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Orthotopic Rat Kidney Transplantation: A Novel and Simplified Surgical Approach

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Cover Letter

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JOHNS HOPKINS
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Dear Editors,

We would like to ask you to consider the attached manuscript titled **'Orthotopic Rat Kidney Transplantation'** for publication in *Jove*. The manuscript, including related data and figures, has not been previously published and is not under consideration for publication elsewhere.

The rat kidney transplant model has served as a useful tool to investigate the immunological phenomena of rejection and tolerance. Our laboratory has more than a decade of experience in performing kidney and liver transplants in rats. Like many surgical procedures, this operation can be performed in a number of different ways, and through trial and error we have successfully developed a unique surgical approach that leads to a 100% survival in syngenic animals.

Existing literature about this procedure is often presented in a summarized fashion. This manuscript compares different approaches and potential pitfalls to rat kidney transplantation and provides readers with a detailed step-by-step protocol in order to perform this procedure successfully. We are convinced it will be of interest to your readership.

Please do not hesitate to contact us directly if you have additional questions.

Thank you in advance for your consideration.

Kind regards,

Zhaoli Sun, M.D. Ph.D.

TITLE:

Orthotopic Rat Kidney Transplantation: A Novel and Simplified Surgical Approach

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KEYWORDS:

Kidney, transplantation, orthotopic, rat, survival, rejection, tolerance

SUMMARY:

The purpose of this manuscript and protocol is to explain and demonstrate in detail the surgical procedure of orthotopic kidney transplantation in rats. This method is simplified to achieve the correct perfusion of the donor kidney and shorten the reperfusion time by using the venous and ureteral cuff anastomosis technique.

ABSTRACT:

Kidney transplantation offers increased survival rates and improved quality of life for patients with end-stage renal disease, as compared to any type of renal replacement therapy. Over the past few decades, the rat kidney transplantation model has been used to study the immunological phenomena of rejection and tolerance. This model has become an indispensable tool to test new immunomodulatory pharmaceuticals and regimens prior to proceeding with expensive preclinical large-animal studies.

This protocol provides a detailed overview of how to reliably perform orthotopic kidney transplantation in rats. This protocol includes three distinctive steps that increase the probability of success: perfusion of the donor kidney by flushing through the portal vein and the use of a cuff system to anastomose the renal veins and ureters, thereby decreasing cold and warm ischemia times. Using this technique, we have achieved survival rates beyond 6 months with normal serum creatinine in animals with syngeneic or tolerant kidney transplants. Depending on the aim of the study, this model can be modified by pre- or posttransplant treatments to study the acute,

chronic, cellular, or antibody-mediated rejection. It is a reproducible, reliable, and cost-effective animal model to study different aspects of kidney transplantation.

INTRODUCTION:

Historically, the first transplant rejection studies were performed by Brent and Medawar using skin transplants in rodents[1]. It soon became clear that skin has distinct immunological features, making it a highly immunogenic organ that is different in rejection from other vascularized solid organs[2]. Rat studies of solid organ transplant rejection are habitually limited to heart, liver, and kidney transplants. Although each of these organs is suitable to study rejection, there are advantages and disadvantages to each of them. Heart transplants are often transplanted into the abdomen and anastomosed to the aorta and vena cava, with the recipient's native heart in place[3]. This does not recreate human clinical, anatomical, and physiological conditions. Additionally, hearts are very sensitive to cold ischemia and have to be reperfused preferentially within 1 h in order to be able to recover their function[4]. Liver transplants are generally considered to be surgically more challenging and time-sensitive to perform. After removing the native liver, the donor liver has to be implanted and reperfused within 30 min as the recipients cannot last longer without a functioning liver[5]. The hepatic artery, portal vein, and especially the bile duct reconstruction requires refined surgical skills. Besides the surgical challenges, the liver is known to possess tolerogenic properties and rodents and humans can become operationally tolerant[6–8]. The kidney, unlike the aforementioned organs, can be transplanted in an orthotopic fashion, is known to be an immunogenic organ with consistent, reproducible rejection episodes (if not immunosuppressed), and allows for prolonged cold ischemia times of several hours. This makes the rat kidney transplant an ideal model for studying allograft rejection and tolerance.

Kidney transplantation (KT) is the preferred choice of treatment for patients with end-stage renal disease. Over the last few decades, short-term survival outcomes after KT have improved dramatically, but long-term survival outcomes are stagnant[9]. Conventional immunosuppressive regimens remain the standard anti-rejection therapy. However, the chronic use of immunosuppressive therapies causes significant morbidity and mortality, such as nephrotoxicity, diabetes, and secondary malignancies[10–12]. In the long-term, chronic antibody- and cellular-mediated rejection threaten graft survival, with limited therapeutic options available.

A major goal in transplantation is the induction of transplant tolerance in order to obviate the need for chronic immunosuppression. The rat KT model is a robust tool to investigate the immunological rejection process and to evaluate new approaches to immunomodulation and transplant tolerance. The rat also serves as a suitable model to study acute and chronic cell- and antibody-mediated rejection[13–17]. This surgical model has proven to be a reliable, reproducible, and cost-effective tool to study various aspects of allograft rejection and tolerance. It is often used to test novel tolerance-inducing protocols prior to undertaking expensive and cumbersome large-animal studies. Performing KT in rats requires extensive surgical training and expertise to reach survival rates of >90%. In this manuscript and in the accompanying instructional video, we provide a step-by-step outline for orthotopic KT in the rat, as successfully performed for many years at our institution.

Prior to starting any procedure, donor and recipient selection is critical and depends on the nature of the experiment. Ideally, donors and recipients should weigh between 220–260 g and be between 8–12 weeks of age. Animals under 220 g have small-diameter arteries, veins, and ureters, making the anastomosis in the recipient particularly challenging. Minor blood loss can cause hypovolemia and lead to death in smaller animals. Animals heavier than 260 g display more fat around their vessels, and vessel isolation will require more operative time and increase the cold ischemia time.

PROTOCOL:

Lewis ($RT1^{1}$) and Dark Agouti (DA) ($RT1A^{a}$) rats were purchased from commercial vendors (see the **Table of Materials**). These fully MHC-mismatched strains are often used to study acute renal allograft rejection. All animals were housed and maintained according to the National Institutes of Health's (NIH) guidelines in a specific pathogen-free facility at the Johns Hopkins University. All procedures were approved by the institutional animal care and use committee.

