A Modified Surgical Technique for Kidney Transplantation in Mice

Decheng Yin[*,1,2], **Jian Fu**[*,1,3], **Rongjun Chen**[4], **Nelli Shushakova**[5], **Ida Allabauer**[1], **Xin-Yi Wei**[1], **Mario Schiffer**[6], **Diana Dudziak**[7], **Song Rong**[*,5], **André Hoerning**[*,1]

[1] Department of Pediatric and Adolescent Medicine, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg [2] Department of Pediatric Surgery, Chengdu Women's and Children's Central Hospital [3] The Key Laboratory of Hainan Trauma and Disaster Rescue, The first affiliated Hospital of Hainan Medical University [4] Department of Department of Pediatric Kidney, Liver, and Metabolic Diseases, Hannover Medical School [5] Department of Nephrology, Hannover Medical School [6] Department of Nephrology, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg [7] Department of Dermatology, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg

[*] These authors contributed equally

Corresponding Authors

Song Rong
rong.song@mh-hannover.de

André Hoerning
Andre.Hoerning@uk-erlangen.de

Citation

Yin, D., Fu, J., Chen, R., Shushakova, N., Allabauer, I., Wei, X.Y., Schiffer, M., Dudziak, D., Rong, S., Hoerning, A. A Modified Surgical Technique for Kidney Transplantation in Mice. *J. Vis. Exp.* (185), e63434, doi:10.3791/63434 (2022).

Date Published

July 22, 2022

DOI

10.3791/63434

URL

jove.com/video/63434

Abstract

Kidney transplantation in mice is a complicated and challenging surgery procedure. There are very few publications demonstrating the key steps of this operation. Therefore, this article introduces the technique and points out the surgical caveats associated with this operation. In addition, important modifications in comparison to the conventional procedure are demonstrated. Firstly, a patch of the abdominal aorta is cut and prepared so that the proximal bifurcations of the renal artery, including the ureteral artery are transected together with the donor kidney *en bloc.* This reduces the risk of a ureter necrosis and avoids the development of a urinary tract occlusion. Secondly, a new method of the vascular anastomosis is demonstrated that allows the operator to flexibly increase or decrease the size of the anastomosis after renal transplant reperfusion has already been initiated. This avoids the development of vessel strictures and intraabdominal bleeding. Thirdly, a technique that enables the anastomosis of the delicate donor ureter and the recipient bladder that does not cause a trauma is shown. Adopting this protocol can shorten the operation time and reduces the damage to the recipient's bladder, thereby significantly increasing the operation success rate for the recipient mice.

Introduction

Since Sakowitz et al. developed mouse models of kidney transplantation in 1973 for the first time[1], it has proven as an important experimental tool to study the mechanisms of transplant ischemic injury and alloimmune rejection as well as for developing new treatments aimed to prolong allograft survival and possibly to achieve immunological tolerance.

Copyright © 2022 JoVE Journal of Visualized Experiments

However, the surgical technique has proven to be complex and very demanding, sometimes having complications such as vascular anastomotic strictures leading to prerenal non-immunological kidney transplant failure[2], postrenal failure caused by ischemia and subsequent necrosis of the transplanted ureter, strictures of the anastomosis of the transplanted ureter and/or the recipient's urine bladder leading to a disruption of the urinary outflow. All of these are reasons why renal transplantation in mice has not been further developed and is therefore not widely used. Establishing an effective and long-term stable mouse kidney transplantation model without vascular and urinary tract complications still has irreplaceable significance for many studies in the transplant field with focus on the renal immune mediated but also infectious diseases[3]. In addition, compared with other organ transplants in murine models such as lung, heart, and intestinal transplantation[4,5], the mouse kidney transplantation model offers a chance for studying long-term survival even in the setting of major histocompatibility antigen disparity[3,6]. It has also been shown that in the same setting of donor-recipient strain combinations different organ transplants such as heart or kidney are characterized by different dynamics and onsets of allograft rejection[3]. Furthermore, from the nephrological point of view, it is a more suitable model for studying parenchymal mediated immune regulatory mechanisms in the context of acute and chronic rejection events than simple skin transplant experiments.

On the basis of previous reports on the surgical technique of kidney transplantation in mice[3,7,8,9], we here demonstrate the following reliable improvements that have been successfully applied during the past 10 years within our group[10,11,12]: Firstly, the ureteral artery is safely conserved as the renal artery is resected *en bloc* together with the respective part of the abdominal aorta. Second, a new, simple, and rapid technique of a knotless vascular anastomosis in which the final stitch of anastomosis is not tied with the end of the upper tie like the traditional approach but remains free. This technique enables to increase or decrease the size of the anastomosis after renal reperfusion to avoid vessel stricture and intraabdominal bleeding. Third, 21 G and 30 G syringe needles were used as an auxiliary puncture guiding tool in order to implant the donor ureter into the recipient's bladder wall reducing the damage to the recipient's bladder and facilitating the formation of stricture free anastomosis.

