

# Comparison of Full-Thickness Bladder Tissue Effects in Onlay versus Tubular Ureteral Reconstruction: A Study in a New Zealand Rabbit Model

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## Research Article

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# Abstract

## Objective

To explore the use of full-thickness bladder as a novel autologous material and comparing its efficacy in Onlay Reconstruction versus Tubularized Reconstruction. Approximately 75% of benign ureteral strictures are iatrogenic. Even without complete disruption of ureteral continuity, these strictures can cause many complications. Current, reconstruction is favored for its durable outcomes, its application lacks a standardized consensus due to limitations in graft materials and surgery.

## Methods

Using 2.5 kg New Zealand rabbits, we reconstructed 1.5 cm and 2.5 cm ureteral stricture segments. Postoperative ureterograms assessed urinary leakage. Reconstructed areas were evaluated for morphological changes and fibrosis using HE and Masson staining.

## Results

No postoperative urinary leakage occurred in any rabbits, and all remained healthy during the 6-week observation. The tubularized and 1.5 cm Onlay reconstructions healed well, but a diverticulum developed in the 2.5 cm Onlay area. HE staining revealed good vascular distribution in all reconstructed sites, though urothelial cells were not fully preserved in the Onlay reconstruction areas. Masson staining indicated varying fibrosis levels, with the Onlay area exhibiting the most severe fibrosis, which increased with longer repair lengths.

## Conclusion

Full-thickness bladder wall is a safe, effective autologous graft material with less postoperative complications. Both tubularized and Onlay reconstruction effectively achieve ureteral reconstruction.

## 1. Introduction

Ureteral stricture represents a significant clinical challenge in urology. Although it may not always compromise the continuity of the ureter, it frequently leads to urinary drainage obstruction, which can subsequently cause infection, hydronephrosis, and in severe cases, renal function impairment that may ultimately necessitate a nephrectomy [1, 2]. Current therapeutic modalities primarily include endoscopic management and open ureteral reconstruction [3, 4]. While endoscopic interventions are minimally invasive and offer a fast recovery, their long-term efficacy is often suboptimal [5]. In contrast, ureteral reconstruction is considered a more reliable and durable solution, as it effectively restores ureteral patency. However, the success of the procedure is highly dependent on the location and length of the

stricture, as well as the choice of reconstructive material and surgical, with no current consensus on the optimal approach [6]. For mid- or upper-ureteral strictures less than 2 cm in length, ureteral end-to-end anastomosis is generally considered a straightforward and viable surgical option [7]. However, managing long segment strictures in the middle or upper ureter remains a significant challenge, especially in complex cases. The emergence of autologous tissue graft repair offers a novel solution for these complex cases, allowing for effective reconstruction while significantly reducing surgical morbidity.

Currently, free autologous utilizing buccal mucosa, appendix, and gastrointestinal segments are employed for ureteral reconstruction, yet each presents inherent limitations. Specifically, harvesting buccal mucosa may cause irreversible impairment of oral function [8]. Gastrointestinal tissues, due to their mucus-secreting properties and capacity for electrolyte absorption from urine, often lead to complications such as mucocele formation, infection, and hyperchloremic metabolic acidosis [9]. Moreover, the long-term efficacy and complication rates associated with using the appendix as a reconstructive material have not been extensively studied [10]. Recently, full-thickness bladder tissue has emerged as a promising alternative for ureteral reconstruction, primarily due to its natural resistance to urinary degradation, favorable pliability, and regenerative potential [11]. Being embryologically homologous to the ureter, bladder tissue shares a similar muscular structure and urothelial barrier function. While ureteroneocystostomy, with or without a psoas hitch or a bladder flap, is a well-established technique for short-segment (< 5 cm) distal ureteral strictures and can even manage long-segment strictures (12–15 cm), clinical experience with using full-thickness bladder tissue for repairing mid- or upper-ureteral or long-distance defects remains limited [12, 13].

The shape and size of the graft during the reconstruction process, and the surgical method, directly affect the repair of the reconstructed area [14]. Onlay Reconstruction is distinguished by its ability to preserve the vascularized native ureteral plate. This technique involves making a longitudinal incision through the stricture segment and then inlaying a Onlay graft to augment the luminal caliber and relieve the obstruction [15, 16]. It is technically less demanding as it avoids complete stricture resection, thereby minimizing the number of anastomoses and the risk of anastomotic leakage. In contrast, Tubularized Reconstruction involves tailoring the selected graft into a tubular conduit, which is then anastomosed to the ureter to restore continuity. While this technique may afford a greater reconstructive length, it is a more complex procedure, involving a greater number of suture lines and anastomoses, which may lead to a higher risk of urinary leakage [17, 18]. Both techniques have distinct advantages and disadvantages, and there is no current consensus on their application. Although some surgeons propose that Onlay reconstruction is better suited for shorter strictures and tubularized reconstruction for longer defects, a definitive consensus regarding the optimal length and operation has yet to be established.