1. Donor procedure

1.1. Prepare and autoclave all surgical instruments to be used in this procedure as a means of sterilization and use disposable sterile gloves to prevent infectious complications.

1.2. Anesthetize the donor rat by isoflurane inhalation (induction at 3%–4% and maintenance at 1%–2%) for the rest of the procedure. Give all donor and recipient animals preemptive buprenorphine subcutaneously at 0.1 mg/kg body weight for analgesia.

1.3. Now, place the rat in a supine position and immobilize the limbs with sterile masking tape.

1.4. Use a mechanical clipper to remove hair from the abdominal area.

1.4.1. Apply an eye lubricant and use sterile gauze soaked in povidone-iodine, followed by gauze soaked in isopropyl alcohol, to sterilize the surgical field.

1.4.2. Prior to the first incision, make sure that the rat is adequately anesthetized by checking the absence of the toe pinch withdrawal reflex.

1.5. Using scissors, start off by making a large longitudinal midline skin and muscle incision from the symphysis pubis to the xiphoid, and enter the peritoneal cavity.

1.6. Insert two retractors on either side of the abdominal wall in order to expose the intra-abdominal cavity.

1.7. Cover the intestine with a moist sterile gauze and shift it to the right lateral side of the abdomen, exposing the aorta, vena cava, and left kidney. Apply 1 mL of preheated saline with a 1 cc syringe to keep the intestines and the abdominal organs moist and at a normal temperature.

1.7.1. Apply a second moist gauze to cover and mobilize the stomach and spleen cranial to the kidney and a small moist gauze to cover the exposed kidney (**Figure 1A**).

1.8. Use microsurgical dissecting forceps to isolate and mobilize the left renal artery and vein from the connective tissue and each other. Isolate the left renal vein by cauterizing the left gonadal vein and isolate the left renal artery by cauterizing the adrenal artery. After that, mobilize the aorta and vena cava superior and inferior of the left renal pedicle by dissecting the connective tissue with dissecting forceps (**Figure 1B**).

1.9. Divide and mobilize the ureter from the connective tissue using dissecting forceps, and make a diagonal incision at a length of 2 cm measured from the renal pelvis, using microscissors. Insert a polyamide cuff (see **Table of Materials**) halfway into the ureter and secure the cuff by placing a knot with 8-0 silk suture (**Figure 1C**).

NOTE: It is important not to remove all fat and connective tissue from the ureter, as they provide protection against obstruction caused by adhesions, and their removal may cause ureteral necrosis. Pay extra attention to preserving the vessel supplying oxygen to the ureter.

1.10. Mobilize the left kidney by separating it from the perinephric fat using dissecting forceps or microscissors. Leave the adipose capsule of the kidney attached and use that site for handling the kidney.

1.10.1. Expose the inferior vena cava.

1.11. Administer 200 units of heparin using a syringe with a 27 G needle through the penile vein. Pressure the site of injection with a cotton swab for at least 1 min to prevent bleeding.

1.12. Identify the portal vein (pv) and inferior vena cava (ivc) (**Figure 1D**). Flush the kidney by injecting 50 mL of cold saline mixed with 500 units of heparin into the portal vein using a 16 G needle (**Figure 1E**). Before flushing, cut the inferior vena cava at the infrahepatic level and the portal vein caudal at the needle insertion site to allow the blood to exit the circulation. Start flushing the kidney by gradually infusing the saline solution. Observe a change of color of the kidney from dark red to a uniform grey and pale color (**Figure 1F**).

1.13. After flushing, ligate the renal artery and vein proximal to the aorta and vena cava and place the flushed kidney in a Petri dish in cold saline on ice. **Figure 2A** represents the schematic overview of the donor procedure.

1.14. Once the kidney is in cold saline, fix and immobilize the kidney by using a clamp that is attached to the perirenal fat.

1.15. Pull the renal vein gently over the cuff (see the **Table of Materials**) and fix the cuff by placing three knots using 8-0 silk suture (**Figure 2B**).

NOTE: Pay special attention to the orientation of the vein while securing it in place. Rotated veins cause an obstruction of the blood flow and lead to thrombosis.

2. Recipient procedure

2.1. Repeat steps 1.1–1.11 from the donor procedure.

2.2. Place two atraumatic micro-vessel clamps on the left renal artery and vein proximal to the aorta and vena cava (**Figure 3A**).

2.3. Ligate the recipient renal vein proximal to the inlet of the kidney. Flush the renal vein with heparinized saline to remove all the remaining blood out of the vessel.

2.4. Slide the ligated renal vein over the cuffed renal vein previously positioned in the donor kidney and secure it with an 8-0 silk suture (**Figure 3B**). Maintain the same positional orientation when securing the renal vein over the cuff.

2.5. Ligate the ureter at the level of the lower pole of the left kidney. Mobilize the kidney from the perinephric fat.

2.6. Ligate the renal artery proximal to the inlet of the recipient kidney. Flush it with heparinized saline to remove any excess blood in the vessel. Perform an end-to-end anastomosis of the renal artery with 8 to 10 interrupted sutures using a 10-0 nylon suture (**Figure 3C**). Maneuver the artery by using the adventitial layer.

2.7. Remove the vessel clamps to reinitiate the reperfusion of the kidney. Start by removing the clamp on the vein followed by the clamp on the artery (**Figure 3D**). Use a sterile cotton swab to lightly pressure any oozing areas around the anastomosis region. A few minutes should be sufficient to achieve a patent anastomosis.

2.8. Briefly observe the kidney to assess for adequate perfusion. Immediately after reperfusion, the kidney should change color and gradually regain its natural dark red color after a few minutes (**Figure 3E**). Visible peristalsis of the ureter and on-site urine production are sometimes observed.

2.9. Finish by inserting the exposed tip of the ureteral cuff into the recipient ureter and secure the recipient ureter with an 8-0 silk tie (**Figure 2C** and **Figure 3F**).

2.10. In order to keep the donor and recipient ureters in position, tie off the ends of each ureter side to each other.

2.11. Optionally, the right kidney can be nephrectomized by tying off the right renal artery and vein with a 4-0 silk suture and removing the kidney.

