GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Modified Establishment Method of a Rat Kidney Allograft Model

Yu-Hsing Lin [1, 2, #], Ya-Ling Cyue [4, #], Pi-Hsin Chen [4, #], Ya-Peng Wang [4], Tsung-Han Wu [4], Shih-Yi Guo [4], Keng-Chia Hsu [4], Jhih-Yun Wang [4], Chwei-Jang Chiou [1, 2], Tung-Hsuan Tsai [1], Chia-Ying Lin [4], Yu-Ying Fang [4] and Shao-Wen Hung [3, 4, *]

[1] *Department of Pet Healthcare, Yuanpei University of Medical Technology, Hsinchu 300, Taiwan*
[2] *Department of Animal Healthcare, College of Medical and Health Care, HungKuang University, Taichung 433, Taiwan*
[3] *Department of Nursing, Yuanpei University of Medical Technology, Hsinchu 300, Taiwan*
[4] *Division of Sustainable Agriculture, Agricultural Facilities and Environment Research Center, Agricultural Technology Research Institute, Hsinchu 300, Taiwan*
[#] *Contributed equally to this work.*

GSC Biological and Pharmaceutical Sciences, 2026, 35(03), 075-081

Publication history: Received on 02 May 2026; revised on 08 June 2026; accepted on 10 June 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.35.3.0214

Abstract

Kidney transplantation is an established therapeutic option for patients with end-stage renal disease. However, immune rejection and the adverse effects associated with long-term immunosuppressive therapy remain major challenges affecting graft survival and patient outcomes. With the rapid development of cell therapy, regenerative medicine products, and immunomodulatory agents, reliable and reproducible preclinical animal models are essential for evaluating the safety and efficacy of novel therapeutic strategies. The rat kidney allograft model is a valuable platform for studying acute and chronic rejection, antibody-mediated rejection, immune regulation, and transplant tolerance. This study aimed to establish a standardized rat kidney allograft model and surgical workflow for future preclinical evaluation of immunosuppressive drugs, cell therapy products, and other immunomodulatory interventions. In methods, Sprague-Dawley rats were used as donor and recipient animals. A standardized surgical procedure was established, including donor kidney procurement, renal artery and vein preparation, ureteral catheterization, heparinized saline perfusion, cold preservation of the donor kidney, recipient kidney transplantation, vascular reperfusion, ureteral reconstruction, and postoperative care. General anesthesia, analgesia, aseptic surgical procedures, and postoperative monitoring were incorporated into the protocol. Potential outcome measures for future efficacy studies were also proposed, including graft function, histopathological changes, immune cell infiltration, inflammatory cytokines, and graft survival. In results, a complete rat kidney allograft surgical workflow was successfully established, including donor nephrectomy, graft perfusion, vascular and ureteral reconstruction, reperfusion assessment, and postoperative care. Successful reperfusion was evaluated by immediate color change of the graft, restoration of renal perfusion, ureteral peristalsis, and possible urine production. The model provides a practical platform for assessing graft function, immune rejection, inflammatory responses, and therapeutic effects of immunomodulatory products. In conclusion, the rat kidney allograft model established in this study provides a clinically relevant preclinical platform for investigating transplant rejection, immune regulation, and transplant tolerance. This model may support the development of cell therapy products, immunosuppressive agents, and other immunomodulatory strategies before progression to large-animal studies or clinical trials.

Keywords: Allograft; Cell therapy; Immunomodulation; Preclinical animal model; Rat kidney transplantation; Transplant rejection

* Corresponding author: Shao-Wen Hung

1. Introduction

Kidney transplantation is one of the most effective therapeutic approaches for patients with end-stage renal disease. Compared with long-term dialysis, kidney transplantation generally provides improved quality of life and better long-term outcomes. Despite advances in surgical techniques, immunosuppressive regimens, and post-transplant care, immune rejection remains a major determinant of graft survival. Although current immunosuppressive drugs effectively reduce the risk of acute rejection, long-term use is associated with infection, nephrotoxicity, metabolic complications, malignancy, and other adverse effects. Therefore, strategies that induce transplant tolerance, reduce dependence on chronic immunosuppression, and preserve long-term graft function remain important goals in transplantation medicine [1-2].