In this report, we also compared the traditional, widely used technique with the modified one that is established in our laboratory and found no significant difference in the degree of renal tubular atrophy and kidney transplant interstitial tissue fibrosis. In previous studies, we additionally compared the results of this new technique with the conventional method in terms of local bleeding, thrombosis, time for performing the vessel anastomosis and survival rate. We found improvements such as significant reductions of local thrombosis events (1.1% versus 6.6%), a reduced time for the anastomosis procedure, and a highly reproducible kidney syngeneic graft long-term survival (95% versus 84% with the classical approach)[10].

Protocol

All animal experiments were conducted according to the guidelines from the directive 2010/63/EU of the European Parliament on protection of animals used for scientific purposes (Animal ethics card: Lower Saxony Ministry of Food and Drug Safety, #33.9-42502-04-11/0492). Conduct procedures using sterile surgical instruments and

Copyright © 2022 JoVE Journal of Visualized Experiments

consumables (autoclaved) and try to keep the operating area as sterile as possible.

NOTE: C57BL/6J male mice served as donors and recipients (syngeneic transplant model) while Balb/c mice served as kidney allograft recipients (model for studying acute allograft rejection model[9]). Mice were aged between 8-12 weeks, weighed ~25-30 g at transplantation and were housed under standard conditions. Data reported in this manuscript were generated by four surgeons experienced in mice surgery.

1. Preparatory steps

1. For surgery, use a set of microscopic instruments, including a micro-scissor, micro-forceps, a needle holder, micro hemostatic clamps, and an electrosurgical pen. Perform sutures using 7/0er, 10/0er, or 4/0er nylon monofilament.
2. For anesthesia, place the mouse into the box for inhalation of isoflurane (2%) for about 40-60 s in order to induce unconsciousness.
3. Once the mouse is anesthetized, weigh the mouse.
4. According to the mouse´s weight, apply an intraperitoneal injection of ketamine (100 mg/kg) + xylazine (10 mg/kg) + acepromazine (2 mg/kg) to anesthetize the mouse[13]. Confirm that the mouse is anesthetized by observing a lack of response to a toe pinch.
5. When anesthesia has taken effect, clip the abdominal fur. Then, fix the mouse on the operation table by loosely immobilizing the limbs with a sterile masking tape.
6. Disinfect the mouse's abdomen after placing the mouse on the operation table. Perform disinfection using alternating scrub of povidone iodide (iodophor) and alcohol, three times (use concentric pattern, start scrub in the middle of the abdomen and move outward), and then properly drape the mouse using a fenestrated surgical towel.
7. Apply eye ointment and maintain sterility throughout the procedure.
   NOTE: Antibiotics are not recommended throughout the procedure as these substances may influence immunological responses.

2. Donor operation procedure

1. Use scissors to cut the skin and perform a cross abdominal incision of about 3-4 cm. Cut the muscles of the abdominal wall. Cover and cautiously move away the viscera with a saline imbibed gauze.
2. Use a cotton swab to gently remove the intestines, stomach, and spleen toward the right side (from point of view of the mouse), cover and cautiously move away the viscera with a saline imbibed gauze.
3. Use micro forceps to expose the left kidney, aorta, and inferior vena cava (IVC).
4. Use an electrosurgical pencil to cauterize the left lumbar veins, including their underlying branches and other small vessels along with the left adrenal vessel, carefully.
5. Use micro scissors and forceps to dissect the left ureter and cautiously mobilize it from the surrounding tissue. Clean cut it close to the urinary bladder. Mobilize the aortic region between the left and right renal arteries approximately 2 mm in length.
6. Use micro forceps to separate the infrarenal inferior vena cava (IVC) and aorta, and then use curved forceps to pass under the aorta to place a loose tie of 7-0 silk suture around this vessel.

7. Cross clamp the area of the aorta below the right renal artery and the inferior vena cava (IVC) using two 5 mm microvascular clamps.

8. Transect the left renal vein from the vena cava.

9. Use a syringe to flush the aorta with 1 mL of heparin saline solution (60 U/mL).

10. Use micro forceps to tighten the ligature applied at step 2.5. Then, cut the aorta below the ligature as well as below the proximal clamp. With this, the proximal bifurcations of the renal artery (please note that the arterial opening must be cut neatly, otherwise it will affect the anastomosis) and the ureteral artery are included and transected *en bloc*. Prepare carefully, so that the delicate ureteral artery is completely preserved.

11. Use the electrosurgical pencil and forceps to free the left kidney and associated vessels completely by cautiously cauterizing all vessel surrounding tissue. Remove the kidney and store it in saline solution at 4 °C.

12. Euthanize the anesthetized donor mouse by decapitation.

3. Recipient operation procedure

1. Perform the initial surgical steps (including anesthesia and sterilization, see steps 1.1 to 1.7) as described for the donor mouse.

2. Use scissors to open the abdomen *via* a median incision (about 2.5 cm in length), and then cover the abdominal organs with a wet gauze using saline solution.

