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LETTER TO THE EDITORS

A novel mouse renal transplant technique

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Vascularized organ transplants in the mouse can be used to dissect aspects of immune response and injury based on a wide ranging availability of genetically modified strains. Frequently utilized cardiac transplant models in the mouse allow an in-depth analysis, however are limited as the heart is usually grafted heterotopically. Kidney transplants in the mouse, in contrast, although technically more demanding, can serve as an excellent model in a physiological and clinical relevant system.

Mouse kidney transplants were initially described in 1973 [1] and have been refined thereafter [2,3]. However, the urinary continuity has remained a challenging part of the procedure. Unlike in rat renal transplants, a direct end-to-end anastomosis of the ureter is difficult to achieve given the small diameter of the mouse ureter. Direct implantation of the ureter into the recipient bladder wall has been associated with post-transplant hydronephrosis in the absence of a physiological anti-reflux system [4].

More frequently, a bladder-to-bladder anastomosis with a bladder patch preserved on the distal donor ureter has been utilized to prevent reflux [2,3,5]. Alternatively, a technique mimicking the clinically applied extravesical ureteroneocystostomy has been reported recently [6]. However, these techniques require extensive training and the reproducibility may be limited.

Here, we describe a novel and simplified technique for a urethral reconstruction in a mouse renal transplant model, which employs direct implantation of the terminal part of ureter together with a very small bladder patch. Male C57Bl/6 mice (Charles River Laboratories, age 8–14 weeks, weighting 25–30 g) were used in this model and anesthetized by continuous isoflurane inhalation (Aerrane; Baxter, Deerfield, IL, USA). All surgeries were performed under a surgical microscope (SMZ800; Nikon, Kawasaki, Japan) using standard microsurgical instruments. Renal artery reconstruction was achieved by end-to-side anastomosis between donor aorta and recipient aorta, and venous reconstruction was completed by end-to-side anastomosis between donor renal vein and recipient infrarenal vena cava as described before [3].

During the procurement procedure, the ureter was dissected down to the bladder thereby preserving the ureter–bladder junction including a small bladder patch of 2 mm in diameter. The renal graft was removed thereafter from the abdomen and kept in normal saline at 4 °C until transplantation.

Following the completion of arterial and venous anastomosis, a 21-gauge needle is inserted into the bladder and exteriorized through the opposite wall. A fine curved forcep is passed through the openings and the distal portion of the ureter including a small bladder patch (2 mm Ø) is pulled into the recipient's bladder. The ureter is now gently retracted to assure that the small bladder patch is abutting the bladder wall and two 11–0 nylon sutures grasping solely the adventitia of terminal ureter and the edge of bladder fixate the distal ureter and seal the small opening of the bladder. The opening on the opposite side of the bladder is closed with another stitch (Fig. 1). Contralateral kidneys were removed on day 4.

We have performed a total of 75 renal transplants in mice using this technique. Time for the donor procedure, vessel and ureteral anastomosis, and for the recipient procedure averaged 22.1 ± 4.7, 26.6 ± 5.0, 8.8 ± 1.9, and 57.1 ± 6.6 min, respectively for the last 50 procedures. Our success rate has been 88% with a long-term survival >100 days. Kidney transplants were evaluated histologically by day 30 and documented an absence of hydronephrosis. Analysis of the distal transplant ureter including the bladder patch documented a normal structure and the absence of ischemic injury or necrosis (Fig. 2).

The surgical complexity of mouse kidney transplants remains a hurdle to establish this attractive model in a reproducible fashion. The urinary continuity represents a critical aspect of the procedure, particularly when aiming for long-term studies. In contrast to previous reports which have described that the ureter slipped out of the bladder utilizing techniques that have not included a distal bladder patch [4], we have not observed a dislocation of the distal ureter. Moreover, the ureter reconstruction time (8.8 ± 1.9 min) using our technique is comparable to the direct implantation of the ureter into the recipient bladder method described by Han *et al.*, and significantly faster than a bladder-to-bladder anastomosis [4]. In our hands, this technique proved to be reliable and reproducible. The relatively short learning curve, particularly for