In recent years, cell therapy and immunomodulatory products have emerged as promising approaches for improving transplant outcomes. Regulatory T cells, chimeric antigen receptor regulatory T cells, hematopoietic stem cell-based therapies, and bone marrow-derived multipotent adult progenitor cells have been investigated in preclinical and clinical studies for the prevention or control of transplant rejection. The therapeutic effects of these products are largely mediated through immune regulation, suppression of inflammatory responses, induction of immune tolerance, and reduction of conventional immunosuppressive drug requirements [3-5].

Because cell therapy and immunomodulatory products have complex biological activities, appropriate disease-relevant animal models are required for preclinical evaluation. Compared with in vitro immune assays, organ transplantation models allow integrated assessment of vascular reconstruction, organ reperfusion, graft function, immune rejection, tissue injury, and systemic immune responses. Among available models, the rat kidney allograft model has been widely used to study acute rejection, chronic rejection, antibody-mediated rejection, immunosuppressive drug efficacy, and tolerance-inducing strategies. It is particularly useful as an intermediate platform before conducting more complex and costly large-animal studies [6-7].

However, kidney transplantation in rats is technically demanding. It requires microsurgical skills, vascular reconstruction, ureteral reconstruction, organ perfusion, ischemia control, and intensive postoperative care. Without a standardized surgical procedure and appropriate training, non-immunological causes of graft failure, such as thrombosis, vascular leakage, ureteral obstruction, or infection, may compromise the reliability of experimental results. Therefore, this study aimed to establish a standardized rat kidney allograft model as a preclinical platform for evaluating cell therapy products, immunosuppressive agents, and other immunomodulatory strategies [8].

2. Materials and Methods

2.1. Animals and Housing

Sprague-Dawley (SD) rats suitable for kidney transplantation studies were used as donor and recipient animals. Animals were obtained from a qualified laboratory animal supplier and housed in an animal facility that met institutional animal care standards. Before surgery, animals were observed for general health status. Rats with obvious illness, injury, or conditions unsuitable for surgery were excluded. All animal procedures should be performed in accordance with an approved institutional animal care and use protocol. The study design should follow the principles of the 3Rs: replacement, reduction, and refinement. Appropriate anesthesia, analgesia, aseptic technique, and postoperative care should be provided to minimize pain and distress.

2.2. Experimental Animals

Adult male SD rats (12 weeks old) with specific pathogen-free conditions were used for this study, were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan). These SD rats were fed with standard laboratory diet (No. 5001, LabDiet®; PMI Nutrition International, St. Louis, MO, USA) and distilled water ad libitum during the experimental period. The environment was maintained room temperature (24-27°C) and 60%-70% humidity with a photoperiod of 12-hr light/12-hr dark cycle. The study will begin after a week acclimation. The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC-111013 approved by the IACUC ethics committee.

2.3. Experimental Design

The 30 SD rats were divided into as donor group (n = 15) and recipient group (n = 15). The surgical induction in both groups was performed. Animal behaviors and survival time were monitored during the experiment.

2.4. Surgical Instruments and Materials

The surgical procedure required microsurgical instruments, micro-dissecting forceps, micro-scissors, atraumatic microvascular clamps, sutures, intravenous catheter tubing, sterile saline, heparin, sterile gauze, a surgical microscope or dissecting microscope, sterilization equipment, and postoperative care materials. All surgical instruments were sterilized before use, and the surgical field was maintained under aseptic conditions.