3. Carefully preserve the infrarenal aorta and inferior vena cava (IVC) and make sure every large vessel branch is cauterized. Use the electric cautery as well to dissect the left ureter carefully at a position proximal to the kidney pelvis. Then, remove the left kidney.

4. Use micro forceps and cotton buds to expose the abdominal aorta and inferior vena cava and detach them from the surrounding adipose tissue (approximately over 4 mm in length).

5. Use two microvascular clamps and position them proximally and distally on both the inferior vena cava and the abdominal aorta simultaneously.

6. Use a micro needle holder to guide a 10/0 monofilament (made of synthetic fiber with a smooth surface) suture needle, which is placed through the aorta wall in a proximal to distal manner.

7. Achieve an elliptical arteriotomy of approximately 1 mm with a gentle upward traction of the suture, while cutting directly below the lower face of the needle with fine, curved scissors.

8. Use micro scissors to cut the inferior vena cava (IVC) longitudinally with sufficient length of approximately 1.5 mm. Position this incision slightly below its aortic counterpart.

9. Perform the donor and recipient aorta anastomosis in an end-to-side manner. Place the donor kidney on the right side of the recipient's inferior vena cava aligning the cuff of the donor's abdominal aorta with the anastomosis of the recipient's abdominal aorta.

10. Use a micro needle holder and two separate 10-0 sutures to stitch the proximal and distal ends of the anastomosis.

11. After tying, leave the two long sutures, including the needle, in place. Sew the left side of the aortal wall of the anastomosis continuously with two evenly spaced stitches in a distal-proximal direction.

Copyright © 2022 JoVE Journal of Visualized Experiments

12. After the last stitch, guide the suture through a partial thickness of the vessel wall above the upper stay suture tie.
13. Use micro forceps to simultaneously exert gentle traction to the short end of the lower suture tie.
NOTE: In this new knotless technique, the last stitch is not tied to the short end of the upper tie.
14. Use micro forceps to turn over the transplanted kidney to its normal position. Now continuously sew the right wall of the aortal anastomosis using three stitches in a proximal to distal manner.
NOTE: Compared with the conventional surgical technique[7,8] the last suture is merged with the distal tie nearby. Do not tie it to the end of the lower suture, cut it to leave a free length of 2-3 mm instead.
15. Perform the venous anastomosis using the same suturing procedure as previously described with the difference that four to five stitches are needed for each side of the anastomosis. The final stitch is left as a free end of similar length similar to the aortal anastomosis described above.
16. After completing both anastomoses, use a dry swab to exert gentle pressure toward the sutured area for about 10-20 s.
17. Use a clip applicator forceps to remove both clamps, first the lower then the upper. Rinse the abdominal cavity with 0.9% sodium chloride at a temperature of 38.0 °C.
18. Observe the reperfusion of the transplanted kidney.

4. Ureteral implantation

1. Use a micro needle holder to penetrate through the recipient's urine bladder with a 10/0 suture (straight needle) and insert it into a 21 G needle lumen for guidance (see **Supplementary Figure 1a**).
2. Now guide the 21 G needle to stitch a hole at the place of the previous needle application (**Supplementary Figure 1b**).
3. Pull out the 21 G needle (**Supplementary Figure 1c**).
4. Use a micro needle holder and 10/0 suture to stitch (no tie) the trimmed ureter end and perforate the bladder with this 10/0 suture again at the place of its entry (**Supplementary Figure 1d**).
5. Use a micro needle holder to tow the 10/0 filament and the ureter into the urine bladder through the constructed hole (**Supplementary Figure 1e**).
6. Use a micro needle holder and another 10/0 suture to anastomose the donor's ureter to the recipient's urine bladder. Here, connect the outer membrane of the ureter to the outer membrane of the bladder wall, and perform intermittent sutures with 3 to 4 stitches. Finally, pull out the traction suture (**Supplementary Figure 1f**).
7. Use forceps to place the intestines back into the abdominal cavity. Perform two-layer sutures (first the abdominal muscles followed by the skin) to close the abdominal wound using a 4/0 filament.
8. Place the transplanted mice into an oxygen and temperature-controlled chamber for recovering after surgery.
9. For postoperative analgesia, directly give Metamizol 200 mg/kg per os after operation.
Four and 16 h after operation give Metamizol 200 mg/kg per os plus Carprofen (5mg/kg) s.c. In the further follow up, apply Carprofen (5 mg/kg) s.c. to the transplanted mice every 24 hours for three consecutive days after

operation[13]. If there are any signs of an insufficient analgesia buprenorphine 0.05 mg/kg is additionally given every 8 h s.c.

5. Contralateral nephrectomy and sacrifice of the recipient mouse

NOTE: Perform contralateral nephrectomy of the recipient mouse 5 days after transplantation.

