A new technique for ureteral reconstruction using lingual mucosa grafts in a beagle model

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Research

Keywords: lingual mucosa graft, ureteral stricture, ureteral defect, ureteral reconstruction, ureteroplasty

DOI: https://doi.org/10.21203/rs.3.rs-29043/v1

Abstract

Background The ideal technique for ureteral reconstruction has not been established yet. We report our initial experiment to investigate the feasibility of ureteral reconstruction using lingual mucosa graft (LMG) and to evaluate the histological changes of the engrafted LMG in beagles.

Methods Twelve male beagle dogs were randomly divided into groups A, B and C (n = 4). A ventral ureteral defect was created by excising half of the ureteral wall. The length of the defect was 3 cm, 6 cm and 10 cm in groups A, B and C, respectively. The LMGs were harvested and employed to repair the ureteral defects in an onlay fashion. Two dogs per group were sacrificed after 6 months, with an additional 2 dogs per group sacrificed after 12 months. Intravenous urography (IVU) and macroscopic examination were performed to evaluate renal function and ureteral patency. Histological changes in the engrafted LMGs during the tissue incorporation process were assessed by histological analysis.

Results There were no postoperative complications. Only one dog in group C developed a mild stricture near the proximal anastomosis. In the remaining 11 animals, IVU showed normal renal function and a wide ureteral caliber without stricture or fistula. The diameter of the LMG-reconstructed ureter was greater than that of the proximal and distal ureter (each p value < 0.01). The LMGs survived in situ with newly formed capillaries. The epithelium of the lingual mucosa resembled the urothelium in postoperative 12 months.

Conclusions This new technique for ureteral reconstruction using LMGs is feasible. This approach is a promising alternative clinical treatment for curing long ureteral strictures.

Background

Ureteral strictures or defects can develop due to congenital or secondary causes, including open or endoscopic surgery, stones, trauma, radiotherapy, infection, retroperitoneal fibrosis, tumor or idiopathic causes. More than 57% of ureteral strictures are caused by iatrogenic lesions or impacted stones (1). With the widespread use of endoscopic techniques and laparoscopic gynecological operations, iatrogenic lesions have significantly contributed to the increasing incidence of ureteral stricture (2, 3).

Depending on the location and length of a ureteral stricture, many methods of surgical treatment have been described, such as ureteroureterostomy, ureteroneocystostomy with or without a psoas bladder hitch or a Boari flap, transureteroureterostomy, kidney autotransplantation, ileal interposition, and even nephrectomy (4–6). Although short ureteral strictures can be treated with direct anastomosis, long strictures of the upper ureter are currently treated by ureteric replacement with intestinal segments or renal autotransplantation. Both procedures are elaborate, time-consuming and associated with serious complications (7, 8).

Successful use of buccal mucosal grafts in the repair of urethral strictures has led to interest in a similar technique for ureteral reconstruction (9). Thus far, several case reports have described ureteroplasty using

buccal mucosal grafts to treat ureteral strictures with successful results (10–15). However, buccal mucosal grafts are associated with a risk of donor site morbidity (16–18). Furthermore, the buccal mucosa alone might be insufficient for complicated lengthy ureteral strictures that require a larger supply of graft tissue.

Lingual mucosa has the same tissue features as buccal mucosa (19, 20) (thick epithelium, high content of elastic fibers, rich capillaries and high compatibility in wet environments). Compared to buccal mucosa, LMGs are easy to harvest, have minimal donor site complications and provide lengthy grafts (20–22). Therefore, LMGs may be an ideal donor material for substitution ureteroplasty.

We first put forward the hypothesis of ureteral reconstruction using LMG. However, there are some concerns about this technique: What length of ureteral defect can be repaired by this procedure? Can the LMG survive in situ? What histologic changes may occur in the LMG during the tissue integration process? To answer these questions, we conducted an animal experiment using a dog model.

Methods

The experimental design and animal handling was assessed and approved by the Institutional Animal Care and Use Committee of Tongji Medical College of Huazhong University of Science and Technology, in accordance with the ARRIVE guidelines.

Surgical technique

Twelve male beagle dogs (laboratory animal center, Tongji Medical College, China) weighing 10-12 kg were randomly divided into groups A, B and C (n=4). General anesthesia was administered by intravenous injection of 3% pentobarbital (1 ml/kg, Boster Biological Technology, Wuhan, China). Left laparotomy was carried out to expose the left ureter. After the retroperitoneum was opened, the ureter was identified and incised longitudinally, and then the ventral wall (approximately half of the ureteral circumference) of the upper ureter was removed to produce a defect. The length of the defect was 3 cm, 6 cm and 10 cm in groups A, B and C, respectively. An 18-cm 3Fr Double-J ureteral catheter(Dakang Medical Instrument, Guangzhou, China) was inserted in the ureter (Fig. 1A).