2.12. Remove all gauzes from the abdominal cavity, return all the organs to their natural position,

squirt 1 mL of saline over the intestines to keep them moist, and close the abdomen by using a 4-0 absorbable suture on the rectus muscle and a 4-0 silk suture to close the skin layer in an interrupted fashion.

3. Postoperative care

3.1. Place the animal in a clean cage with access to ad libitum water and food and allow for recovery on a 37 °C heating pad.

3.2. Inject 0.1 mg/kg buprenorphine subcutaneously for analgesia and monitor the animal for recovery. Antibiotics are not routinely administered, as infectious complications are rare.

3.3. Observe the recovery for 1–2 h before returning the animal back to the animal facility. Inspect the animal 2x–3x a day for the first 24 h, followed by a daily inspection. Pay attention to signs of pain and distress, oral intake, and urinary output.

3.4. Remove the stitches 7–10 days after the operation.

REPRESENTATIVE RESULTS:

We performed syngeneic (*N* = 5) and allogeneic kidney transplants (*N* = 5). Animals with a syngeneic transplant achieved long-term survival without any immunosuppressive treatment. Animals that received an allogeneic transplant without immunosuppression rejected their graft and succumbed to renal failure with a median survival of 8 days (**Figure 4A**). Mean serum creatinine increased modestly in the syngeneic group while it increased by 14-fold in the allogeneic group (0.5 mg/dL versus 7.0 mg/dL, p <0.01) (**Figure 4B**). Upon explantation, the macroscopic view of the syngeneic kidney allograft did not show any abnormalities. The kidney color and internal structures remained intact. In contrast, kidney allografts of rejected animals presented red hemorrhagic patches with the destruction of the internal structures (**Figure 4C**). Hematoxylin and eosin stains of syngeneic grafts showed thin glomerular capillary loops with normal numbers of endothelial and mesangial cells. Rejected allografts displayed destroyed glomerular structures with signs of inflammation and tubulitis (**Figure 4D**). To confirm T-cell-mediated rejection, we performed CD8+ staining. While syngeneic allografts showed very few positive CD8+ T cells, the rejected allografts showed a significantly higher number of CD8+ cells in and around the glomeruli and tubuli (**Figure 4E**), confirming T-cell-mediated rejection.

FIGURE AND TABLE LEGENDS:

Figure 1: Donor nephrectomy. (**A**) Upon opening the abdomen, the left kidney is isolated with moist gauzes. (**B**) The left renal artery and vein are isolated and mobilized from the surrounding fat. (**C**) The ureter is ligated, cuffed, and secured with a single silk suture. (**D**) The portal vein (pv) and inferior vena cava (ivc) are identified and the kidney is perfused through the portal vein. (**E**) Perfusion is successfully executed as the right kidney and liver are becoming pale by flushing the animal's portal vein. (**F**) Successful perfusion demonstrates a pale kidney and vessels ready for transplantation.

Figure 2: Schematic overview of the kidney transplant procedure. (**A**) Schematic overview of the donor procedure. (**B**) Schematic overview of a cuffed donor vein. (**C**) Schematic overview of anastomosis of the recipient and the cuffed donor vein and anastomosis of the ureters.

Figure 3: Kidney transplantation in the recipient. (**A**) The recipient's artery and vein are mobilized from the surrounding fat and clamped following separation. (**B**) The donor kidney is introduced, and the veins are connected via the cuff technique and secured with an 8-0 suture. (**C**) The arteries are sutured in an end-to-end fashion. (**D**) The clamps are removed. (**E**) The kidney is reperfused and recovers its natural color without any bleeding. (**F**) Finally, the ureters are anastomosed by using the previously placed cuff and secured with an 8-0 suture.

Figure 4: Kidney transplant survival. (**A**) The Kaplan-Meyer figure demonstrates the survival of rats with syngeneic or allogeneic kidney transplants over time. (**B**) Measurement and comparison of serum creatinine in rats with syngeneic or allogeneic kidney transplants compared to nontransplanted animals. (**C**) Macroscopic overview of explanted kidneys of syngeneic (top) and allogeneic (bottom) kidney transplant at day 8. Animals were perfused with saline prior to explanting. The last two panels show a microscopic overview of (**D**) hematoxylin and eosin staining and (**E**) CD8+ of syngeneic (top) and allogeneic (bottom) kidney explants. The images are taken under 200x magnification. *Results were considered statistically significant if $p < 0.05$.

Figure 5: Required surgical instruments. (**1**) Straight scissors. (**2**) Fine scissors. (**3**) Micro-spring scissors 1 (ureter ligation). (**4**) Micro-spring scissors 2. (**5**) Micro-spring scissors 3. (**6**) Small-animal surgical retractors. (**7**) Forceps. (**8**) Microforceps, straight, smooth. (**9**) Dissecting forceps, curved. (10) Micro-needle holder. (**11**) Needle holder. (**12**) 8-0 braided silk suture without needle. (**13**) 4-0 silk suture. (**14**) Micro-vessel clamps (one pair). (**15**) Micro-vessel clamp applier. (**16**) Fine-tip clamp. (**17**) Heparin. (**18**) Vessel clamp (medium size). (**19**) Vessel clamp (large). (**20**) Sterile cotton swabs. (**21**) 10-0 micro-suture with needle. (**22**) Sterile gauze. (**23**) Heparinized saline flush syringe. (**24**) 60 cc syringe with needle. (**25**) 10 cc syringe. (**26**) 1 cc syringe. (**27**) 25 G 5/8 inch needles. (**28**) 19 G needles. (**29**) Trimmer. (**30**) Bipolar cautery system. (**31**) Tape. (**32**) Petri dish with 0.9% normal saline. (**33**) 60 cc syringe with 50 cc heparinized saline for perfusion. (**34**) 10 cc syringe with 5 cc heparinized saline flush. (**35**) Ureter cuff. (**36**) Vein cuff.

