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 mouse renal allograft model applied in the preclinical research

Yu-Hsing Lin [1, 2, #], Pi-Hsin Chen [4, #], Keng-Chia Hsu [4, #], Shih-Yi Guo [4], Ya-Ling Cyue [4], Ya-Peng Wang [4], Tsung-Han Wu [4], Jhih-Yun Wang [4], 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.*

GSC Biological and Pharmaceutical Sciences, 2026, 36(01), 110–117

Publication history: Received on 25 May 2026; revised on 04 July 2026; accepted on 06 July 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.36.1.0252

## Abstract

Kidney transplantation is an established therapeutic option for patients with end-stage renal disease. However, immune rejection and complications associated with long-term immunosuppressive therapy remain major challenges affecting graft survival and clinical 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 mouse kidney allograft model is a valuable platform for studying acute and chronic rejection, antibody-mediated rejection, immune regulation, ischemia-reperfusion injury, and transplant tolerance. Compared with rat models, mouse models provide additional advantages for mechanistic studies because genetically modified, congenic, and inbred strains can be used to investigate specific immune pathways involved in graft rejection or tolerance. This study aimed to establish a standardized mouse kidney allograft model and surgical workflow for future preclinical evaluation of immunosuppressive drugs, cell therapy products, and other immunomodulatory interventions. The standardized procedure included donor kidney procurement, graft perfusion, preservation of vascular structures, recipient kidney transplantation, vascular reconstruction, urinary reconstruction, graft reperfusion assessment, and postoperative care. General anesthesia, analgesia, aseptic surgical procedures, temperature support, and postoperative monitoring were incorporated into the protocol. Potential outcome measures for future efficacy studies include graft function, serum creatinine, urine analysis, histopathological changes, immune cell infiltration, inflammatory cytokine profiles, graft survival, and overall animal survival. Successful reperfusion may be assessed by immediate color change of the graft, restoration of renal perfusion, absence of major bleeding or thrombosis, and subsequent recovery of recipient activity. The established model provides a practical platform for evaluating graft function, immune rejection, inflammatory responses, and therapeutic effects of immunomodulatory products. In conclusion, the mouse kidney allograft model provides a clinically relevant and mechanistically powerful 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 larger-animal studies or clinical trials

**Keywords:** Allograft; Cell therapy; Immunomodulation; Mouse kidney transplantation; Preclinical animal model; Transplant rejection; Transplant tolerance

* 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 [1-6].

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, bone marrow-derived cell therapy, and other immunomodulatory products have been investigated 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 [7-12].

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 mouse kidney allograft model is particularly useful because genetically modified and inbred mouse strains can be used to dissect the roles of specific immune cells, cytokines, chemokines, and signaling pathways in graft rejection and tolerance.

However, kidney transplantation in mice is technically demanding. It requires advanced microsurgical skills, careful vascular reconstruction, urinary reconstruction, organ perfusion, ischemia control, and intensive postoperative care. Non-immunological causes of graft failure, such as thrombosis, vascular leakage, anastomotic stenosis, ureteral obstruction, ischemia-reperfusion injury, or infection, may compromise interpretation of experimental results. Therefore, this study aimed to establish a standardized mouse kidney allograft model as a preclinical platform for evaluating cell therapy products, immunosuppressive agents, and other immunomodulatory strategies [13-18].

## 2. Materials and Methods

### 2.1. Animals and Housing

Adult male BALB/c mice suitable for kidney transplantation studies were used as donor and recipient animals. Depending on the experimental purpose, syngeneic combinations may be used for technical establishment and ischemia-reperfusion injury studies, whereas allogeneic strain combinations may be used for rejection and immunomodulation studies. 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. Mice with obvious illness, injury, abnormal body condition, 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 replacement, reduction, and refinement. Appropriate anesthesia, analgesia, aseptic technique, temperature support, and postoperative care should be provided to minimize pain and distress.

### 2.2. Experimental Animals

Adult male BALB/c mice (12 weeks old) with specific pathogen-free conditions were used for this study, were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan). These BALB/c mice 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-110096 approved by the IACUC ethics committee.