For LMG harvesting, the tongue was pulled out with a traction suture in the tip to expose its ventral surface. The required graft was measured and marked. To minimize bleeding and to facilitate graft harvesting, diluted epinephrine (1:100,000) was injected into the subepithelial layer of the tongue. The LMG was harvested and tailored to the length of the ureteral defect, with a width of 0.8 cm (Fig. 1B). The attached subepithelial muscle and adipose tissue remnants were removed to create an ultra-thin patch graft. Then, the graft was stored in 4℃ saline solution for use. The donor site was carefully examined for bleeding and closed with 3-0 Vicryl(Ethicon, Cincinnati, OH, USA) in a continuous running suture (Fig. 1C).

The graft was placed over the ureteral defect in an onlay fashion, and the epithelium of the LMG became the ureteral lumen (Fig. 1D). The longitudinal edges were anastomosed using 6-0 Vicryl in running

sutures, while interrupted sutures were used for proximal and distal anastomosis (Fig. 1E). After the nontransected augmented ureteroplasty was completed, the omentum was mobilized and wrapped around the reconstructed ureteric segment (Fig. 1F). Proximal and distal anastomosis was marked by nonabsorbable sutures(PROLENE, Ethicon, USA). Finally, the abdominal incision was closed.

Postoperatively, the animals had free access to water and feed and received 1.0 g of cefoperazone intramuscularly daily for 5 days. The Double-J ureteral catheter was removed 6 weeks after surgery.

Functional investigation

IVU was performed after 8 weeks, 6 months and 12 months to evaluate renal function and patency of the reconstructed ureter.

Macroscopic examination

Animals were anaesthetised by intravenous injection of 3% pentobarbital and
killed through air embolism. Two dogs per group were sacrificed after 6 months for short-term examination with a further 2 dogs per group sacrificed after 12 months for long-term analysis. The left ureter and kidney were resected for macroscopic observation. Then, we used a vernier caliper to measure the diameter of the proximal and distal ureter, along with the diameter of LMG-reconstructed ureter within the area outlined by the mark sutures.

Histological analysis

The reconstructed ureteric segments were fixed in 10% buffered formalin. Sections (5 μm) were cut and prepared for histological analysis. Hematoxylin-eosin, Masson's trichrome, and immunohistochemical (IHC) staining using uroplakin-II (UP-II, Santa Cruz, CA, USA) were utilized to reveal the morphology of the grafts, in addition to changes in the epithelium and subepithelial tissue of the LMG. IHC staining using CD31(Santa Cruz, CA, USA) was performed to observe the neovascularization of the LMG.

Statistics

We compared the diameters of the LMG-reconstructed ureter and the proximal and distal ureter. All data are shown as the mean ± SD. Statistical analysis was performed with a t-test using SPSS®, with $p < 0.05$ considered statistically significant.

Results

All dogs started oral feeding one day postoperatively and survived their intended survival period.

Functional investigation

IVU showed normal renal excretory function of both kidneys without renal pelvis dilation. The contrast medium passed through the reconstructed segment freely without any signs of stricture or contrast leak in all animals (Fig. 2A-C), except for one dog in group C that developed a mild ureteral stricture near the proximal anastomosis without hydronephrosis at 6 months postoperation (Fig. 2D).

Macroscopic examination

In all animals, macroscopic examination showed normal kidney morphology without renal pelvis dilation, and the LMG was well incorporated into the ureteral wall (Fig. 3A-C). Only one dog in group C exhibited proximal ureteral dilation. There were no tongue complications, such as bleeding, hematomas, or ulcers (Fig. 3D).

We measured the diameter of the proximal and distal ureter, as well as the LMG-reconstructed ureteric segment in all animals, except for the dog exhibited proximal ureteral stricture in group C. At both 6 and 12 months after surgery, the LMG-reconstructed ureter showed an increased ureteral diameter compared to the proximal and distal ureter (each p value < 0.01). In addition, the diameter of the proximal ureter did not differ significantly from that of the distal ureter (each p value > 0.05) (Fig. 4).

Histological analysis

At 6 months postoperation, HE staining showed that the typical squamous epithelium of lingual mucosa could be discerned by differences in appearance from the urothelium, and the anastomosis of the LMG with the native ureter was obvious (Fig. 5A).

The UP-II antibody is a special urothelium marker. IHC staining with the UP-II antibody showed that almost half of the ureteral inner wall was positive, whereas the squamous epithelium of the lingual mucosa was negative in postoperative 6 month. Thus, it was simple to identify the two different types of epithelium. Moreover, there was intermittent expression of uroplakin on the surface of the LMG (Fig. 5B).