DISCUSSION:

In this manuscript, we describe the surgical method for orthotopic KT in rats in detail, including all the necessary equipment needed to perform this procedure (**Figure 5**). In 1965, Fisher and Lee published the first report on KT in rats, which became the start of an exciting investigative field[18]. Since then, many modifications have been introduced to improve the reproducibility of this model. It has served as an effective animal model for studying ischemia-reperfusion injury and renal transplant rejection and tolerance, thanks to the availability of several inbred and outbred strains with partial and full MHC mismatch combinations[19]. The rat KT model can serve as a tool to test hypotheses prior to extending investigations to swine and nonhuman primate models of KT. Options to study kidney transplant rejection or tolerance in rodents are limited. The kidney transplant model in mice is technically very challenging and requires a long training period to

achieve survival rates of >80%[20]. Another limitation of the mouse model is the spontaneous renal allograft acceptance without the need for immunosuppression in about 30% of the recipients. However, other organ transplants in mice, such as skin and heart, are rejected within 10 days, suggesting that the rejection of kidney allografts in fully MHC-mismatched mice is weak and not representative of the clinical situation[21]. However, if the technical challenge can be overcome, mice models are preferred for mechanism studies of allograft rejection because of the availability of genetically modified knock-in or knock-out mice.

KT in rats can be performed in a number of ways. We will discuss a few advantages and disadvantages of these various methods. Irrespective of the preferred technique, it is always critical to reduce warm ischemia time and to avoid irreversible injury to the graft and recipient.

Right versus left kidney

The abdominal anatomy of rats is very similar to that of humans. The left kidney is located superior compared to the right kidney because of the anatomical position of the liver. One of the advantages of using the left kidney is the length of the vessels. Generally, the left renal artery and vein are twice the length of the right renal vessels. This is especially beneficial when performing anastomosis where the length of vessels is not a limiting factor. However, reports exist of right-sided donor kidney retrieval and transplantation[22,23]. Approaches using both kidneys for transplantation have also been described[24].

Flushing donor kidney via the portal vein

One of the key steps of this procedure is the donor kidney perfusion. Perfusion is necessary to remove all donor blood from the vessels and kidney and to cool the organ down to slow biological deterioration. There are various methods described for perfusing the kidney. We have experimented with flushing the kidney in different ways and concluded that flushing the kidney through the portal vein offers advantages and consistently leads to complete perfusion of the kidney and vessels. The conventional approaches described in the literature entail flushing the donor kidney after ligating the renal artery and vein or retrograde through the infrarenal aorta[24–28]. These approaches may lead to endothelial damage and renal vasoconstriction because of increased local pressures or to incomplete perfusion due to low perfusion pressure[29,30].

By flushing the kidney through the portal vein, the pressure is managed by the heart. During the perfusion, the heart is still active and pumps the perfusion fluid in a normal fashion to the aorta and kidney with pulsatile flow, preventing damage to capillaries and glomeruli due to shear pressure flow. When transplanting kidneys en bloc or using the right kidney for transplantation, this method is suitable to achieve uniform perfusion and to harvest both kidneys at the same time.

Arterial and venous anastomosis

One of the most critical steps in the rat KT model is performing a reliable microvascular anastomosis in a time-efficient manner. The donor renal artery can be anastomosed to the recipient's renal artery or the aorta. Anastomosing the donor vessels to the aorta and inferior vena cava causes ischemic injury to the recipient's organs. In this protocol, we demonstrate the

end-to-end anastomosis of the renal arteries, as it avoids ischemic injury to other organs. During the arterial anastomosis, it is important not to damage the endothelial surface of the lumen when handling the vessel. For the vein anastomosis, we use a cuff technique to reduce the warm ischemia time and shorten the operative procedure. This has proven to be a very reliable and durable method to ensure adequate venous flow. To ensure adequate venous flow, it is imperative for the veins not to be kinked or twisted when these are secured together. Alternatively, an end-to-end or an end-to-side vein anastomosis is possible, depending on the surgeon's preference. Ideally, the arterial and venous vessel anastomosis should take between 20–30 min.

Pahlavan et al. summarized the complications of each type of technique based on a literature screening[31]. One of the main complications that may occur after any microsurgical vessel anastomosis is thrombosis. Ligation and adequate flushing of the recipient vessels significantly reduce thrombosis formation, and it is certainly not a complication frequently observed. Other complications are leakage or rupture of the anastomosis after reperfusion. This is related to inadequate microsurgical technique or inadequate handling of the vessels.

Ureteral anastomosis

The ureter has to be handled with utmost care, especially during isolation of the ureter in the donor. Injury to the periureteric structures can cause ureteral ischemia leading to strictures and obstruction and, in the worst-case scenario, ureteral necrosis. The literature reports different methods for ureteral anastomosis. End-to-end, cuff-assisted end-to-end, bladder patch, and bladder insertion are the most commonly used[19,32,33]. In previous studies, we have used a cuff with oblique edges on both ends to facilitate the entry into the ureter on both ends. We did not observe any urine leakage or blood clot formations. However, long-term complications (>30 days) of this technique include hydronephrosis and occasionally nephrolithiasis, which can be explained by stricture formation, dislocation, or obstruction of the cuff due to ureteral stones. This finding is consistent with other reports and our own findings of performing ureteral anastomosis with a cuff. Ureteral complications are often noticed postoperatively after significant injury to the kidney, are unsalvageable, and require the animal to be euthanized.

Postoperative care and survival

The postoperative care of transplanted animals requires adequate pain management and detailed observations of the animals' overall activity, weight observations, and urine production. Common early postoperative complications include bleeding from the arterial or venous anastomosis, urine leakage, ureteral obstruction, or delayed graft function because of prolonged ischemia time. Animals with these complications show modest activity and usually remain in a hunched-back position with no urinary output and nutrient intake. Generally, it is favorable to administer up to 1–5 mL of saline to the animals postoperatively to speed up their recovery and prevent dehydration. Animals that receive no immunosuppression can survive between 7 to 10 days, which allows for a sufficient therapeutic window to test novel drugs or other methods. If animals are adequately immunosuppressed (1.0 mg/kg/day FK506 subcutaneously) or tolerant, they can be monitored long-term past 6 months as previously reported[13]. The rat kidney transplant model allowed the definition of the mechanisms of tolerance induced by using a

unique approach of stem cell mobilization prior to confirming this phenomenon in large animals[34]. Rat KT has provided crucial information to investigators for decades, and it will continue to do so in the future.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure NR1