### 2.3. Experimental Design

The 60 BALB/c mice were divided into as donor group (n = 30) and recipient group (n = 30). The surgical induction in both groups was performed. Animal behaviors and survival time were monitored during the experiment. The model may be established in two stages. In the first stage, syngeneic transplantation can be used to optimize surgical procedures, reduce technical failure, and confirm graft perfusion and survival. In the second stage, allogeneic transplantation can be used to evaluate immune rejection, graft injury, and therapeutic effects of immunomodulatory products. Donor and recipient mice are assigned according to the experimental design. General condition, body weight, survival, graft function, and clinical signs are monitored after transplantation. Depending on the purpose of the study, the contralateral native kidney may be removed several days after transplantation to allow functional assessment of the transplanted kidney.

### 2.4. Surgical Instruments and Materials

The procedure requires microsurgical instruments, micro-dissecting forceps, micro-scissors, atraumatic microvascular clamps, fine sutures, sterile saline, heparinized perfusion solution, sterile gauze, a surgical microscope or dissecting microscope, sterilization equipment, warming devices, and postoperative care materials. All surgical instruments should be sterilized before use, and the surgical field should be maintained under aseptic conditions (Fig. 1).

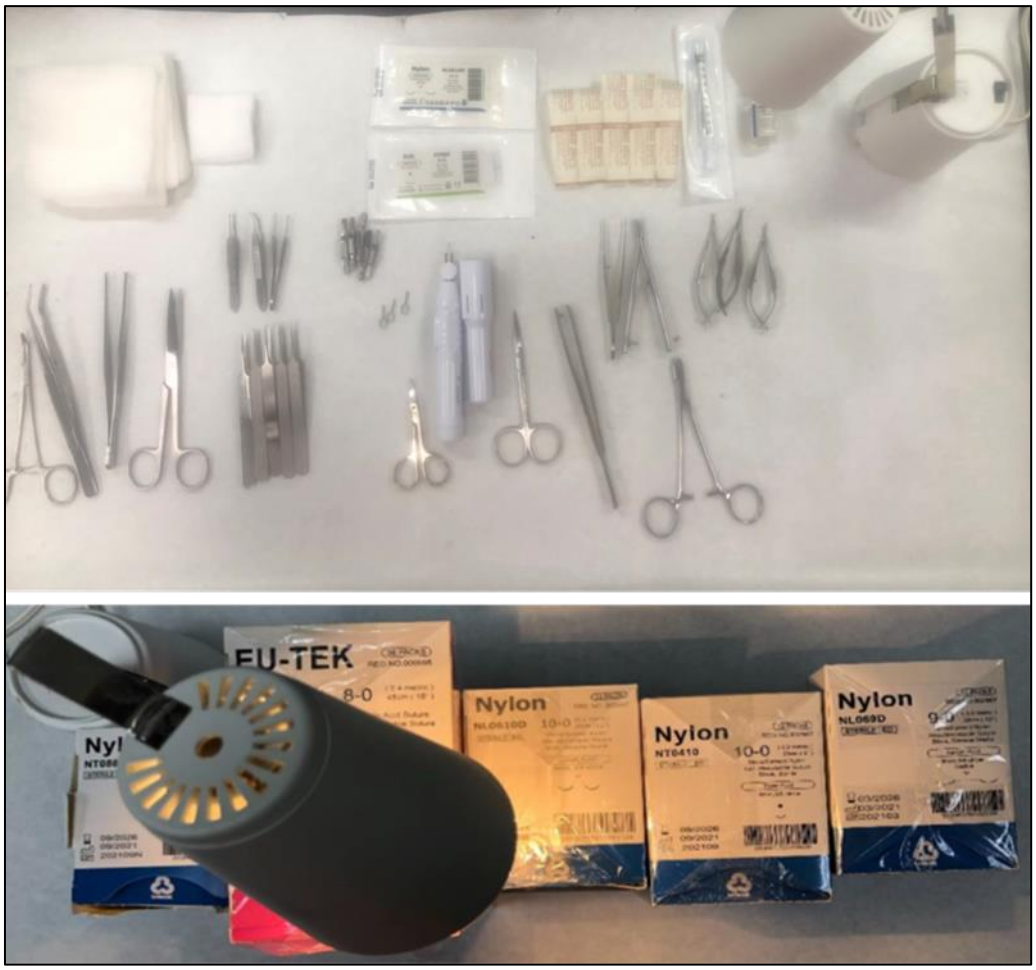


**Figure 1** Surgical instruments and materials included microsurgical instruments, micro-dissecting forceps, and micro-scissors, atraumatic microvascular clamps, sutures, and a dissecting microscope etc

### 2.5. Anesthesia and Analgesia

Mice are anesthetized with Zoletil™ 50 or isoflurane before surgery according to the approved animal protocol. The depth of anesthesia should be monitored throughout the procedure by respiratory pattern, reflex responses, and overall physiological stability. Analgesics should be administered before or after surgery according to institutional animal care guidelines. Ophthalmic protection, warming support, and fluid support should be provided when appropriate.

### 2.6. Donor Kidney Procurement

After anesthesia, the donor mouse is placed in the supine position, and the surgical area is prepared under aseptic conditions. A midline abdominal incision is made to expose the abdominal cavity. The intestines are gently moved aside and covered with sterile moist gauze to expose the left kidney, abdominal aorta, inferior vena cava, renal vessels, and ureter.

The left kidney is carefully isolated under microscopic visualization. The adrenal and gonadal vessels are ligated or divided as needed. The ureter is dissected with preservation of its blood supply. Depending on the urinary reconstruction method, a small bladder patch containing the ureterovesical junction may be harvested with the donor ureter, or the donor ureter may be prepared for direct implantation into the recipient bladder.

