$. A paired t-test was used to compare the serum creatinine between day 0 and week 12 after transplantation.

Results

Isograft transplantation was conducted in 20 mice using the cuff technique. One animal was excluded from this study due to anatomic variant vessels and another one died three day after transplantation, which was considered as technical failure although no vascular thrombosis was found. Total

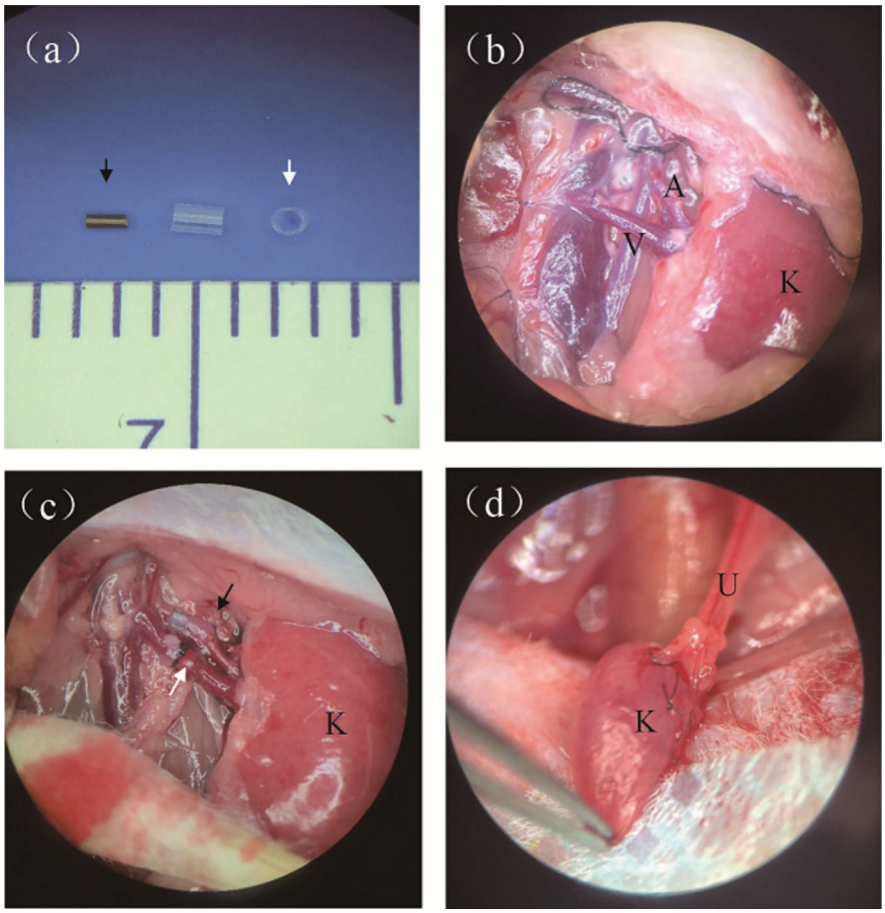

Figure 2. Mouse kidney transplantation using the cuff technique. (a) Preparation of recipient cuff. Black arrow represents an artery cuff and the white arrow indicates a vein cuff. (b) Donor operation and isolation of renal vessels. Non-essential vessel branches and fats are removed, and the vessel trunks are preserved. Letter A represents the renal artery and V represents the renal vein. (c) After the cuff is placed successfully, the donor kidney is perfused. Black arrow indicates position of the artery cuff and white arrow indicates position of the vein cuff. (d) Ureteral implantation. Letter K represents donor kidney and U represents the ureter.

Table 1. Operation time.

	Mean(SD)	Range
Total operative time, min	77 ± 3	70–83
Donor operative time, min	25 ± 3	21–33
Donor kidney warm ischemia time, sec	11 ± 3	6–18
Donor kidney cold ischemia time, min	24 ± 3	20–32
Recipient operative time, min	52 ± 4	46–62
Vessels separation, min	13 ± 3	10–20
Arterial anastomosis, min	7 ± 1	4–11
Venous anastomosis, min	7 ± 1	4–12
Uretero-bladder anastomosis, min	13 ± 2	9–19
Right kidney resection, min	5 ± 1	4–7
Abdominal closure, min	5 ± 1	3–8

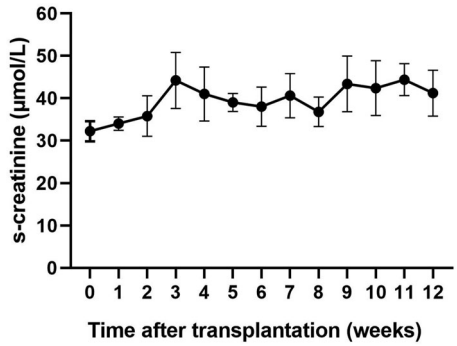

Figure 3. Renal function remains stable over 12 weeks in the isogenic graft recipient mice. Differences were considered as significant at $p < 0.05$. A paired t-test was used to compared the serum creatinine day 0 and week 12 and showed insignificantly increased after transplantation and remained stable over 12 weeks posttransplant.

operative time was 77 ± 3 min (range: 70–83). Donor operation lasted for 25 ± 3 min (range: 21–33) and recipient operation lasted for 52 ± 4 min. The time for vessels separation was 13 ± 3 min, with 7 ± 1 min for arterial anastomosis and 7 ± 1 min for venous anastomosis (Table 1).