1. Perform the contralateral nephrectomy of the transplanted mouse 5 days after transplantation under anesthesia. Ligate and cut the recipient's autologous right renal arteries and veins, remove the right kidney and close the abdominal cavity. The postoperative care and analgesia are the same as described before (see step 4.7).
2. Raise and record the state of the mouse. Provide the transplanted mouse postoperative analgesia, food, and water supply.
3. Four weeks after transplantation, sacrifice half of the transplanted mice and perform H&E staining for their kidney transplants.
4. 12 weeks after transplantation, sacrifice the remaining mice and perform Masson Gold staining of these kidney transplants.

Representative Results

Four weeks after transplantation, both the modified technique as well as the conventional technique displayed moderate signs of renal tubular atrophy[14,15] when compared to the native recipient contralateral kidneys (**Figure 1**). The degree of the renal tubules atrophy demonstrated no significant difference between the two different techniques. Masson Goldner's trichrome staining[14,15] of the kidneys 12 weeks after transplantation uniformly showed obvious signs of interstitial tissue fibrosis when compared to normal non-transplanted kidneys (**Figure 2**).

We previously investigated the outcome of this new knotless technique (n = 175) and compared it to the classical approach (n = 122) in terms of technical aspects of the procedure and intraoperative and postoperative complications (see also **Table 1**)[10]. The modified technique that is shown here was associated with a lower occurrence of intragraft arterial or venous thrombotic events (**Figure 3b**, 1.1% versus 6.6%). The time to perform the anastomosis was significantly less (**Figure 3a**), and a highly reproducible kidney graft long-term survival was achieved (95% versus 84%, $p < 0.001$, **Figure 3c**) as determined by the recipient survival 12 weeks after transplantation. In addition, the application of this modified transplant procedure does not affect the renal allograft function as assessed by serum creatinine during the observation period of 12 weeks[10].

Copyright © 2022 JoVE Journal of Visualized Experiments

	Conventional	New knotless technique
	(n=122)	(n=175)
Operation times		
Time for arterial anastomosis (min)	9.2 ± 0.09	7.5 ± 0.06**
Time for venous anastomosis (min)	9.1 ± 0.10	7.5 ± 0.05**
Complication rates		
Thrombosis	8 (6.6%)	2 (1.1%)*
Local bleeding	4 (3.3%)	1 (0.6%)
Success rate	103 (84.4%)	167(95.4%)**
Rong,S., Lewis AG., Kunter U., et al. A knotless technique for kidney transplantation in the mouse. J Transplant. Epub2012:127215,(2012).		

Table 1: Comparison of this new technique (n = 175) to the conventional technique (n = 122) in terms of technical aspects of the procedure and intraoperative and postoperative complications[10]. Numbers represent the operation times in minutes of each procedure (mean ± SD).

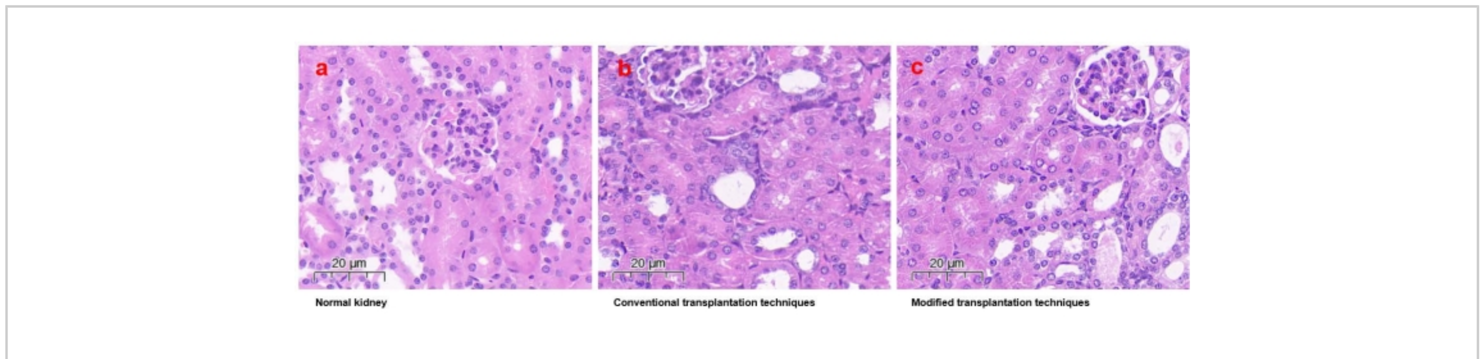

Figure 1: Representative histological results assessing tubular atrophy. HE Staining of kidney transplants 4 weeks after transplantation (40x): (a) normal non-transplanted kidney, (b) conventional technique, and (c) modified technique of a syngeneic renal transplantation. Please click here to view a larger version of this figure.