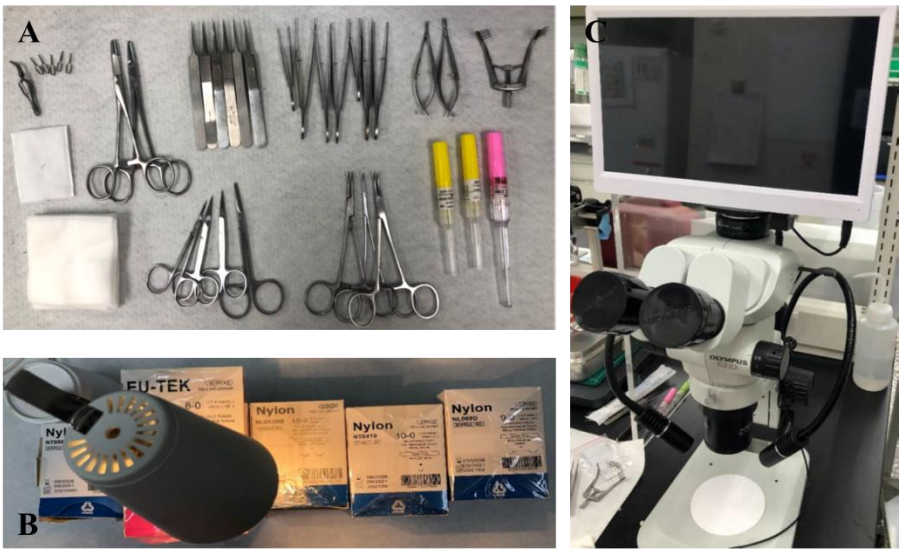

Figure 1 Surgical instruments and materials. (A) Microsurgical instruments, micro-dissecting forceps, and micro-scissors etc. (B) Atraumatic microvascular clamps, sutures (C) a dissecting microscope

2.5. Anesthesia and Analgesia

Rats were anesthetized before surgery. Based on the established protocol, Zoletil 50 or inhaled isoflurane may be used for general anesthesia. Ketoprofen may be administered subcutaneously for analgesia before or after surgery. The depth of anesthesia should be monitored throughout the procedure to ensure the absence of pain responses and to maintain physiological stability.

2.6. Donor Kidney Procurement

After anesthesia, the donor rat was placed in the supine position, and the limbs were fixed. Abdominal hair was removed, and the surgical area was disinfected with povidone-iodine and 75% ethanol. A midline abdominal incision was made through the skin and abdominal wall to expose the abdominal cavity. Retractors were placed to improve visualization. The intestines were covered with sterile moist gauze and gently moved to the right side of the abdomen to expose the abdominal aorta, inferior vena cava, and left kidney. Warm sterile saline was applied to maintain tissue moisture and temperature.

The left kidney was identified and covered with moist gauze. Under microscopic visualization, the left renal artery and renal vein were carefully separated from surrounding connective tissue. The ureter was isolated, obliquely transected, and cannulated using intravenous catheter tubing. The catheter was secured with fine absorbable sutures.

Before removal of the donor kidney, heparinization was performed to reduce thrombus formation. The kidney was then perfused with cold heparinized saline to remove residual blood from the renal vasculature. Excessive perfusion pressure should be avoided to prevent vascular or parenchymal injury. After perfusion, the renal artery and renal vein were ligated near the aorta and vena cava, respectively. The donor kidney was removed and preserved in cold saline until transplantation (Fig. 2A).

2.7. Recipient Kidney Transplantation

The recipient rat was anesthetized, prepared, and disinfected using the same procedure as the donor. After laparotomy, the left kidney, abdominal aorta, inferior vena cava, and ureter were exposed. Atraumatic microvascular clamps were applied near the aorta and vena cava to temporarily interrupt blood flow. The recipient renal vessels and ureter were prepared for connection with the donor graft.

The donor renal vein catheter was inserted into the recipient renal vein and secured with fine sutures. The donor renal artery catheter was then connected to the recipient renal artery and similarly secured. After completion of vascular reconstruction, the vascular clamps were removed to allow reperfusion of the transplanted kidney. Successful reperfusion was indicated by an immediate change in graft color from pale to dark red. Ureteral peristalsis and urine production may also be observed.

After vascular reperfusion, the donor ureteral catheter was inserted into the recipient ureter and secured with fine sutures. The abdominal cavity was inspected for bleeding, vascular leakage, thrombosis, or organ malposition. Sterile saline was applied to maintain moisture, and antibiotics may be used when appropriate to reduce infection risk. The abdominal muscle layer was closed with absorbable sutures, and the skin was closed with interrupted sutures (Fig. 2B).

2.8. Postoperative Care

After surgery, rats were placed in clean cages and monitored during recovery. Warming, food, and water were provided. Postoperative observations included activity, food intake, water intake, body weight, urine output, wound healing, and signs of pain or distress. Analgesics and supportive care were provided as needed. Skin sutures may be removed 7 to 10 days after surgery, depending on wound healing.

Humane endpoints should be clearly defined. Animals showing severe lethargy, persistent anorexia, rapid weight loss, anuria, abdominal distension, wound infection, respiratory distress, or other severe clinical signs should be managed according to the approved animal protocol.