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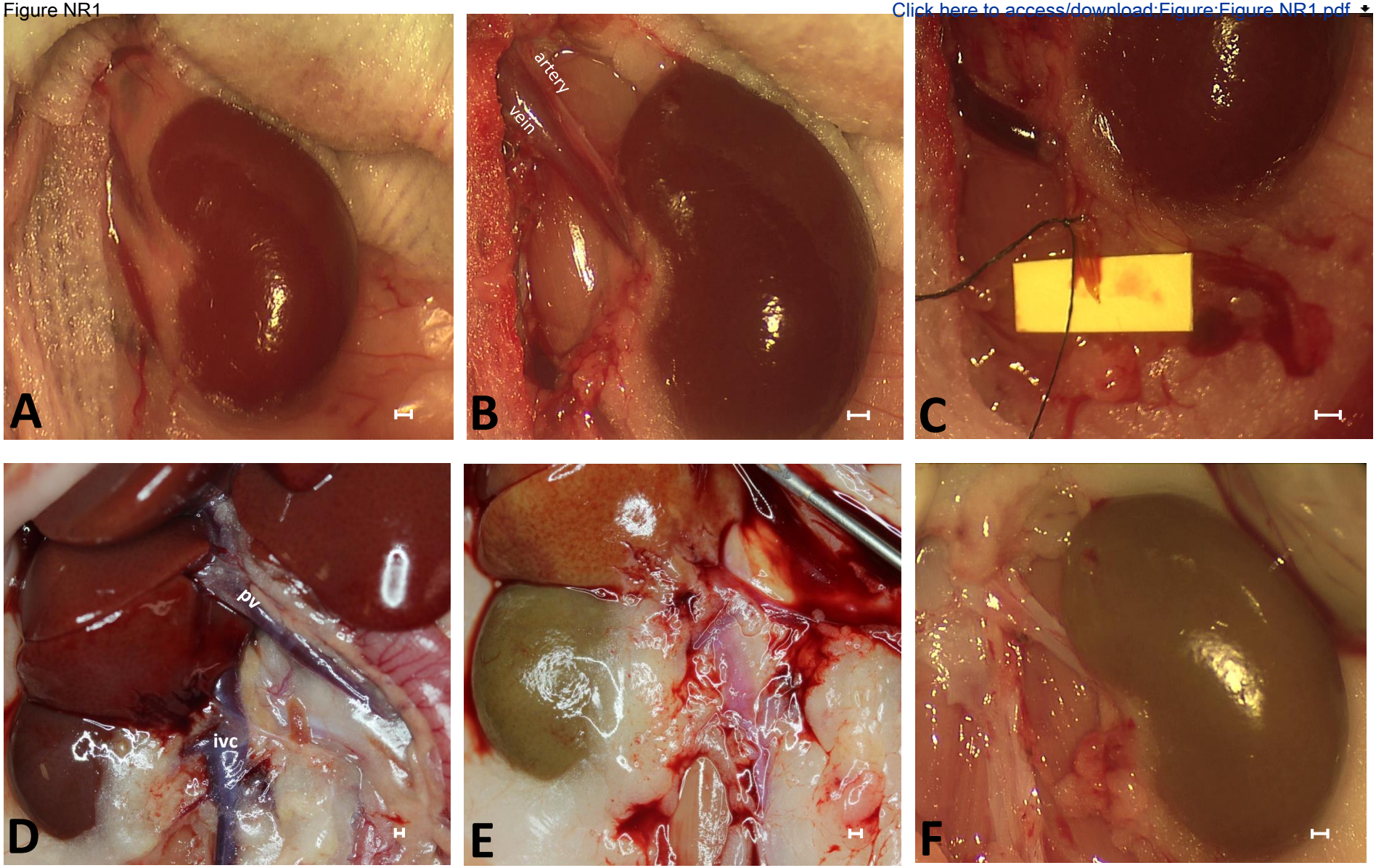

Figure 1. Donor Nephrectomy.