At 12 months, only a fraction of typical squamous epithelium remained. The epithelium morphology over the LMG was highly similar to the morphology of the urothelium (Fig. 5D). The epithelium over the LMG exhibited intermittent expression of uroplakin (Fig. 5E).

Subepithelial fibrosis was also investigated using Masson’s trichrome staining. At 6 months postoperation, the subepithelial tissue of the LMG appeared to be a strong blue, indicating irregular proliferation of collagen fibers (Fig. 5C). At 12 months after surgery, the anastomosis of the LMG with the

native ureter could be discerned by a gap of smooth muscle fibers. A few smooth muscle fibers were deposited, and mature collagen fibers were formed (Fig. 5F).

IHC staining with the CD31 monoclonal antibody showed a few vascular capillaries after 6 months. At 12 months postoperation, newly formed capillaries were dense (Fig. 6A, B).

Discussion

The ultimate outcomes of ureteral strictures are functional obstruction or renal failure in short period if untreated. To preserve renal function and eliminate ureteric obstruction, a multitude of surgical procedures have been described (4–6). For short ureteral strictures, primary ureteroureterostomy is advisable. Distal ureteral strictures can be resolved by direct ureteral reimplantation. Mid-ureteral strictures or long strictures near the pelvic brim can be managed by ureteroneocystostomy using a bladder elongation procedure with a psoas hitch or Boari flap.

Long stricture or defect of the upper ureter present a challenging problem for urologists and require ileal replacement or renal autotransplantation. Both procedures are of considerable magnitude and are associated with complications such as mucous plugging, stone formation, pyelonephritis, metabolic acidosis and vascular complications (7, 8). These issues have prompted urologists to search for a less invasive treatment for upper ureteral strictures. Tissue engineering offers a promising option for urethral reconstruction (23), but tissue-engineered ureters have been confined to pre-clinical investigations. Published research in this area is controversial and scarce (24).

The successful use of buccal mucosal grafts in urethral replacement has led to interest in the use of a similar approach in ureteric reconstruction. Naude first reported the use of buccal mucosa in ureteroplasty in 1999 (12). Recently, several studies have shown its clinical application, with promising results (10, 11, 13–15). However, the harvest of buccal mucosal graft leads to donor site morbidity, such as salivatory duct damage, facial hematoma, neural damage causing paraesthesia, and limited mouth opening (16–18). More importantly, for long ureteral strictures, which require a larger supply of graft tissue, buccal mucosa alone may be insufficient.

LMGs have the same features of buccal mucosal grafts, including resistance to infection, rapid revascularization and compatibility with wet environments (19, 20). The ventral and lateral surfaces of the tongue have no particular functions and can be used for a mucosa graft up to 14–16 cm long. In addition, compared to buccal mucosa, the LMG harvesting procedure is easier and associated with less donor site morbidity (20–22). Therefore, LMGs can be another reliable substituted material, used alone or combined with buccal mucosal grafts for long ureteral reconstruction (25).

In our animal study, macroscopic examination and IVU results demonstrated that the LMGs were well incorporated into the ureteral wall, maintained a wide caliber and had excellent drainage. The ureteral diameter of the proximal ureter did not significantly differ from that of the distal ureter, which verifies the absence of proximal ureteral obstruction. Only one dog in group C developed a mild ureteral stricture near

the proximal anastomosis. Macroscopic examination showed a lack of omentum around the proximal anastomosis. We ascribed the formation of stricture to insufficient omental wrapping. The omentum has an excellent vascular bed, which can offer additional blood supply for the graft (26). After we gained experience and optimized techniques, the other animals in the three groups had excellent outcomes. We concluded that leakage-free anastomosis and proper omental wrapping around the graft are key points for success. In our study, LMGs were found to repair ureteral defects with a length of 10 cm. We believe that if more omentum can be mobilized downward to the pelvis brim in the dog, longer ureteral defects can be repaired.

It is well known that neovascularization is a reliable indicator for the viability of an engrafted tissue. CD31 antibody was used to identify capillaries (neovascularization) because of its specificity as an endothelial marker (27). In this experiment, IHC staining with CD31 showed the formation of new capillaries under the epithelium, confirming that the LMG can survive in situ. We conserved half of the ureteral wall as a ureteral plate, and the ureteral defect was repaired by a graft patch in an onlay fashion. This technique is similar to nontransecting urethral reconstruction (28), with the advantage that the residual ureteral plate preserves blood supply to the graft.

Collagen proliferation or fibrosis in connective tissue under urothelium is a major cause leading to stricture recurrence. Leakage-free anastomosis is essential. In our study, well-formed collagen and smooth muscle fibers around the LMG were observed with Masson’s trichrome staining in postoperative 12 months.