Before removal of the donor kidney, systemic or local heparinization and cold perfusion are performed according to the established protocol to reduce thrombus formation and remove residual blood from the renal vasculature. Excessive perfusion pressure should be avoided to prevent vascular or parenchymal injury. The donor kidney is removed en bloc with appropriate vascular structures, including the donor aortic cuff and renal vein or venous patch, and preserved in cold sterile solution until transplantation.

### 2.7. Recipient Kidney Transplantation

The recipient mouse is anesthetized, prepared, and disinfected using the same aseptic procedure. After laparotomy, the abdominal aorta, inferior vena cava, recipient kidney region, and urinary bladder are exposed. The native kidney on the transplantation side is removed or prepared according to the selected surgical approach. Atraumatic vascular control is applied to temporarily interrupt blood flow at the recipient vascular reconstruction site.

For vascular reconstruction, the donor aortic cuff is anastomosed to the recipient abdominal aorta, and the donor renal vein or venous patch is anastomosed to the recipient inferior vena cava or renal vein, depending on the selected technique. A cuffed renal vein method may also be considered to simplify venous reconstruction and reduce venous anastomosis-related complications. During anastomosis, careful attention should be paid to vessel orientation, luminal patency, suture tension, and avoidance of twisting, stenosis, bleeding, or thrombosis.

After completion of vascular reconstruction, the clamps are gradually released to allow reperfusion of the transplanted kidney. Successful reperfusion is indicated by rapid color change of the graft from pale to pink or red, restoration of vascular filling, and absence of major bleeding or venous congestion.

Urinary reconstruction is then performed. Depending on the selected protocol, the donor ureter with a small bladder patch may be anastomosed to the recipient bladder, or the donor ureter may be implanted into the bladder. The abdominal cavity is inspected for bleeding, vascular leakage, thrombosis, ureteral obstruction, or organ malposition. The abdominal wall and skin are closed in layers using appropriate sutures.

### 2.8. Postoperative Care

After surgery, recipient mice are placed in a warmed recovery environment and monitored until fully awake. Postoperative observations include activity, posture, grooming, food intake, water intake, body weight, urine output, wound healing, and signs of pain or distress. Analgesics, antibiotics, fluid support, and nutritional support may be provided according to the approved protocol.