Scale bar = 1mm

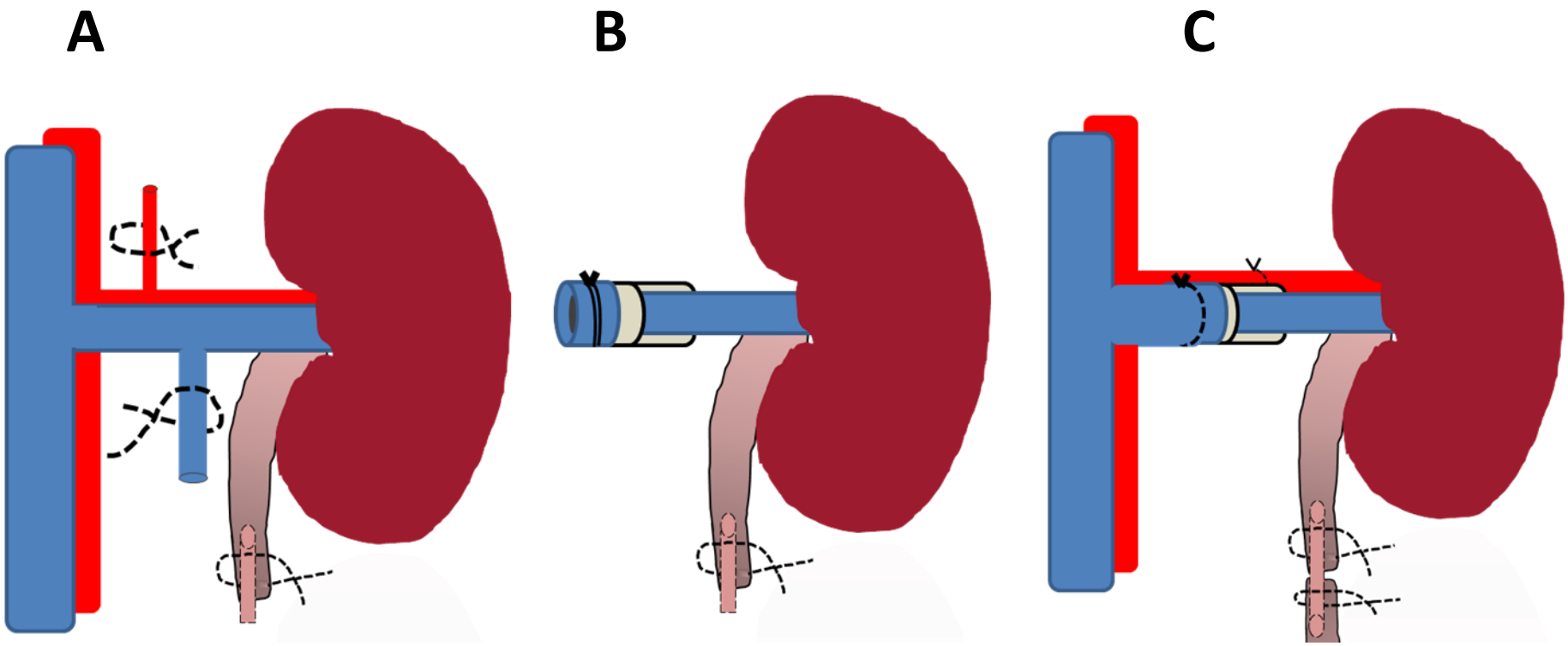

Figure 2. Schematic Overview of the Kidney Transplant Procedure

Figure NR3

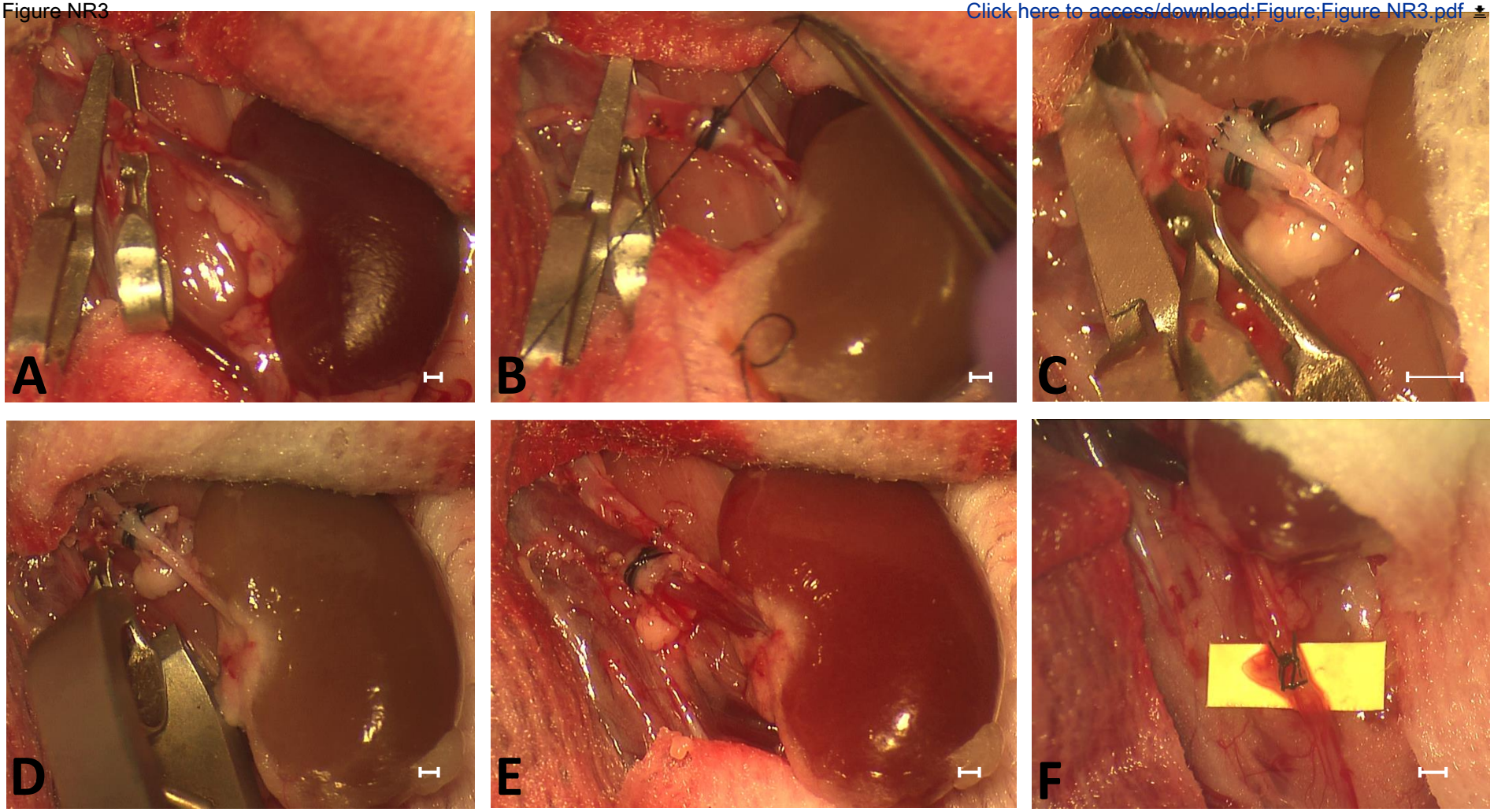

Figure 3. Kidney Transplantation in the Recipient.