To observe changes in the engrafted lingual mucosa, we performed histological analysis. Fairbanks et al. described the process in their experimental study with bladder mucosal grafts in rabbits. Their hypothesis was that the bladder mucosa underwent partial degeneration, followed by regeneration of the urethral epithelium from the graft margins (29). In the study of colonic mucosal grafts for urethral reconstruction performed by Yuemin Xu, they found that the colonic mucosa underwent metaplasia from unilaminar cylindrical epithelium to urethral epithelium (30). In our study, histological examination showed that the LMG survived inside the ureteral lumen and maintained its typical squamous epithelium at 6 months postoperation. At the same time, the expression of uroplakin on the surface of the LMG, as identified by IHC staining with UP-II, was intermittent, which may exclude the possibility of urothelium crawling from the graft margins. At 12 months after surgery, only a fraction of typical squamous epithelium remained. Most importantly, it appeared that this fraction of squamous epithelium was not completely exposed to urine. The epithelium of the LMG resembled urothelium, with intermittent expression of uroplakin. Based on these findings, we are more inclined to accept the hypothesis of metaplasia. However, further study is needed to investigate the mechanism of histological changes after the LMG is exposed to urine.

One limitation of this experimental study is that it is based on a very small series, with only 4 subjects in each group and a follow-up of only 1 year. Perhaps a larger group of subjects and a longer follow-up period would further demonstrate the viability of this surgical technique.

Conclusions

In summary, we developed a dog model for ureteral reconstruction using LMGs. We found that LMGs can repair a 10-cm ureteral defect and demonstrated the morphological changes of the engrafted LMG. Due to the advantages of easy harvesting and less donor site morbidity, we suggest that LMGs may be an ideal material for ureteral substitution in the treatment of long upper ureteral strictures.

Abbreviations

LMG
Lingual mucosa graft; IVU:Intravenous urography; IHC:Immunohistochemical; UP-II:Uroplakin-II

Declarations

Ethics approval and consent to participate

All procedures concerning animals in this study were approved by the Institutional Animal Care and Use Committee of Tongji Medical College of Huazhong University of Science and Technology, in accordance with the ARRIVE guidelines, as well as the EU Directive 2010/63/EU for animal experiments.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by the National Natural Science Foundation of China (grant number 81671216) to Bing Li. We are grateful to the funding for its financial support in the animal operation and analysis of data.

Authors' contributions

Conception and design : YX ,BL; Animal surgery: LS, QP; Drafting of the manuscript: YX; Acquisition of data and Statistical analysis: XH; Critical revision of the manuscript: BL. All authors have read and approved the manuscript.

Acknowledgements

None.