Because mice are vulnerable to hypothermia after microsurgery, temperature support during the early postoperative period is essential. Skin sutures may be removed after adequate wound healing. Humane endpoints should be clearly defined. Animals showing severe lethargy, persistent anorexia, rapid body weight loss, anuria, abdominal distension, wound infection, respiratory distress, severe pain, or other serious clinical signs should be managed according to the approved animal protocol.

### 2.9. Recipient Contralateral Nephrectomy

To allow functional assessment of the transplanted kidney, the remaining native kidney may be removed several days after transplantation, after the graft has recovered from ischemia-reperfusion injury. This staged approach reduces the risk of early postoperative mortality and allows serum creatinine or other renal functional markers to better reflect graft function.

## 3. Results

### 3.1. Establishment of the Mouse Kidney Allograft Surgical Workflow

A standardized surgical workflow for mouse kidney allograft transplantation was established. The workflow included donor kidney procurement, cold perfusion, preservation of vascular structures, recipient vascular preparation, vascular reconstruction, urinary reconstruction, reperfusion assessment, and postoperative care. This workflow provides a practical foundation for training microsurgical personnel and establishing a reproducible preclinical transplant platform (Figs. 2-3).

Successful graft reperfusion was assessed by immediate color change of the transplanted kidney, restoration of renal perfusion, absence of major bleeding or thrombosis, and postoperative recovery of recipient activity. The model can be further used to evaluate graft function, histopathological injury, immune cell infiltration, and inflammatory responses (Fig. 4). In addition, the survival time of the recipient BALB/c mice (n = 30) with recipient kidney transplantation was between 15 days and successful proportion is between 40-50% (data not shown).

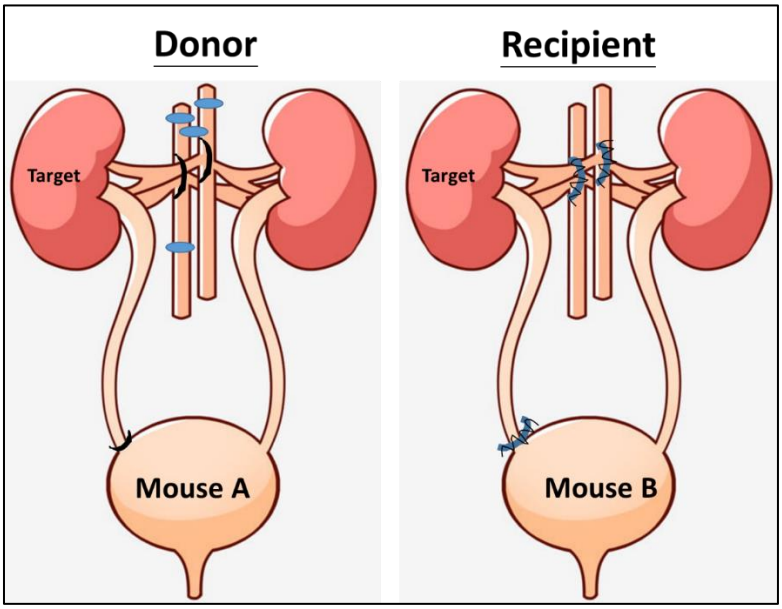


**Figure 2** Surgical procedure for establishment of the mouse kidney allograft model. Donor kidney procurement. After anesthesia and abdominal exposure, the kidney was flushed with heparinized saline via the portal vein, harvested after vascular and ureteral transection, and prepared with a venous fixation sleeve. 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