Considering that buccal mucosa, appendix, or digestive tract tissues may lead to complications such as infection and electrolyte imbalances, and that there is no consensus on the use of Onlay and tubularized reconstruction, this study aims to explore the feasibility of reconstructing the ureter using full-thickness bladder tissue that closely matches the ureter, as well as the adaptability of the repair length using full-thickness bladder tissue. Additionally, the study evaluates the effectiveness of Onlay-type and tubular-

type surgical approaches in reconstructing mid-to-upper ureteral strictures of varying lengths. The goal is to provide a new autologous Onlay method for reconstructing the mid-to-upper ureter and to offer surgical recommendations for using full-thickness bladder Onlayes in ureteral reconstruction.

## **2. Methods**

### **2.1. Material**

HE staining kit and Masson trichrome staining solution were purchased from Solarbio science & technology company (Bei Jing). Optimal cutting temperature compound was purchased from sakuraus Company (USA).

### **2.2. Animal and surgery**

New Zealand rabbits (Experimental Animal Center of Chongqing Medical University, male, 10 weeks old, 2.3 kg) were anesthetized with 20% ethyl carbamate (5ml/kg) and placed supine with their abdomen exposed. Cut open from the upper end of the pubic symphysis to the lower end of the left kidney, exposing the bladder and left ureter. Then, a ureteral stent is implanted and appropriate bladder tissue is cut and soaked in physiological saline. Under the guidance of ureteral stent, incise or remove the stenosis segment, and perform Onlay Urethroplasty and Tubularized Incised Onlay Urethroplasty respectively. Finally, suture the bladder and lower abdomen.

Four groups in total: the 1.5 cm tubularized group, the 1.5 cm onlay group, the 2.5 cm tubularized group, and the 2.5 cm onlay group. 6 pieces per set, preoperative assessment of kidney condition in New Zealand rabbits to exclude animals with abnormal indicators.

### **2.3. Urography**

New Zealand rabbits were placed in the supine position after anesthesia. Then, 20ml of 60% iohexol solution was injected into the ear margin vein. Take DR after injected iohexol solution, and take contrast DR after waiting for 5-10min (Additional scan settings were as follows: 53 KVP, 20 ms).

### **2.4. Frozen section**

New Zealand rabbits were euthanized by air embolism and ureteral reconstruction tissue was collected. Fixed with 4% paraformaldehyde for 1 day, dehydrated with 30% sucrose solution until the tissue sinks to the bottom. After trimming the tissue, embedding is performed. The tissue for Onlay Urethroplasty is cut horizontally, and the tissue for Tubularized Incised Plate Urethroplasty is cut vertically.

### **2.5. HE staining**

Firstly, wash the frozen sections with running water for 5 minutes and let them air dry. Next, perform hematoxylin staining for 10 minutes and wash off the staining solution with running water. Afterwards, stain with eosin for 1 minute and wash the dye solution with running water. Then 95% ethanol (I) for 2–3 seconds, 95% ethanol (II) for 2–3 seconds, 100% ethanol (I) for 2–3 seconds, and 100% ethanol (II) for 1

minute. Finally, xylene (I) was used for 1 minute, xylene (II) was used for 1 minute, and neutral gum was used to seal and observe under a microscope.

## **2.6. Masson staining**

Clean the slices with running water for 5 minutes and air dry them slightly. Stain with Lichun Red solution for 10 minutes, wash with weak acid for 1 minute. Then wash with phosphomolybdic acid solution for 1 minute and weak acid solution for 1 minute. Directly add aniline blue solution for 1 minute and weak acid for 1 minute. Finally, dehydrate anhydrous ethanol three times, each time for 5–10 seconds. Xylene transparent for 3 times, 1–2 minutes each time. Seal with neutral gum and observe under a microscope.