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Figures

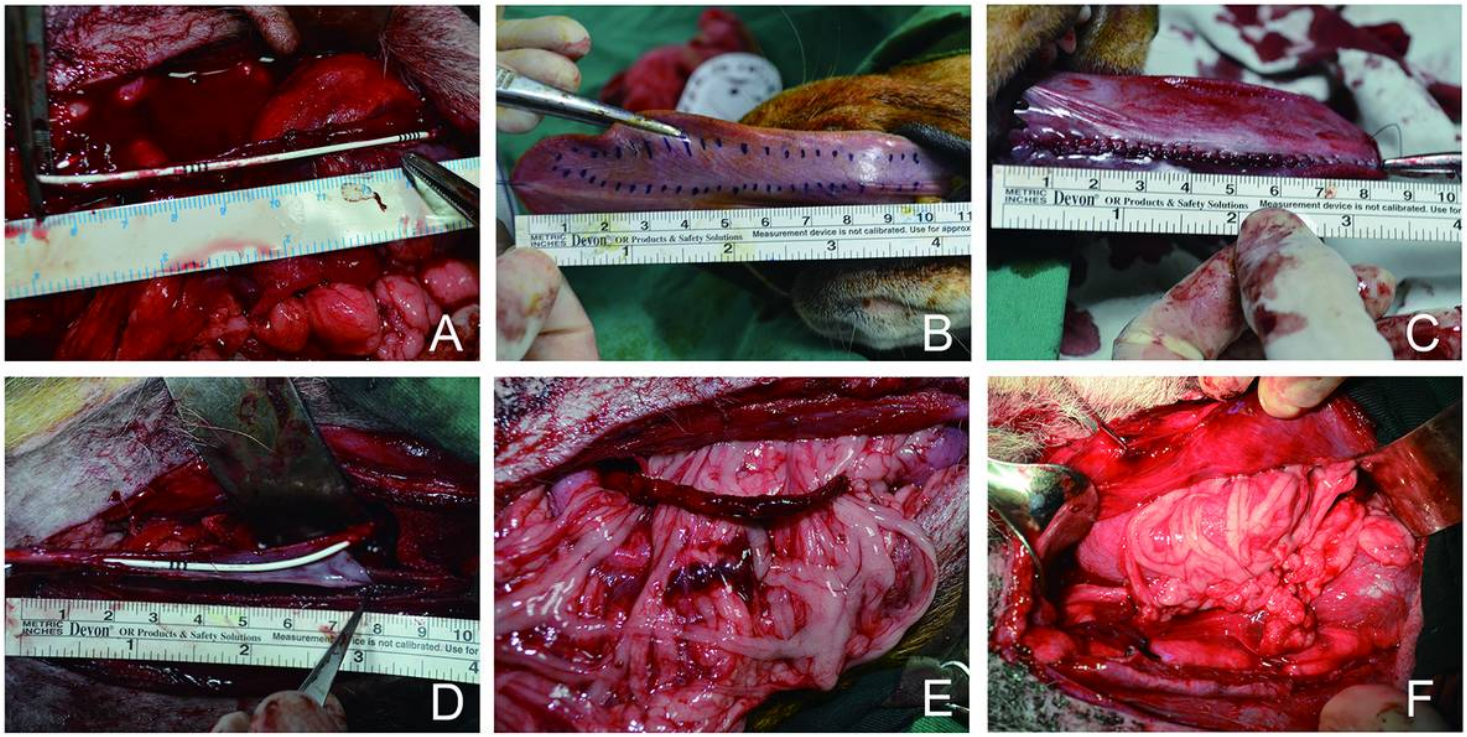

Figure 1

Nontransected onlay-augmented ureteroplasty with a LMG. (A) A ventral ureteral defect was created. (B) The required LMG was marked. (C) The donor site was closed. (D) The LMG was anastomosed with the ureter in an onlay fashion. (E) The ureteroplasty was completed. (F) The omentum was wrapped around the reconstructed ureteric segment.

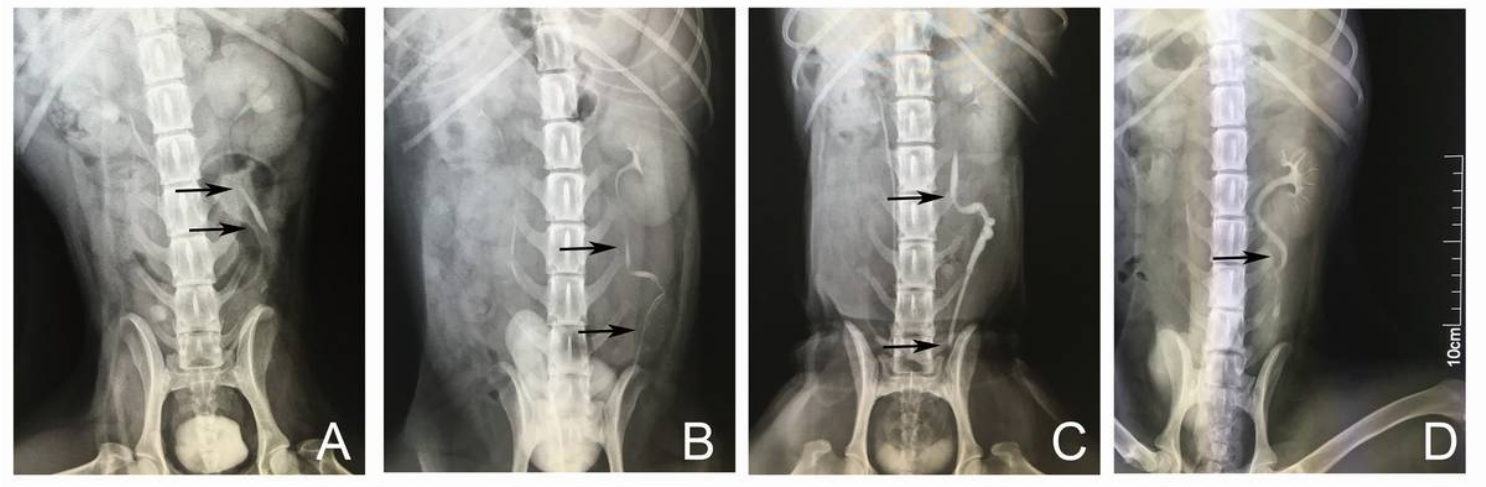

Figure 2

IVU. (A-C) In groups A (3 cm), B (6 cm) and C (10 cm), ureterograms showed that the reconstructed ureter had a wide caliber without renal pelvis dilation or stricture (black arrow). (D) One dog in group C developed a mild ureteral stricture near the proximal anastomosis (black arrow).