Copyright © 2022 JoVE Journal of Visualized Experiments

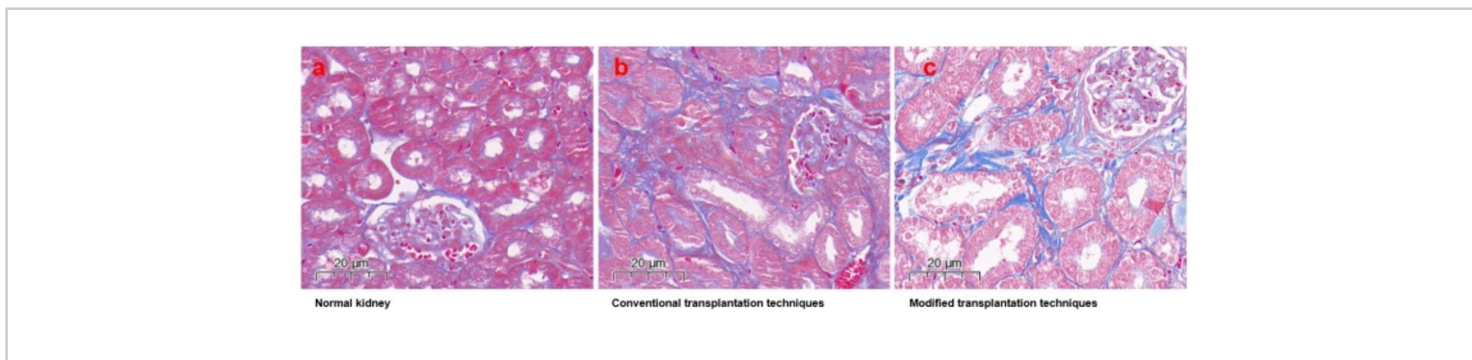

Figure 2: Representative histological results assessing interstitial fibrosis. Masson Goldner's trichrome staining 12 weeks after transplantation (40x) of (a) normal non-transplanted kidney, (b) conventional technique, and (c) modified technique of a syngeneic renal transplantation. Please click here to view a larger version of this figure.

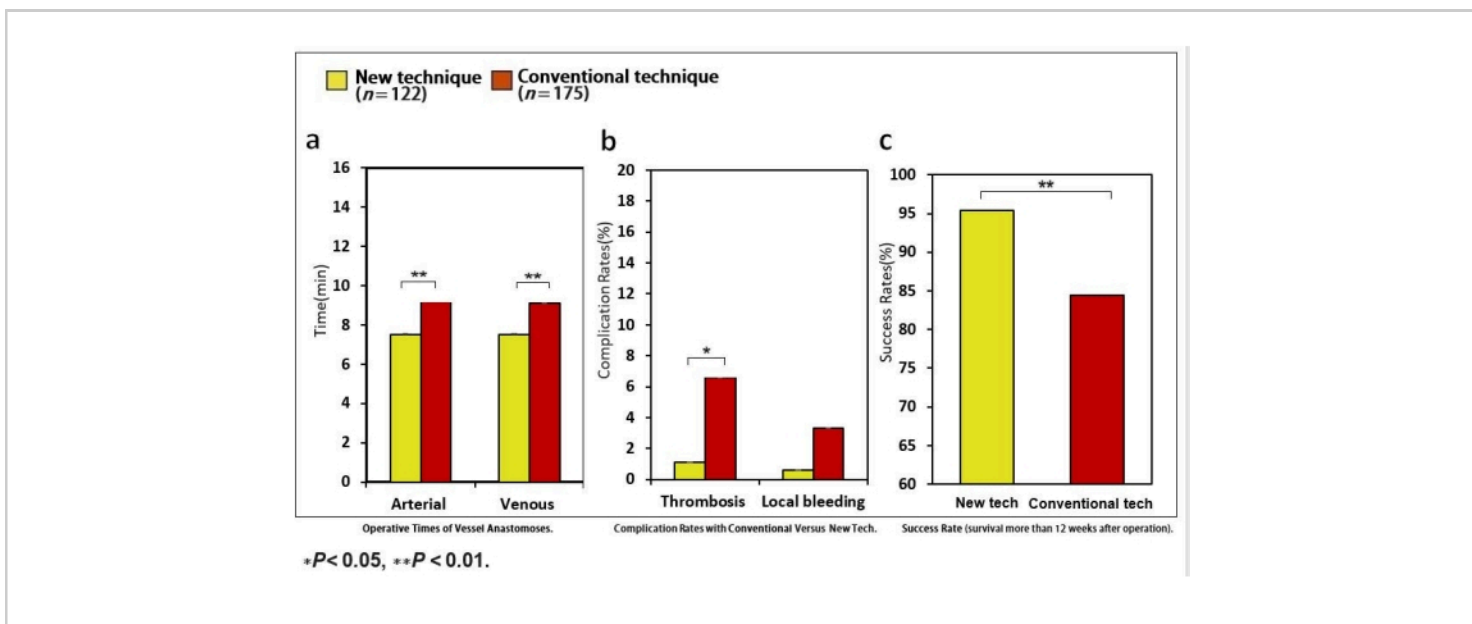

Figure 3: Comparison of operation times of vessel anastomoses, frequency of complications, and success rates in between the modified and the conventional technique[10] The bargraphs in (a) depict the operation time needed to perform the vessel anastomosis; the bargraphs in (b) depict intragraft thrombosis events and local bleeding problems; while the bargraphs in (c) demonstrate a higher success rate of the new knotless technique according to the survival greater than 12 weeks posttransplant after additional explantation of the native contralateral kidney. Please click here to view a larger version of this figure.