Scale bar = 1mm

Figure NR4

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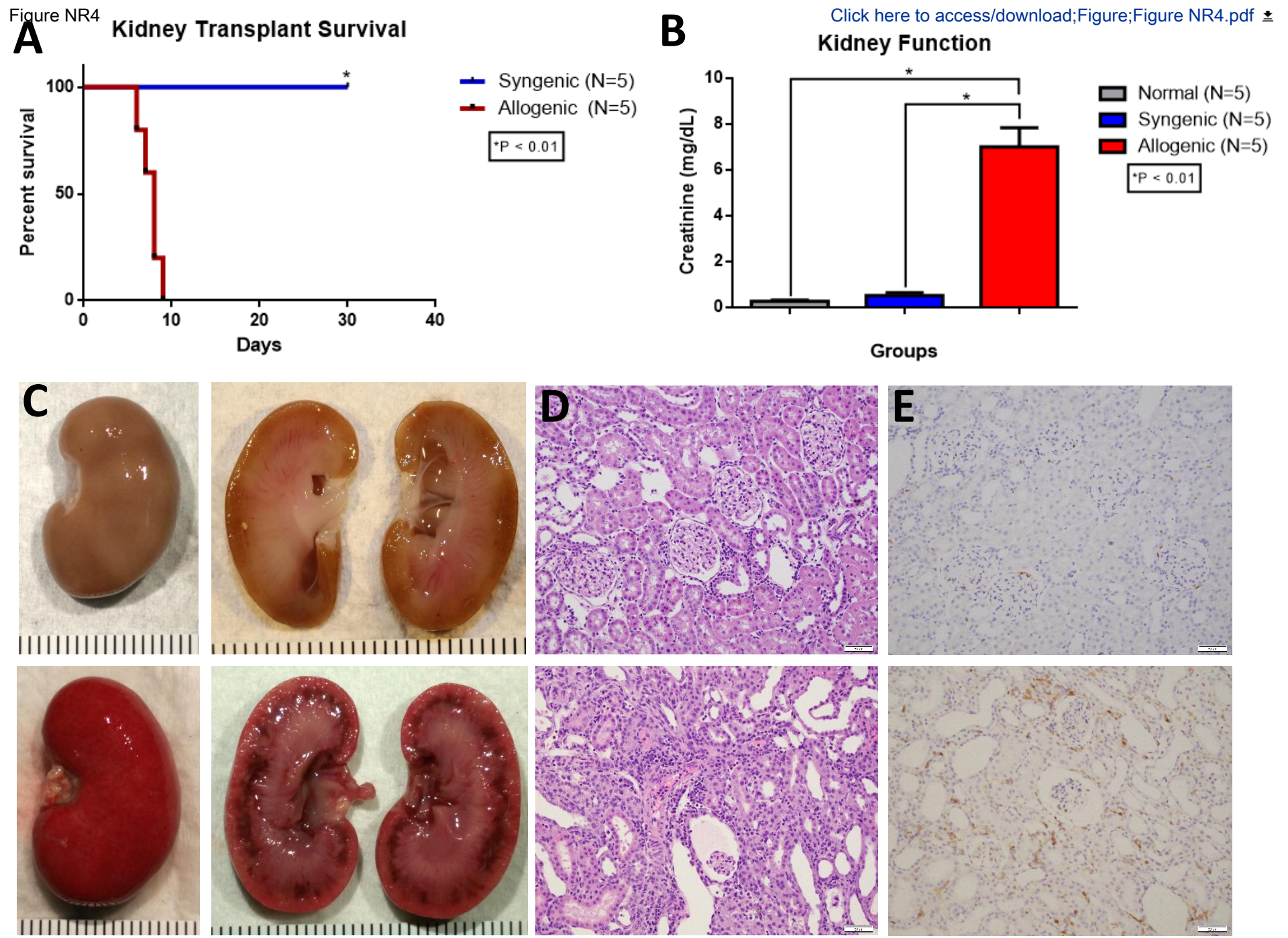

Figure 4. Kidney Transplant Survival

Figure NR5

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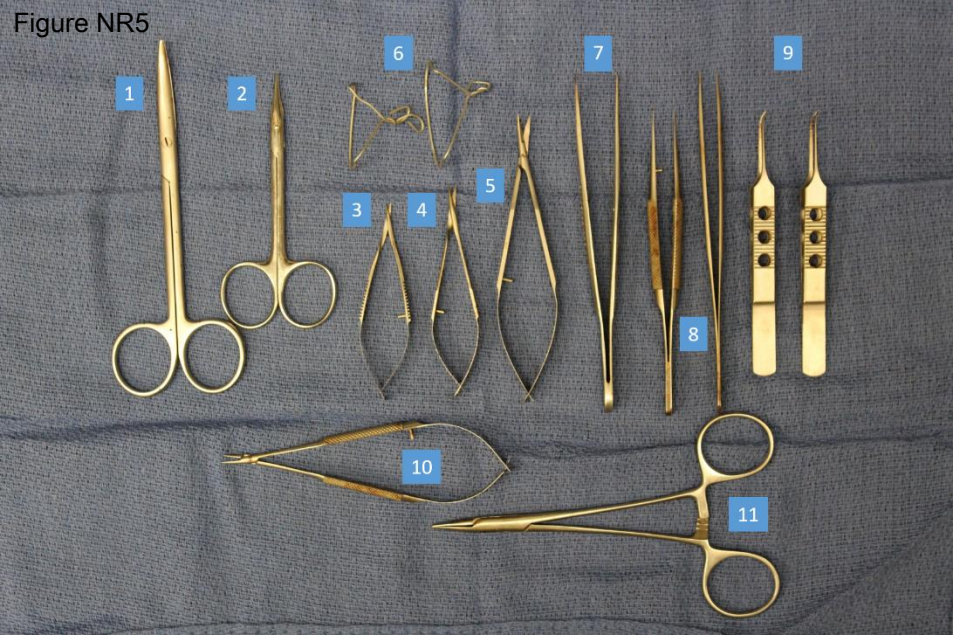