3. Results

3.1. Establishment of the Rat Kidney Allograft Surgical Workflow

A standardized surgical workflow for rat kidney allograft transplantation was established. The procedure included donor kidney procurement, ureteral catheterization, graft perfusion, cold preservation, recipient vascular preparation, renal artery and vein reconstruction, ureteral reconstruction, reperfusion assessment, and postoperative care. This workflow provides a practical foundation for training microsurgical personnel and establishing a stable preclinical transplant platform (Fig. 2).

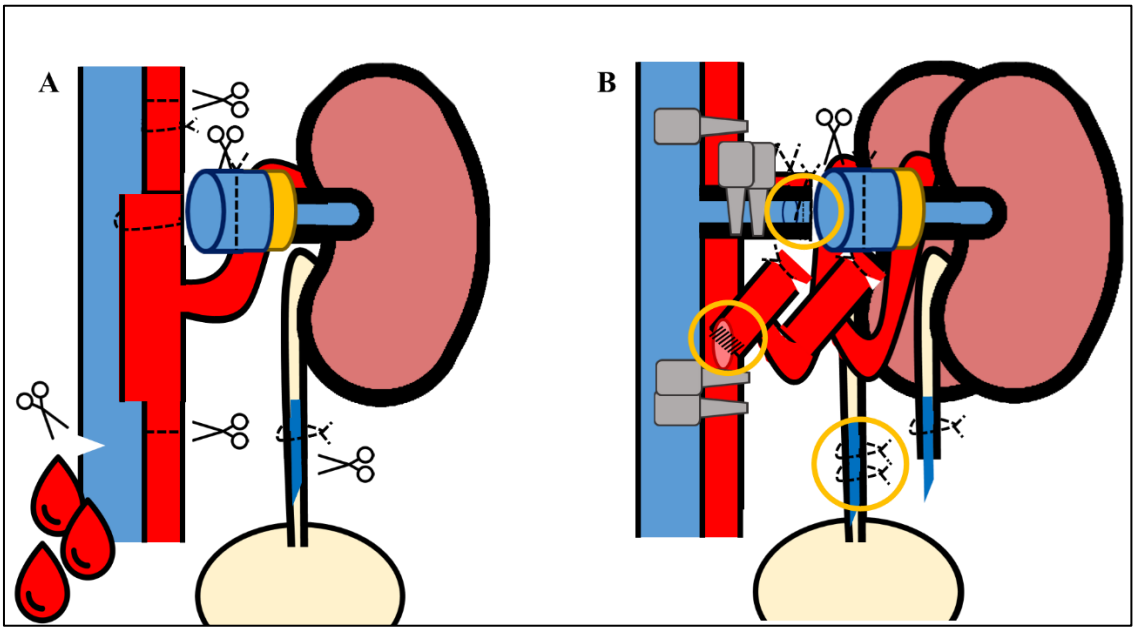

Figure 2 Surgical procedure for establishment of the rat kidney allograft model. (A) Donor kidney procurement. After isoflurane anesthesia and abdominal exposure, the kidney was flushed with heparinized saline via the portal vein, harvested after vascular and ureteral transection, and prepared with a venous fixation sleeve. (B) Recipient kidney transplantation. After vascular occlusion, an aortic opening was created for arterial anastomosis, and the donor renal vein was inserted into the recipient renal vein and secured with a vascular sleeve

3.2. Establishment of Intraoperative Criteria for Reperfusion Assessment

After removal of the vascular clamps, successful reperfusion was assessed by the gross appearance of the transplanted kidney. A successfully reperfused graft typically changed from a pale color to a natural dark red color within minutes. Ureteral peristalsis and urine production may also be observed. These intraoperative findings provide useful indicators for determining the technical success of the transplantation procedure (Fig. 3). In addition, the survival time of the SD rats (n = 15) with recipient kidney transplantation was between 7-14 days (data not shown).