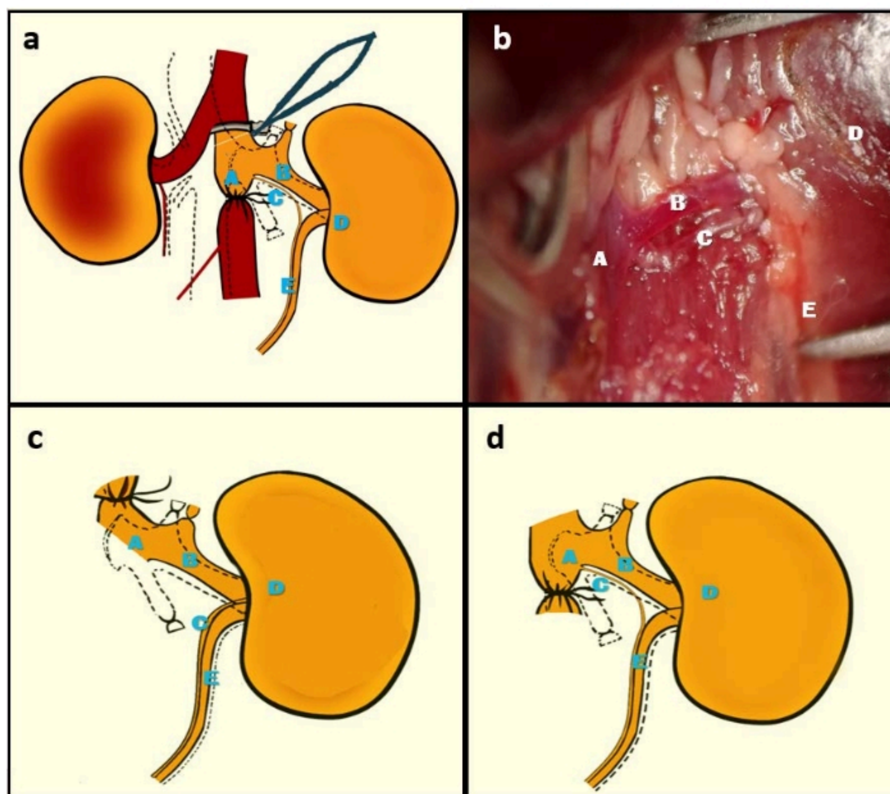

Figure 4: Overview of the anatomical structure (upper panels a and b) and resection lines of the aorta and renal artery for both the conventional (c) and the modified technique (d). (A) Abdominal aorta, (B) Renal artery, (C) Ureteral artery, (D) Kidney, (E) Ureter. The venous vessels (V. cava, including the Vv. renales) are depicted as dotted lines. Please click here to view a larger version of this figure.

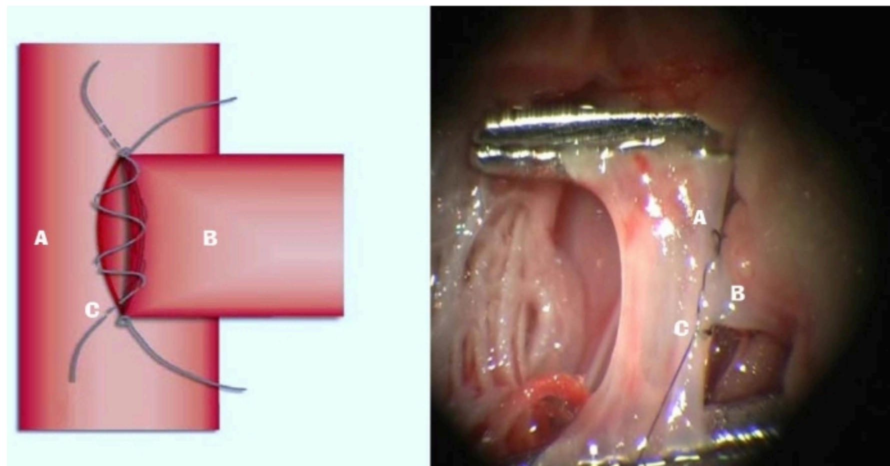

Figure 5: Exemplary demonstration of a knotless stitching of the artery vessel anastomosis showing (A) the abdominal aorta, (B) the renal artery, and (C) the knotless stitching technique where the last stitch of the anastomosis is not tied. Please click here to view a larger version of this figure.

Supplementary Figure 1: Anastomosis of the donor ureter with the recipient's urine bladder. (**a**) Penetrate through the recipient's urinary bladder with a 10/0 monofilament and insert it into a 21 G needle's lumen, (**b**) Guide the 21 G needle to perform a hole located at the previous needle perforation, (**c**) pull out the 21 G needle, (**d**) stitch the trimmed ureter end with the 10/0 suture and perforate the bladder with the 10/0 suture again at the place of its entry, (**e**) then, tow the 10/0 suture and the ureter into the urine bladder through the constructed hole, (**f**) and anastomose the donor's ureter to the recipient's bladder with another 10/0 suture. Finally, pull out the traction suture. Please click here to download this File.