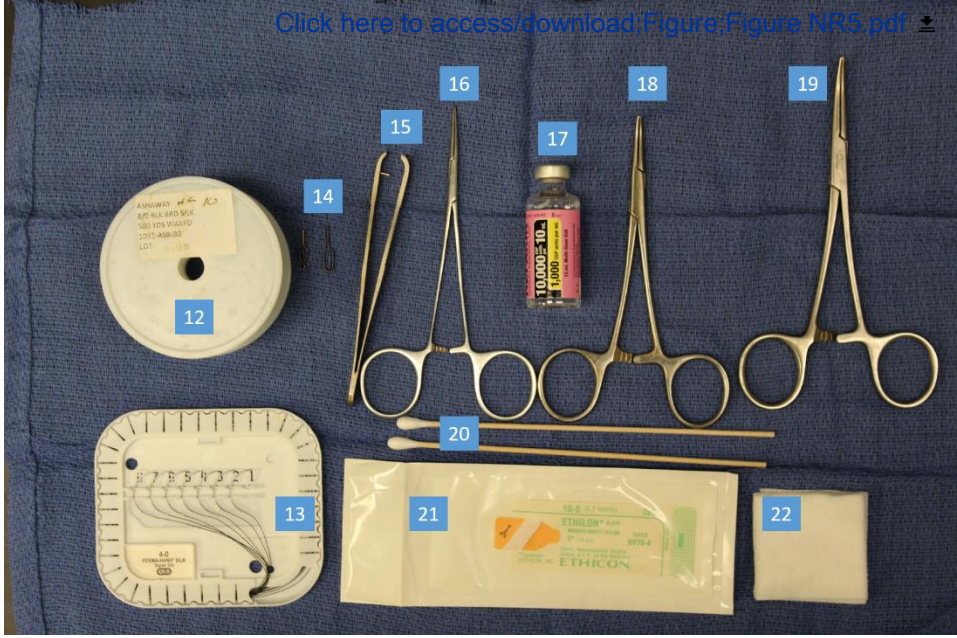

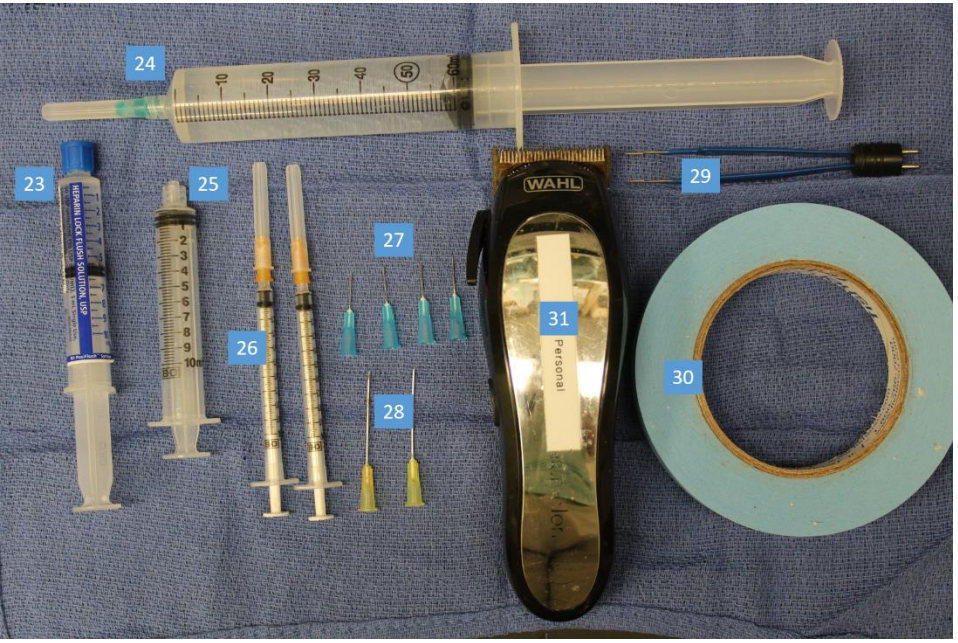

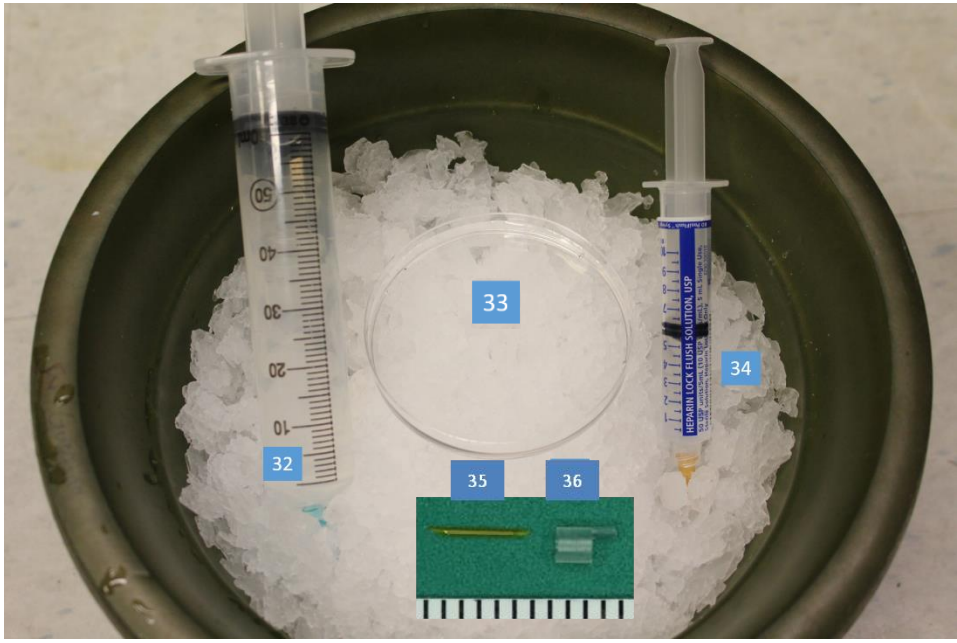

Figure 5. Required Surgical Instruments

Table of Materials

Name of Material/ Equipment	Company	Catalog Number
Buprenorphine HCL	Reckitt Benckiser Healthcare UK	NDC12496-0757-5
Dissecting forceps, curved	Zhenbang, China	
Heparin sodium injection USP	Sagent Pharmaceuticals	NDC25021-400-10
Micro-forceps, straight, smooth	Jingzhong, China	WA3010
Micro needle holder	Jingzhong, China	WA2010
Micro vessel clamps	Jingzhong, China	WA40120
Micro spring sciccor 1	ROBOZ	RS-5620
Micro spring sciccor 2	F.S.T.	91501-09
Micro spring sciccor 3	Zhenbang, China	
Prograf (Tacrolimus/FK506)	Astellas	
Rats	Charles River & Taconic Biosciences	LEW/Crl & DA-M
Shaver	Wahl	79600-2101
Suture 4-0	Ethicon	J304H
Suture, 4-0	Ethicon	683G
Suture, 10-0	Ethicon	2820G
Syringes & Needles	BD	
Thread, 8-0	Ashaway	75290
Ureteral cuff	Microlumen	160-1
Venous cuff	Intramedic BD	7441

Comments/Description

11cm Flat handle

8.5cm Vannas,curved

Polymide Tubing, Diameter 0.41 mm
PE-200 Non-radiopaque polyethylene tubing ID: 1.4 mm, OD: 1.9 mm

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Author(s): Ali R. Ahmadi, Le Qi, Kenichi Iwasaki,Jinny Huang, Wei Wang,Russell N. Wesson, Andrew M. Cameron, Zhaoli Sun

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January, 7th 2019

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Please do not hesitate to contact us if you have any remaining questions.

Kind regards,

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