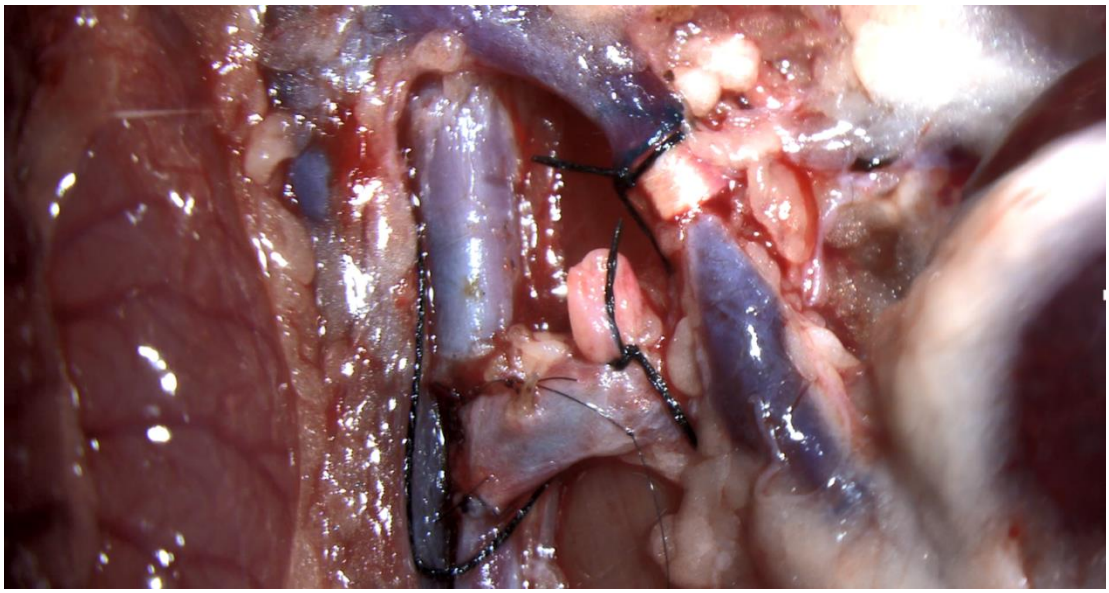

Figure 3 Successful establishment of the rat kidney allograft model

4. Discussion

The rat kidney allograft model is technically challenging but highly valuable for transplantation research. Compared with skin graft or heterotopic heart transplantation models, kidney transplantation provides direct functional readouts of a solid organ graft. Renal function can be assessed using serum biochemical markers, urine analysis, and histopathological evaluation. Therefore, this model is particularly suitable for studying kidney transplant rejection and for testing therapeutic strategies intended to preserve graft function [4-6, 9].

For the development of cell therapy products, the selected preclinical model does not always need to match the exact target organ used in the clinical indication. However, the model should reasonably reflect the expected mechanism of action of the product. If the therapeutic product primarily acts through immune modulation, such as regulatory T cell expansion, CAR-Treg therapy, bone marrow-derived cell therapy, or other immunosuppressive cellular products, preclinical studies should evaluate whether the product reduces rejection, modulates inflammatory responses, prolongs graft survival, and improves graft function. The rat kidney allograft model allows simultaneous assessment of organ function and immune responses, making it more informative than isolated in vitro immune assays [10-11].

Several limitations should be considered. First, this model requires advanced microsurgical skills. Technical failure due to thrombosis, vascular leakage, ureteral obstruction, ischemic injury, or infection may confound interpretation of immune-mediated rejection. Second, immune responses in rats do not fully recapitulate human transplant immunology. Therefore, findings from this model should be interpreted carefully and, when necessary, validated in additional models. Third, when the model is used for product efficacy testing, appropriate controls are essential, including syngeneic controls, allogeneic controls, immunosuppressive drug controls, and treatment groups [12-13]. Future studies should further quantify surgical and biological outcomes, including operative time, cold ischemia time, warm ischemia time, reperfusion success rate, postoperative survival, serum creatinine, blood urea nitrogen, graft histopathology scores, immune cell infiltration, cytokine profiles, and graft survival. Comparisons among syngeneic transplantation, untreated allogeneic transplantation, and immunosuppression-treated allogeneic transplantation would help validate the sensitivity of this model for detecting rejection and tolerance.

The rat kidney allograft model established in this study provides a clinically relevant preclinical platform for evaluating cell therapy products, immunosuppressive agents, and other immunomodulatory strategies. Beyond assessing surgical feasibility, this model enables integrated evaluation of graft function, survival, histopathological injury, immune cell infiltration, inflammatory and anti-inflammatory cytokine responses, antibody-mediated rejection markers, and the distribution or persistence of administered therapeutic cells. These multidimensional endpoints allow comprehensive assessment of both graft outcomes and immune regulatory mechanisms. Given the increasing development of cell therapy and advanced biomedical products, the establishment of this model may help address the current lack of domestic preclinical platforms for organ transplantation, transplant rejection, and transplant tolerance research, thereby supporting academic and industrial product development and facilitating future translation to large-animal studies or clinical trial planning. Overall, the rat kidney allograft model established in this study provides a foundational preclinical platform for the development of cell therapy products, immunosuppressive agents, and transplant immunomodulatory strategies. Integration of standardized surgical procedures with functional, histological, and immunological endpoints will improve the scientific reliability and regulatory relevance of future preclinical studies.