## **3. Result**

### **3.1 Using Full-Thickness Bladder Tissue for Tubularized Reconstruction Effectively Repairs Ureteral Strictures**

To explore the feasibility of using full-thickness bladder tissue as a repair material, we established a New Zealand rabbit model of tubularized reconstruction. During surgery, after excising 1.5 cm and 2.5 cm strictured segments from the mid-upper ureter, pre-prepared full-thickness bladder tissue was tubularized and an end-to-end anastomosis was performed with the proximal and distal ureteral ends (Fig. 1). Imaging evaluation revealed no urinary leakage on urography (Fig. 3A). Six weeks post-surgery, the reconstructed ureter was observed to have an intact morphology; aside from some thickening, there were no significant shape changes, indicating good integration between the bladder and ureter tissue (Fig. 1).

In summary, this study successfully utilized full-thickness bladder tissue to perform tubularized reconstruction of different lengths (1.5 cm and 2.5 cm). No postoperative complications such as urine leakage were observed, suggesting that full-thickness bladder tissue can serve as a safe and effective autologous material for ureteral reconstruction.

### **3.2 Using Full-Thickness Bladder Tissue for Onlay Reconstruction Effectively Repairs Ureteral Strictures**

Onlay reconstruction preserves part of the ureteral tissue, maintaining both the ureteral plate and its blood supply. Oral mucosa is commonly used as a Onlay material in this type of surgery, but it can cause complications such as difficulty opening the mouth. Moreover, many researchers believe that Onlay reconstruction is more suitable for short-segment repairs and not ideal for long-segment strictures. Based on this, our study used full-thickness bladder tissue Onlayes of different lengths (1.5 cm and 2.5 cm) to perform Onlay reconstruction and observed the reconstruction outcomes.

Under anesthesia, New Zealand rabbits were placed supine for open abdominal surgery. A ureteral stent was implanted, and the bladder and mid-ureter were exposed. Appropriate-sized full-thickness bladder

tissue Onlayes were harvested, the strictured segment (1.5 cm or 2.5 cm) was incised, and the bladder tissue Onlay was sutured over the incision. Six weeks postoperatively, the reconstructed ureteral tissue was evaluated. The animals showed no abnormal signs, and ureteral thickening was observed. In the 2.5 cm Onlay repair group, a prominent lumen was noted (Fig. 3). The urography showed a ureteral stent inside the bladder, and the contrast agent did not diffuse into the abdominal cavity, indicating that the bladder tissue Onlay reconstruction did not cause urine leakage (Fig. 3B).

Notably, a diverticulum was observed in the 2.5 cm Onlay repair area, whereas no morphological changes were seen in other groups. This phenomenon may be related to the characteristics of the full-thickness bladder tissue. Collectively, these results confirm that bladder tissue Onlay reconstruction effectively reconstructs the ureter without postoperative complications such as urine leakage or obstruction.

### **3.3 Both Tubularized and Onlay Reconstruction have Vascular Regeneration**

To assess the tissue structure and vascular regeneration of the reconstructed ureters, HE staining was performed. No significant morphological changes or damage were observed in the reconstructed area, indicating good compatibility between the bladder Onlay and the host ureter tissue (Fig. 4A). However, compared with tubularized, the transitional epithelial cell boundary of Onlay reconstruction at the Onlay-ureter anastomosis was blurred and difficult to clearly identify. Regardless of whether Onlay or tubularized repair was used, no tissue necrosis was observed in any reconstructed area, and all showed good blood supply (green circle).

Masson staining (Fig. 4B) was used to evaluate fibrosis after ureteral reconstruction. Different degrees of fibrosis were observed between Onlay and tubularized repair groups. In the 1.5 cm tubularized reconstruction group, numerous red-stained muscle fibers were seen in the mucosal and muscular layers, while blue-stained collagen fibers were mainly distributed in the lamina propria and extensively infiltrated the muscular layer, with a density significantly higher than that of normal ureter. In the 2.5 cm group, no blue-stained collagen fibers were found in the mucosal layer, but the arrangement of muscle and collagen fibers in the lamina propria and muscular layers was highly disorganized, with fibrosis markedly more severe than in the 1.5 cm tubularized group. In the Onlay reconstruction groups, red-stained muscle fibers and blue-stained collagen fibers were widely distributed throughout the tissue, with collagen fibers far exceeding muscle fibers in content. Compared to the 1.5 cm Onlay group, the 2.5 cm Onlay group showed broader distribution and greater amounts of collagen fibers. Overall, fibrosis was significantly more pronounced after Onlay reconstruction than after tubularized reconstruction.