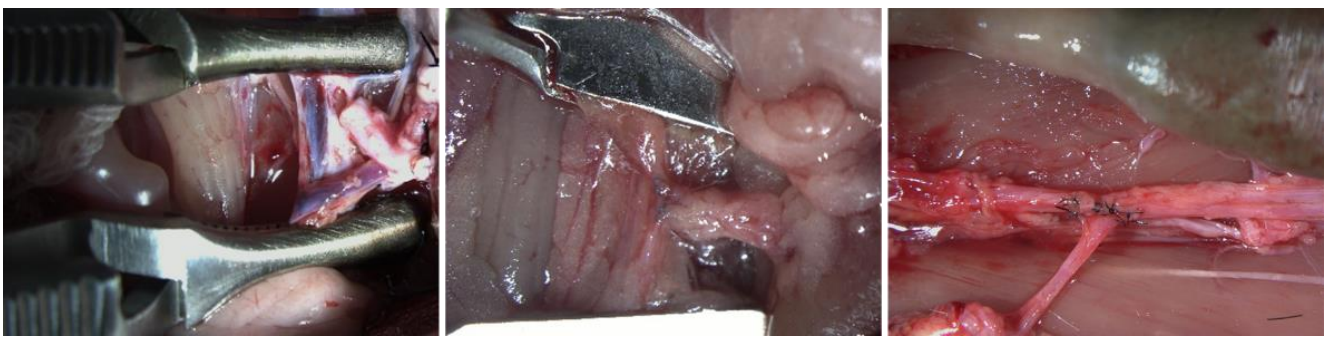

**Figure 3** Successful procedure establishment of the mouse kidney allograft model

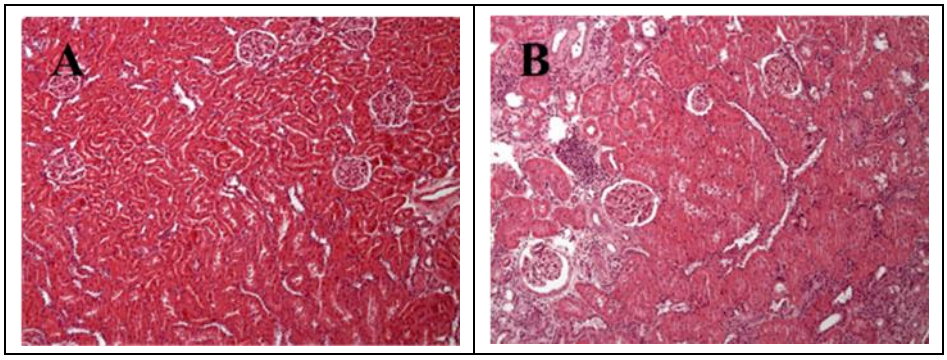


**Figure 4** Histopathological injury in transplanted kidney after kidney transplantation. (A) the donor normal kidney; (B) the recipient transplanted kidney. H and E staining

## 4. Discussion

The mouse 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, survival, and histopathological evaluation. Therefore, this model is particularly suitable for studying kidney transplant rejection and for testing therapeutic strategies intended to preserve graft function [1-7; 19-22].

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-$T_{reg}$ 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 mouse kidney allograft model allows simultaneous assessment of organ function and immune responses, making it more informative than isolated in vitro immune assays [6-12; 23-26].

Several limitations should be considered. First, this model requires advanced microsurgical skills. Technical failure due to thrombosis, vascular leakage, anastomotic stenosis, ureteral obstruction, ischemic injury, or infection may confound interpretation of immune-mediated graft injury. Second, the small size of mouse vessels increases the difficulty of vascular reconstruction and requires careful control of operative time, tissue moisture, temperature, and bleeding. Third, postoperative monitoring and supportive care are critical for reducing non-immunological mortality [13-17; 27-28].

Despite these limitations, the mouse kidney allograft model provides important advantages for mechanistic and translational studies. The availability of genetically modified mouse strains enables detailed investigation of immune pathways involved in rejection and tolerance. When combined with functional, histological, immunological, and molecular analyses, this model can serve as a powerful platform for evaluating immunosuppressive agents, cell therapy products, and other immunomodulatory interventions [29-30].

## 5. Conclusion

A mouse kidney allograft model and standardized surgical workflow were established as a preclinical platform for transplantation research. This model enables integrated evaluation of graft function, immune rejection, inflammatory responses, histopathological injury, and therapeutic efficacy. With appropriate microsurgical training, ischemia control, and postoperative care, the mouse kidney allograft model may support the development of immunomodulatory therapies, cell therapy products, and tolerance-inducing strategies for kidney transplantation.