5. Conclusion

Animal models serve as the critical bridge between in vitro studies and human applications, providing essential insights into rejection mechanisms, immunomodulation, and graft function. Small animal models are best suited for mechanistic and pharmacological studies, whereas large animal models offer clinically relevant platforms for translational research. Selecting the appropriate model is pivotal to deriving meaningful conclusions and ensuring effective translation of cell-based therapies into clinical practice.

This study successfully established a standardized rat kidney allograft model, including donor kidney procurement, graft perfusion, vascular reconstruction, ureteral reconstruction, reperfusion assessment, and postoperative care. The model is suitable for investigating transplant rejection, immune regulation, and transplant tolerance. It may serve as a preclinical platform for evaluating cell therapy products, immunosuppressive drugs, and other immunomodulatory strategies. Future incorporation of renal function tests, histopathological scoring, immune cell profiling, and cytokine analysis will further enhance the translational and regulatory value of this model.

Compliance with ethical standards

Acknowledgments

All authors thank the Council of Agriculture in Taiwan (Executive Yuan) [grant numbers 110AS-1.6.1-ST-m2] for supporting this study.

Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC-111013 approved by the IACUC ethics committee.

References

[1] Liu F, Kang SM. 2007. Heterotopic heart transplantation in mice. Journal of Visualized Experiments 6: e238.

[2] Ruzza A, Vespignani R, Czer LS, Robertis MDe, Wu GN, A. Trento A. 2010. Heterotopic heart transplantation in rats: improved anesthetic and surgical technique. Transplant Proceedings 42: 3828-3832.

[3] Oldani G, Lacotte S, Morel P, Mentha G, Toso C. 2012. Orthotopic liver transplantation in rats. Journal of Visualized Experiments 65: e4143.

[4] Orlando G, Soker S, Wood K. 2009. Operational tolerance after liver transplantation. Journal of Hepatolgy 50: 1247-1257.

[5] Lodhi SA, Lamb KE, Meier-Kriesche HU. 2011. Solid organ allograft survival improvement in the United States: the long-term does not mirror the dramatic short-term success. American Journal of Transplantation 11: 1226-1235.

[6] Kasiske BL, Snyder JJ, Gilbertson D, Matas AJ. 2003. Diabetes mellitus after kidney transplantation in the United States. American Journal of Transplantation 3: 178-185.

[7] de Mattos AM, Olyaei AJ, Bennett WM. 2000. Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. American Journal of Kidney Diseases 35: 333-346.

[8] Shrestha B, Haylor J. 2014. Experimental rat models of chronic allograft nephropathy: a review. International Journal of Nephrology and Renovascular Disease 7: 315-322.

[9] Wang JJ, Hockenheimer S, Bickerstaff AA, Hadley GA. 2009. Murine renal transplantation procedure. Journal of Visualized Experiments. 29: e1150.

[10] Bickerstaff AA, Wang JJ, Pelletier R P, Orosz CG. 2001. Murine renal allografts: spontaneous acceptance is associated with regulated T cell-mediated immunity. Journal of Immunology 167: 4821-4827.

[11] Pahlavan PS, Smallegange C, Adams MA, Schumacher M. 2006. Kidney transplantation procedures in rats: assessments, complications, and management. Microsurgery 26: 404-411.

[12] Ahmadi AR, Qi L, Iwasaki K, Wang W, Wesson RN, Cameron AM, Sun Z. 2019. Orthotopic rat kidney transplantation: A novel and simplified surgical approach. Journal of Visualized Experiments 147: e59403.

[13] Yu Y, Lia J, Bia Z, Wu C, Yang S, Jiang Q, Deng R, Fu Q, Liu L, Wang C. 2022. Cuff anastomosis of both renal artery and vein to minimize thrombosis: A novel method of kidney transplantation in mice. Journal of Investigative Surgery 35: 56-60.