Discussion

While the skin transplantation model in mice is simple and easy to perform to study alloimmune rejection events, the surgical techniques for investigating more specifically the alloimmune-related inflammatory alterations after heart[16] and kidney transplantation[10] has been proven to be complex and very demanding. From the transplant nephrologist's point of view, the establishment of an effective and long-term stable mouse renal transplantation model has still an irreplaceable significance for many functional and immunological studies. In addition, compared with other organ transplants, the mouse kidney transplantation model can achieve a long-time survival even with certain differences in major histocompatibility antigens implying the opportunity for studying immune regulation mechanisms in the long-term development of both rejection or the identification of prerequisite factors to establish alloimmune tolerance[3].

As described previously, kidney transplantation in mice is a challenging procedure, and the success rates of even experienced surgeons vary widely between 40 and

Copyright © 2022 JoVE Journal of Visualized Experiments

95%[10,17,18,19,20]. With respect to the reports of various research teams around the world on this surgical technique, we have made the following modifications in comparison to the classical approach leading to several improvements.

First, a patch of the abdominal aorta is cut and prepared so that the proximal bifurcations of the renal artery and the ureteral artery are transected, including the donor kidney *en bloc*. This maneuver not only completely preserves and retains the blood supply and function of the donor's ureter by avoiding an injury to the periureteral tissue thus preventing a postoperative hydronephrosis, but it also prevents postoperative strictures of the renal artery (**Figure 4**). Hence, renal transplant ischemia mediated by a stricture of the renal artery or caused by a hydronephrosis mediated by a strictured and ischemic transplant ureter are avoided, representing two of the key aspects to achieve the long-term transplant survival in this model. However, there are anatomic variants for the offspring of the ureteral artery. For example, in some mice the ureteral artery originates from the main trunk of the abdominal aorta instead from the renal artery and the position of this offspring is mostly about 0.2 to 0.5 mm distal from the renal artery's offspring (depicted in **Figure 4**). From our experience, we would estimate the occurrence of the ureteral artery originating directly from the aorta in about 20% of C57BL/6J male mice (unpublished observation), and more seldom in other strains of mice such as BALBc. In some of the reported traditional surgical methods, this important nutritive vessel was sometimes neglected to protect, as it was disregarded and directly ligated or electrocautered.

Especially in these situations of anatomical variants when the offspring of the ureteral artery of the mouse is originating from the main trunk of the abdominal aorta below the offspring of the renal artery, this method of *en bloc* transection and reconstruction of the aortic anastomosis is even more suitable. Experienced surgeons can even decide when to use the traditional or modified *en bloc* anastomosis.

Second, the application of a new, simple, and rapid technique of a knotless vascular anastomosis in which the final stitch of anastomosis is not tied with the end of the upper tie like the traditional approach but remains free instead offers a valuable advantage (see **Figure 5**). This technique still allows to increase or decrease the size of the anastomosis after renal transplant reperfusion has already been initiated. This avoids the development of vessel strictures and intraabdominal bleeding. In addition, the free tails of the anchoring stitches at both ends can be pulled in opposite directions to flexibly adjust and expand the anastomosis to avoid stenosis of the arteries or veins. The stitching technique, therefore, improves the surgery fault tolerance rate and is friendly to novices[20].

Third, to atraumatically perform the anastomosis of the donor ureter and the recipient bladder, 21 G and 30 G syringe needles were used as auxiliary puncture guide tools. In mice, the ureter is quite thin and very delicate to perform an end-to-end anastomosis. Usually, the donor ureter is directly pulled into the bladder using forceps to guide the ureter after perforating the bladder with a syringe needle. We have further improved this method, using a thinner diameter 30 G syringe needle as a guide for the 21 G syringe needle (Seldinger procedure). With this atraumatical technique, the 21 G syringe needle does not penetrate the entire bladder, which reduces the damage to the bladder and the difficulty of the ureteral implantation[17] (**Supplementary Figure 1**).

A critical step of the protocol is the configuration of the arterial opening. In both cases (donor and recipient) these must be cut neatly, otherwise it will affect the quality of the anastomosis. Furthermore, in this new knotless

technique, the last stitch is not tied to the knotted thread. After anastomosis, the surgeon should initially keep the anastomotic stoma small. Then, after reperfusion, pull the thread's ends at the upper and lower ends in order to enlarge it. Another critical step that one needs to be aware of is the positioning of the incision of donor renal artery as the ureteral artery must be identified to be protected.

A major limitation of this technique is-besides the described improvements-that the operator still needs to meet high requirements as the vessel walls are small and very tender. Without intense and persevering practice, the success rate of the surgery will be low.