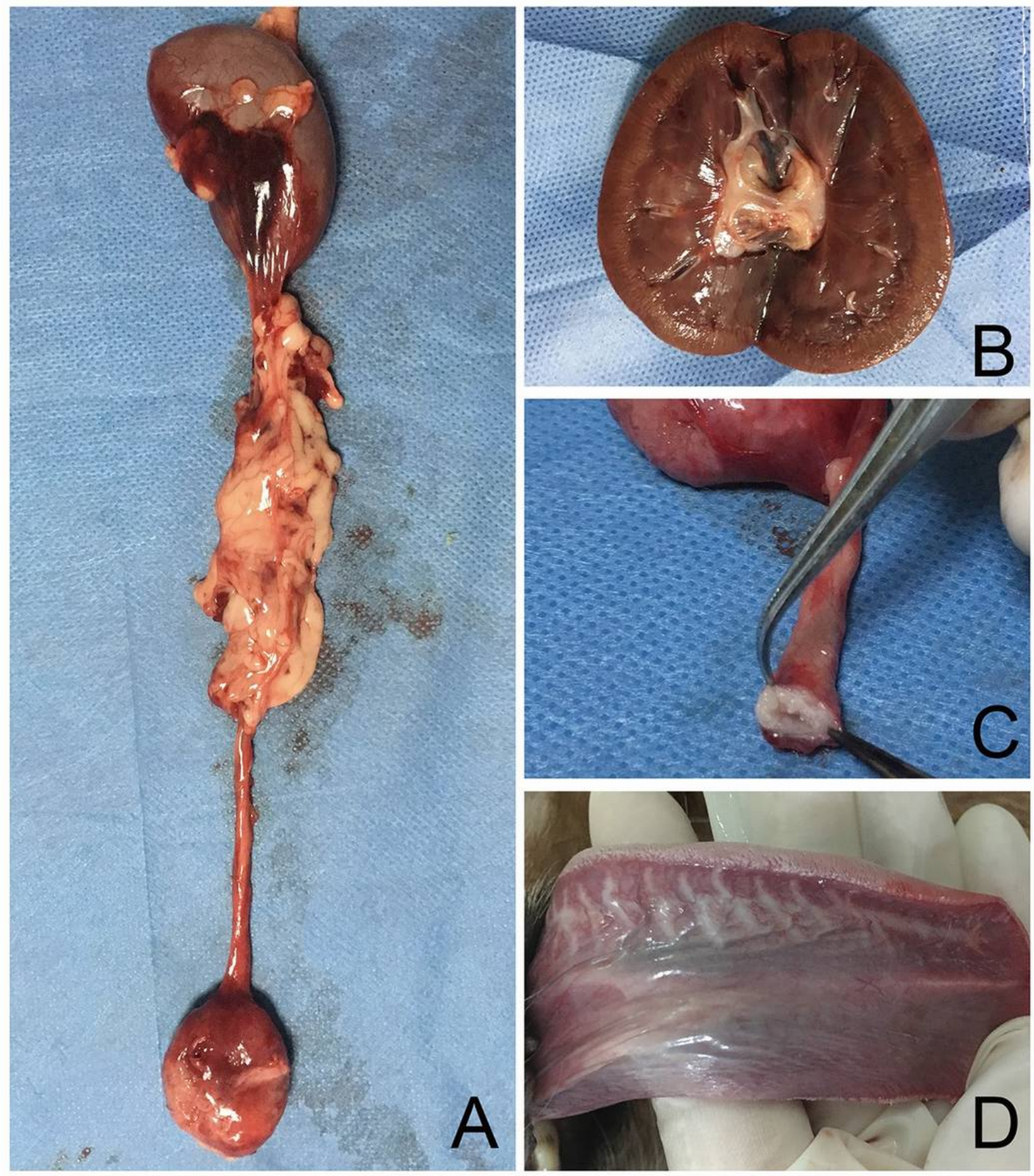

Figure 3

Macroscopic examination. (A) The reconstructed upper urinary tract was harvested. (B) Cross section of the left kidney without hydronephrosis. (C) Cross section of the LMG-replaced ureteric segment. (D) The

tongue donor site healed well.

ureteral diameter (mm)	6 month	12 month
proximal ureter	3.02 ± 0.16	3.15 ± 0.31
reconstructed segment	4.15 ± 0.33 *	4.16 ± 0.39 *
distal ureter	3.05 ± 0.14	3.10 ± 0.16