## **4. Discussion**

Currently, commonly used autologous repair materials include buccal mucosa, oral mucosa, appendix, and digestive tract tissues, but each has its limitations. For example, harvesting oral mucosa may affect oral function; digestive tract tissues, due to their continuous mucus secretion and electrolyte absorption

functions, may lead to complications such as urinary tract infections or hyperchloremic metabolic acidosis[19, 20]; and the long-term efficacy of appendix tissue remains insufficiently studied[21]. Full-thickness bladder wall grafts have emerged as a new research direction in ureteral reconstruction because of their excellent resistance to urine erosion, and potential regenerative capacity[22, 23]. In view of this, the present study aims to explore the feasibility of using full-thickness bladder tissue as a novel autologous Onlay. And conduct a comparison of its reconstructive effects under tubularized and Onlay reconstruction with the goal of providing important references for clinical surgical decision making.

Previous studies have shown that while autologous tissue Onlayes can effectively repair ureteral defects, their long-term efficacy is limited by the source and function of the tissue. As a major component of the urinary system, bladder tissue lacks secretory or absorptive functions, and its transitional epithelial cells are highly homologous to those of the ureter, making it an ideal repair material[24, 25]. The Boari flap procedure has been successfully applied in lower ureteral reconstruction, confirming the potential of bladder tissue in urinary system reconstruction[26]. In this study, we explored for the first time the use of full-thickness bladder tissue to repair mid- and upper ureteral stricture. The results showed satisfactory therapeutic outcomes in both tubular and Onlay surgeries for 1.5 cm short-segment and 2.5 cm long-segment stricture models. Animals were in good condition six weeks postoperatively, with effective maintenance of ureteral morphology and lumen patency, and no urine leakage was observed on urography (Figs. 1 and 2). This contrasts sharply with complications such as inflammation and anastomotic urine leakage commonly reported after intestinal or appendiceal reconstructions, strongly confirming the superiority of bladder tissue as a repair material. Additionally, HE staining showed abundant vascularization in the reconstructed area, which is crucial for tissue survival and functional recovery in long-segment repairs (Fig. 5).

Although full-thickness bladder tissue has natural advantages, consensus has not been reached regarding the choice of reconstruction technique and its compatibility with repair length. Our study found significant histological and pathological differences between the two surgical methods. While Onlay reconstruction also successfully repaired ureteral strictures in the short term without urine leakage (Figs. 3 and 4), we observed obvious diverticulum formation in the reconstructed area when used to repair 2.5 cm long-segment defects[27, 28]. We speculate this may be due to the greater distensibility of bladder tissue compared to the ureter[29]. Pathological staining after Onlay reconstruction surgery showed that the condition of the transitional epithelial cells was worse compared to tubularized reconstruction. More importantly, Masson staining revealed significantly higher fibrosis in the Onlay reconstruction area compared to the tubular method, with fibrosis severity increasing as the repair length extended from 1.5 cm to 2.5 cm (Fig. 6). This aligns with previous studies suggesting that Onlay repair of long-segment strictures may induce fibrosis and diverticulum formation. Although Onlay surgery preserves the blood supply of the “ureteral plate” and is relatively simple to perform, it may provoke excessive fibrotic responses in long-segment repairs due to tissue tension and the large interface between the Onlay and host tissue, potentially threatening long-term outcomes. Therefore, our pathological findings suggest that while Onlay surgery is an effective short-term repair method, it is not suitable for reconstructing ureteral strictures of 2.5 cm or longer.

In summary, full-thickness bladder tissue shows great potential as a novel autologous Onlay in ureteral reconstruction. Tubularized reconstruction effectively repairs ureteral strictures while maintaining low fibrosis levels, making it the preferred option for both long- and short-segment strictures. Although Onlay can achieve morphological reconstruction, its severe fibrotic tendency, especially in long-segment repairs, limits its clinical application. This study is the first to systematically compare the effects of full-thickness bladder tissue ureteral reconstruction under two different surgical methods in an animal model, providing new insights for clinical practice. However, a limitation of this study is the relatively short follow-up period in the animal model. Future research should include longer-term follow-up to comprehensively assess long-term functional recovery and restenosis rates post-reconstruction, combined with functional indicators such as urodynamics, to provide more comprehensive evidence for eventual clinical translation.

## 5. Conclusion

This study demonstrates that full-thickness bladder tissue can be used as a novel free autologous graft for reconstructing mid-upper ureteral strictures. Using this graft allows for the reconstruction of both long and short segment strictures through tubularized and onlay ureteral reconstruction surgeries, with the graft exhibiting good blood supply. Pathological changes and fibrosis in the reconstruction area are more severe in onlay repair compared to tubularized repair. This research provides a new type of autologous graft for ureteral reconstruction and offers detailed references for its clinical application.