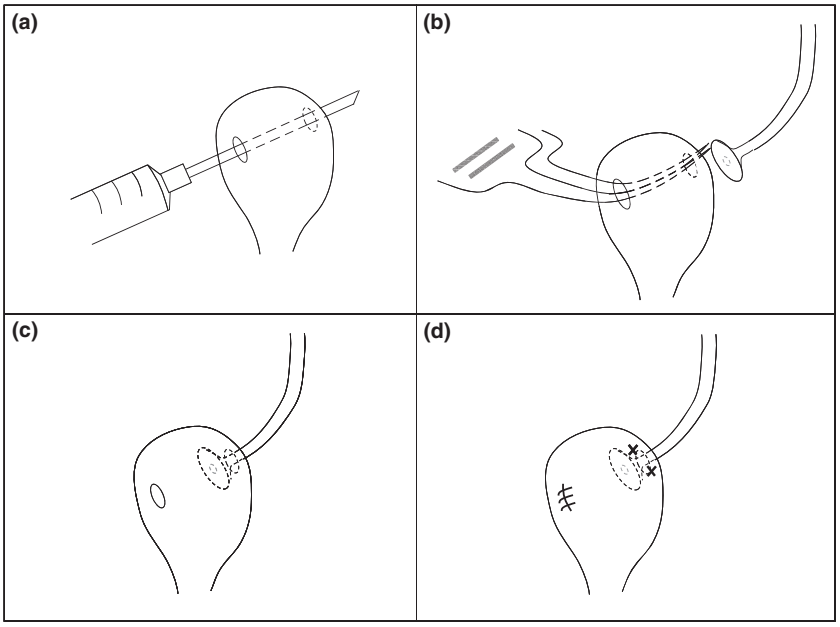

Figure 1 Ureteral anastomosis: (a, b) A 21-gauge needle is forwarded through the bladder wall and the ureter is pulled with a curved forceps; (c) to assure a close contact of the patch with the bladder wall the ureter is gently mobilized backwards; (d) two interrupted sutures through the adventitia fixate the ureter in its position and the contralateral opening is closed.

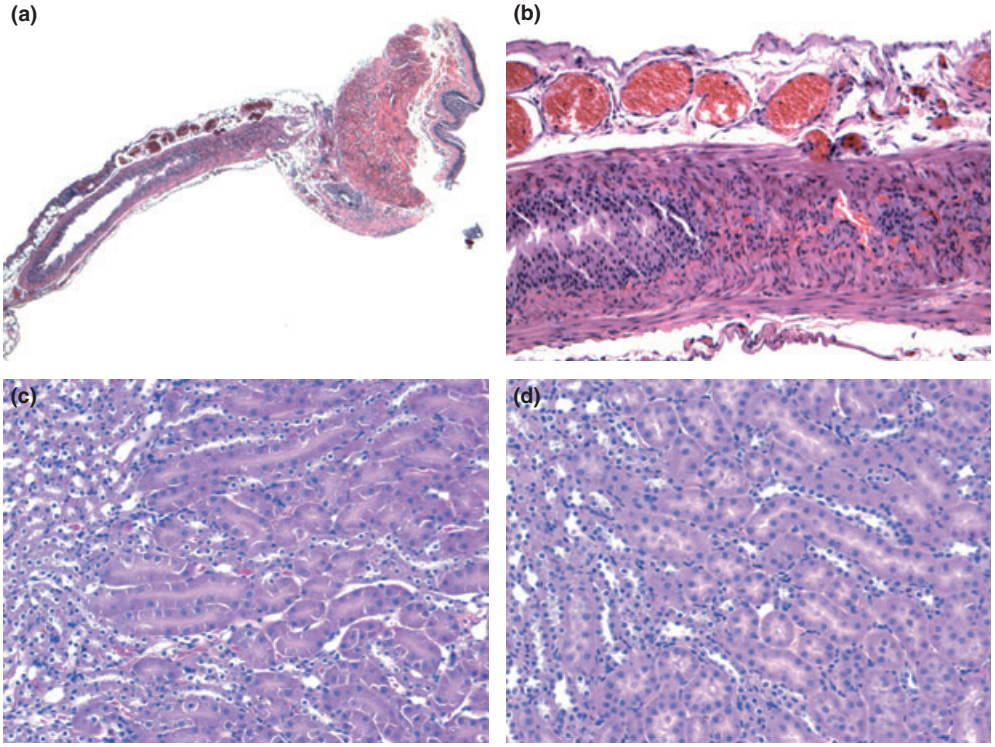

Figure 2 Representative postoperative histology sections (a) The distal transplant ureter including a patch of graft bladder demonstrated a normal structure by week 2 (40×). (b) Under higher magnification (200×) sufficient blood supply in peri-ureteral vessels with good capillary circulation could be documented. (c) Naive control kidneys demonstrate a normal structure (200×). (d) H&E staining of syngeneic renal transplants demonstrated well preserved glomeruli and absence of dilated tubules by day 30 (200×).

those who have previous microsurgical experience, will allow a wider adoption of this model.

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