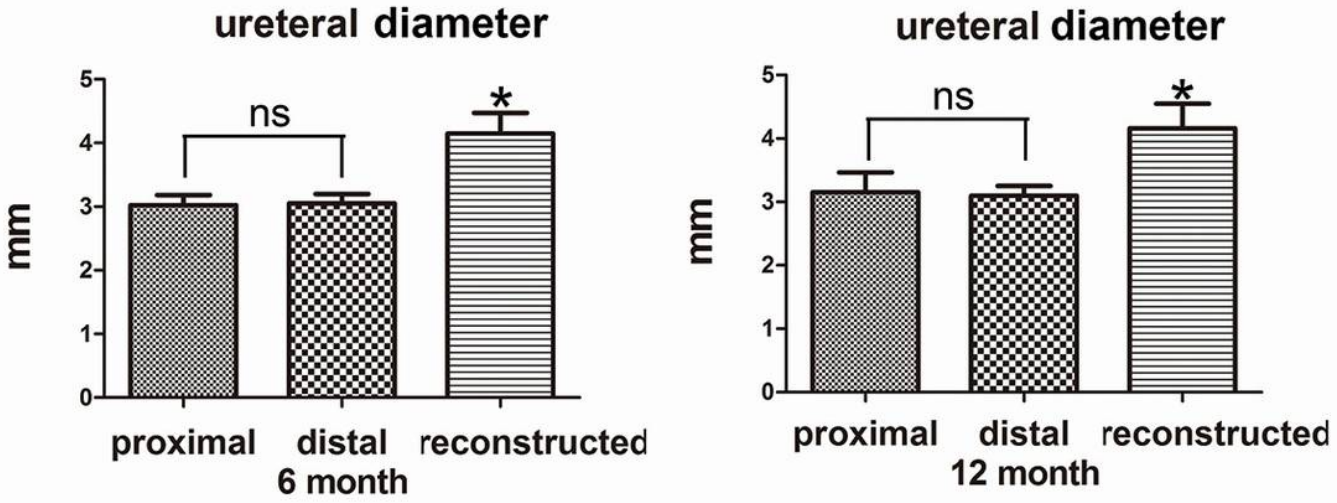

Figure 4

The diameter of the reconstructed segment and of the proximal and distal ureter in the 11 animals at different time points. All data are presented as the mean ± SD. * indicates a significant difference in the ureteral diameter of the reconstructed segment compared with the proximal and distal ureter (each p value < 0.01). “ns” indicates that the diameters of the proximal versus distal ureter were statistically identical (each p value > 0.05).