No urological complications such as visible hydronephrosis were observed during the first 4 days after transplantation. All of the remaining 18 mice survived beyond 12 weeks. Serum creatinine increased insignificantly after transplantation and remained stable over 12 weeks posttransplant (Figure 3). Five recipient mice were sacrificed for histologic examination at 12 weeks after transplantation. No vascular thrombosis was observed at the site of anastomosis. The isografts showed no evidence of acute and chronic lesions such as extinctive ischemic sclerosis and interstitial fibrosis (Figure 4a).

Discussion

Mouse kidney transplantation model has many advantages, including genetic background close to humans and availability of a variety of genetically well-defined strains [13]. However,

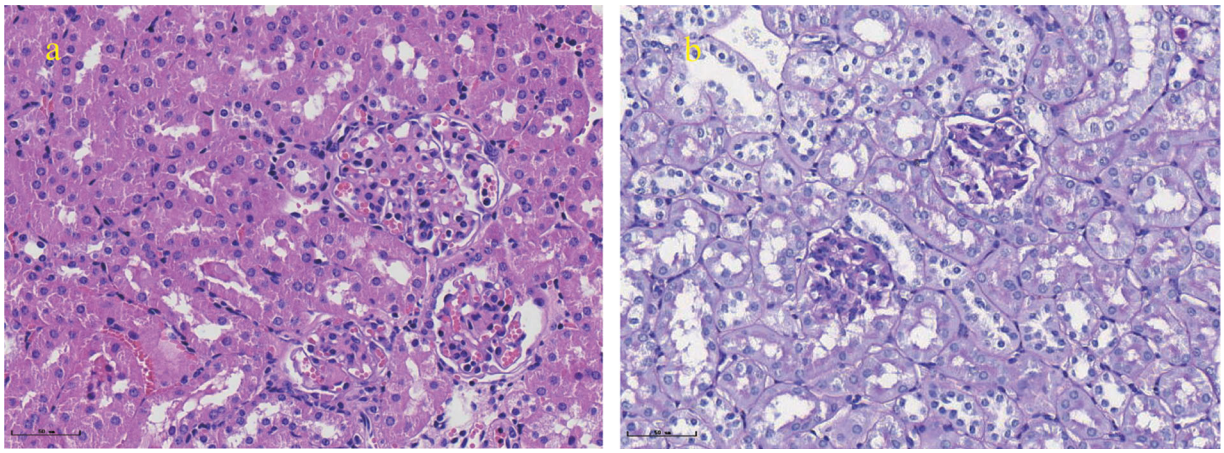

Figure 4. Representative histopathology of the transplanted kidney. At 90 days following transplantation, H&E and PAS sections exhibit no evidence of acute and chronic lesions such as extinctive ischemic sclerosis and interstitial fibrosis. Magnification, 400 x.

the difficulties and complexity of conventional suture techniques associated with vascular anastomosis limit its widespread use [14]. In this work, we developed a novel mouse kidney transplantation model, in which both renal artery and vein were anastomosed using cuffs.

Compared with conventional suture techniques in the literature, our results showed a moderate decrease of mean ischemia time (from 30 mins to 25 mins) [14]. In addition, no animals died and no renal isograft dysfunction was detected during long-term observation. Pathological analysis of renal biopsy showed no chronic lesions such as extinctive ischemic sclerosis and interstitial fibrosis. These evidences demonstrated the innovative cuff methods have no complication of renal artery stenosis or microthrombus which can lead to chronic ischemia injury and the consequent pathological lesions such as glomerulosclerosis, renal tubular atrophy and interstitial fibrosis. We did not notice any signs of vascular thrombosis and speculate that lack of vascular complications could be attributed to sutureless anastomosis of both the renal artery and vein. Lack of exogenous materials at the site of anastomosis may also minimize endothelial cell hyperplasia compared to conventional methods of suture. The cuffs could also function as stents to prevent anastomotic stenosis [15]. Our method avoided blockade of the abdominal aorta and inferior vena cava, and this may benefit recipient mice to rapid recover after surgery. Whether this feature contributed to the success of this model remains to be further investigated.

Using appropriate cuffs is critical. We respectively used seamless capillary and 24G intravenous catheter as the arterial and venous cuffs, which are easy to obtain. Another difficult part of the cuff technique is to dissociate the kidney vessels clearly, especially avoiding large tear of vessels in order to evert the vessels to wrap around the cuffs smoothly. Our experience is that we selected a 10-0 silk thread and ligated the root of the vascular branch as close as possible and avoid electrocoagulation. A large stump could affect the success rate of the cannula. A small amount of heparin solution can be applied onto the cannula before inserting into donor kidney vessels to prevent thrombosis.

Although this technique has advantages compared to the conventional methods, it has a limitation. This technique could not be applied to anatomic variant vessels such as double branch artery as it is very difficult to evert the vessels to wrap around the cuff. Our novel model shows that cuff anastomosis can serve as an important method for establishing renal transplantation model in mice.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This project was supported by the National Natural Science Foundation of China (81870511, 81670680, 81700655, 81300623), Science and Technology Planning Project of Guangdong Province, China (2015B020226002, 2017A020215012), Guangdong Natural Science Foundation (2018A030313016), Guangdong Basic and Applied Basic Research Foundation (2020A1515010884), Key Scientific and Technological Program of Guangzhou City (201903010058, 201803040011), Guangdong Provincial Key Laboratory on Organ Donation and Transplant Immunology (2013A061401007, 2017B030314018), and Guangdong Provincial International Cooperation Base of Science and Technology (Organ Transplantation, 2015B050501002).

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