In summary, this report demonstrates the applicability of a technical modification of the renal transplant procedure in mice. The surgery procedure presented here has proven as a valuable and reliable method that was serving as an essential component of several research publications within the last 10 years[3,19,20]. In comparison to the classical and widely established surgery model, the method demonstrated here provides several important improvements that lead to less complication rates and a longer transplant survival rate in the syngeneic kidney transplant setting[3]. It is important to mention that both techniques (modified and conventional) share the same further complications impacting the recipient's mortality such as decease by visceral damage, leakage of urine, hydronephrosis, infections, etc., which did not differ. In summary, this new surgical technique improves the overall success rate and long-term graft survival making it a reliable tool for studying the alloimmune response after kidney transplantation.

Disclosures

None.

Acknowledgments

We thank Dr. Tiantian Bai team for help with voice over, Miss Mian Pao for her help in medical illustration. This work was supported in part by the German Research Foundation (DFG) to promote international collaborations (HO2581/4-1 to AH) and the National Science Foundation of China (NSFC; #81760291 to FJ).

References

1. Skoskiewicz, M., Chase, C., Winn, H. J., Russell, P. S. Kidney transplants between mice of graded immunogenetic diversity. Transplantation *Proceedings.* **5** (1), 721-725 (1973).
2. Jiang, K. et al. Noninvasive assessment of renal fibrosis with magnetization transfer MR imaging: Validation and evaluation in murine renal artery stenosis. *Radiology.* **283** (1), 77-86 (2017).
3. Tse, G. H. et al. Mouse kidney transplantation: Models of allograft rejection. *Journal of Visualized Experiments.* (92), e52163 (2014).
4. Okazaki, M. et al.Costimulatory blockade-mediated lung allograft acceptance is abrogated by overexpression of Bcl-2 in the recipient. *Transplantation Proceedings.* **41** (1), 385-387 (2009).
5. Chuck, N. C. et al.Ultra-short echo-time magnetic resonance imaging distinguishes ischemia/reperfusion injury from acute rejection in a mouse lung transplantation model. *Transplant International.* **29** (1), 108-118 (2016).
6. Zhang, Z. et al. Pattern of liver, kidney, heart, and intestine allograft rejection in different mouse

Copyright © 2022 JoVE Journal of Visualized Experiments

strain combinations. *Transplantation.* **62** (9), 1267-1272 (1996).

7. Wang, J., Hockenheimer, S., Bickerstaff, A. A., Hadley, G. A. Murine renal transplantation procedure. *Journal of Visualized Experiments.* (29), e1150 (2009).
8. Plenter, R., Jain, S., Ruller, C. M., Nydam, T. L., Jani, A. H. Murine kidney transplant technique. *Journal of Visualized Experiments.* (104), e52848 (2015).
9. Plenter, R. J., Jain, S., Nydam, T. L., Jani, A. H. Revised arterial anastomosis for improving murine kidney transplant outcomes. *Journal of Investigative Surgery.* **28** (4), 208-214 (2015).
10. Rong, S., Lewis, A. G., Kunter, U., Haller, H., Gueler, F. A knotless technique for kidney transplantation in the mouse. *Journal of Transplantation.* 127215 (2012).
11. Kreimann, K. et al. Ischemia reperfusion injury triggers CXCL13 release and B-cell recruitment after allogenic kidney transplantation. *Frontiers in Immunology.* **11**, 1204 (2020).
12. Schmidbauer, M. et al. Diffusion-Weighted imaging and mapping of T1 and T2 relaxation time for evaluation of chronic renal allograft rejection in a translational mouse model. *Journal of Clinical Medicine.* **10** (19), 4318 (2021).
13. Wu, K. et al. Novel technique for blood circuit reconstruction in mouse heart transplantation model. *Microsurgery.* **26**, 594-598 (2006).
14. Haas M. Chronic allograft nephropathy or interstitial fibrosis and tubular atrophy: what is in a name?. *Current Opinion in Nephrology and Hypertension.* **23** (3), 245-250 (2014).
15. Dang, Z., MacKinnon, A., Marson, L. P., Sethi, T. Tubular atrophy and interstitial fibrosis after renal transplantation is dependent on galectin-3. *Transplantation.* **93** (5), 477-484 (2012).
16. Yin, D. et al. Blood circuit reconstruction in an abdominal mouse heart transplantation model. *Journal of Visualized Experiments.* (172), e62007 (2021).
17. Zhang, Z. et al. Improved techniques for kidney transplantation in mice. *Microsurgery.* **16** (2), 103-109 (1995).
18. Mannon, R. B. et al. Chronic rejection of mouse kidney allografts. *Kidney International.* **55** (5), 1935-1944 (1999).
19. Coffman, T et al. Improved renal function in mouse kidney allografts lacking MHC class I antigens. *Journal of Immunology.* **151** (1), 425-435 (1993).
20. Martins P. N. Learning curve, surgical results and operative complications for kidney transplantation in mice. *Microsurgery.* **26** (8), 590-593 (2006).

Copyright © 2022 JoVE Journal of Visualized Experiments