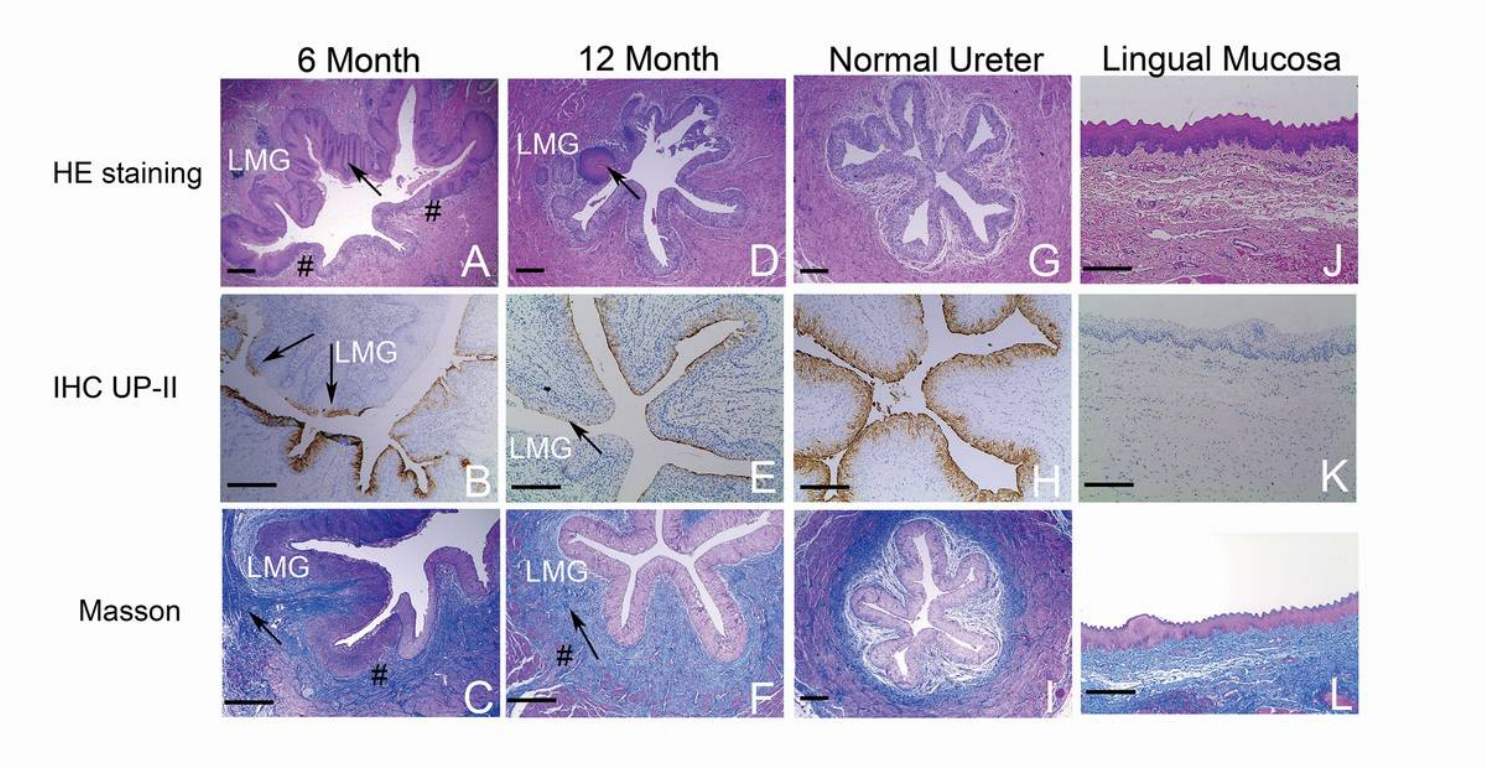

Figure 5

Histological analysis of the LMG at different time points. (A) HE staining showed lingual mucosa in the reconstructed ureter at 6 months postoperation (black arrow). (B) IHC staining with UP-II antibody showed intermittent expression of uroplakin on the surface of the LMG (black arrow). (C) Masson's trichrome staining showed many irregular collagen fibers around the LMG (black arrow). (D) The epithelium over the LMG was greatly similar to urothelium at 12 months, with only a fraction of typical squamous epithelium remaining (black arrow). (E) The epithelium over the LMG exhibited intermittent expression of uroplakin (black arrow). (F) Well-formed collagen and smooth muscle fibers were observed around the LMG (black arrow). (G,H,I) Normal ureter. (J,K,L) Lingual mucosa before engraftment. ("#" indicates anastomosis of the LMG with the native ureter). Bars = 200 μm.

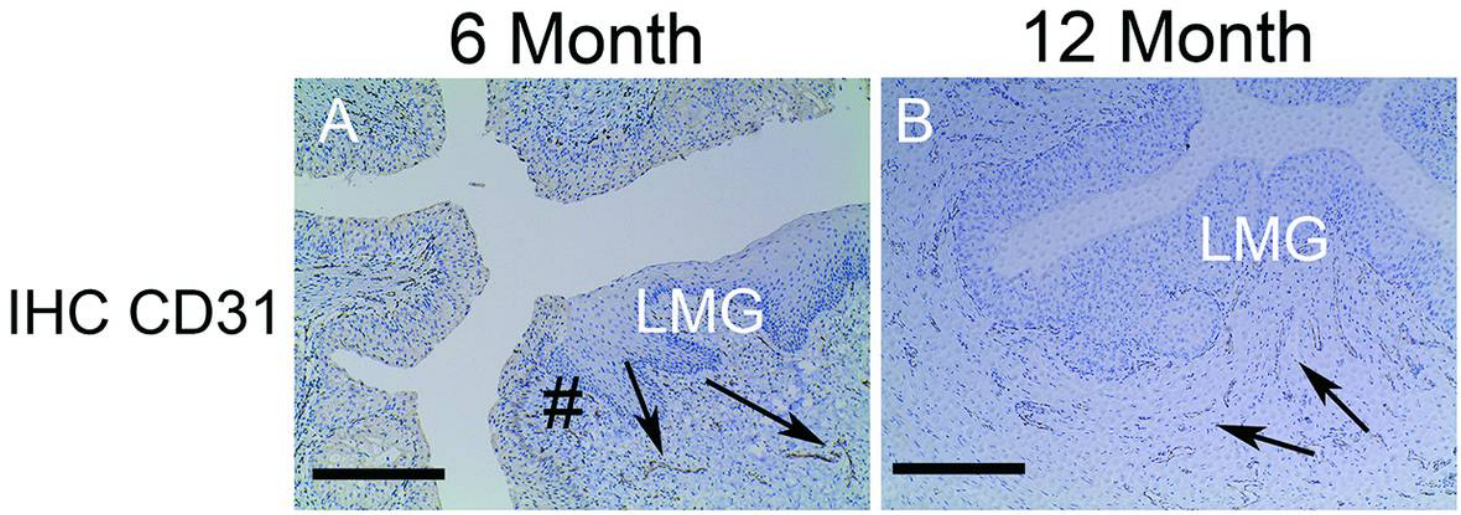

Figure 6

IHC staining with CD31. (A) A few vascular capillaries in the submucosa of the LMG at 6 months postoperation (black arrow). (B) Newly formed capillaries were dense at 12 months (black arrow). Bars = 200 μm.