For future efficacy studies, recommended outcome measures include serum creatinine, blood urea nitrogen, urine output, proteinuria, electrolyte levels, body weight, food and water intake, graft survival, overall survival, and clinical condition. Histopathological evaluation may include tubular injury, interstitial inflammation, vasculitis, glomerular injury, fibrosis, necrosis, and features of acute or chronic rejection.

Immune-related outcome measures may include infiltration of T cells, B cells, macrophages, neutrophils, regulatory T cells, and other immune cell populations. Cytokines and inflammatory mediators such as TNF-α, IL-1β, IL-6, IFN-γ, IL-

10, and TGF-β may be analyzed according to the study objective. For antibody-mediated rejection studies, complement deposition, immunoglobulin deposition, transplant vasculopathy, and donor-specific antibody responses may be considered. For cell therapy studies, the distribution, persistence, and immunomodulatory effects of administered cells may also be evaluated.

## Compliance with ethical standards

*Acknowledgments*

All authors thank the Council of Agriculture in Taiwan (Executive Yuan) [grant numbers 111AS-2.7.2-ST-a1] 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-110096 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.

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

[15] Plenter R, Jain S, Ruller CM, Nydam TL, Jani AH. 2015. Murine kidney transplant technique. Journal of Visualized Experiments 104: e52848.

[16] Chen H, Zhang Y, Zheng D, Praseedom RK, Dong J. 2013. Orthotopic kidney transplantation in mice: technique using cuff for renal vein anastomosis. PLoS ONE 8: e77278.

[17] Lin YH, Cyue YL, Chen PH, Wang YP, Wu TH, Guo SY, Hsu KC, Wang JY, Chiou CJ, Tsai TH, Lin CY, Fang YY, Hung SW. 2026. Modified Establishment Method of a Rat Kidney Allograft Model. GSC Biological and Pharmaceutical Sciences. (in acceptance)

[18] Zhang Z, Schlachta C, Duff J, Stiller C, Grant D, Zhong R. 1995. Improved techniques for kidney transplantation in mice. Microsurgery 16: 103-109.

[19] Kalina SL, Mottram PL. 1993. A microsurgical technique for renal transplantation in mice. Australian and New Zealand Journal of Surgery. 63: 213-216.

[20] Han WR, Murray-Segal LJ, Mottram PL. 1999. Modified technique for kidney transplantation in mice. Microsurgery 19: 272-274.

[21] Ge F, Gong W. 2011. Strategies for successfully establishing a kidney transplant in a mouse model. Experimental and Clinical Transplantation 9: 287-294.

[22] Martins PN. 2006. Learning curve, surgical results and operative complications for kidney transplantation in mice. Microsurgery 26: 590-593.

[23] Wang M, Bai J, Baumann M, Heemann U. 2003. New model for simultaneous heart and kidney transplantation in mice. Microsurgery 23: 164-168.

[24] Rong S, Lewis AG, Kunter U, Haller H, Gueler F. 2012. A knotless technique for kidney transplantation in the mouse. Journal of Transplantation 2012: 127215.

[25] Takahashi N, Boysen G, Li F, Li Y, Swenberg JA. 2007. Tandem mass spectrometry measurements of creatinine in mouse plasma and urine for determining glomerular filtration rate. Kidney International 71: 266-271.

[26] Huang LJ, Reese S, Djamali A. 2012. Contributing factors to complications and surgical success in mouse kidney transplantation. International Brazilian Journal of Urology 38: 395-403.

[27] Wang H. 2012. Small animal models of xenotransplantation. Methods in Molecular Biology 885: 125-153.

[28] Martins PN. 2006. Technique of kidney transplantation in mice with anti-reflux urinary reconstruction. International Brazilian Journal of Urology 32: 713-718.

[29] Zhang Z, Schlachta C, Duff J, Stiller C, Grant D. 1995. Improved techniques for kidney transplantation in mice. Microsurgery 16: 103-109.

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