## Declarations

### Disclosure statement

The authors declare no competing interests.

### Ethical declaration

This animal study was approved by IACUC of Chongqing Medical University (IACUC-CQMU-2024-04040). All experimental procedures were done in accordance with China legislation.

### Declaration of generative AI in scientific writing

The authors did not use generative AI or AI-assisted technologies in the development of this manuscript.

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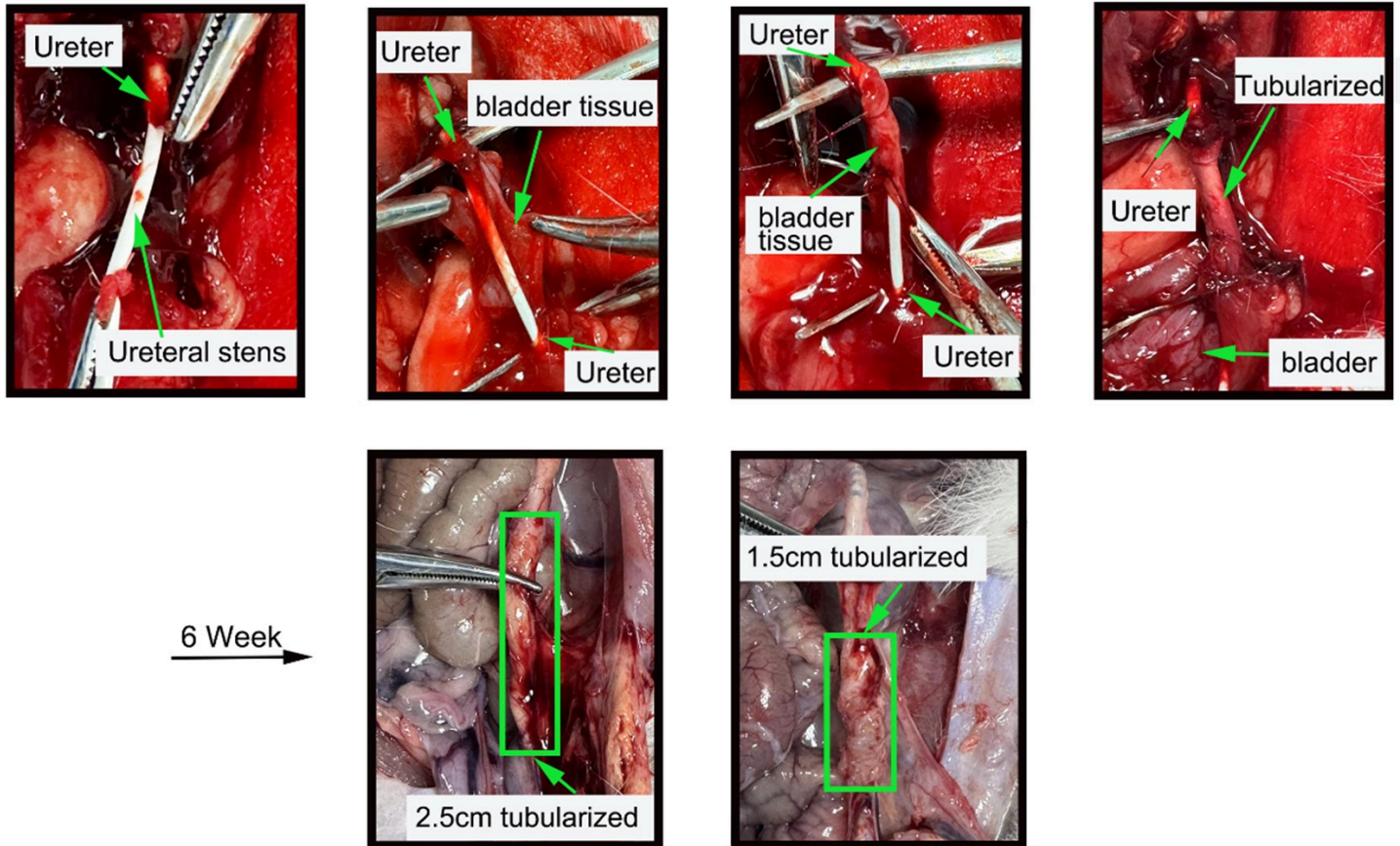
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## Figures

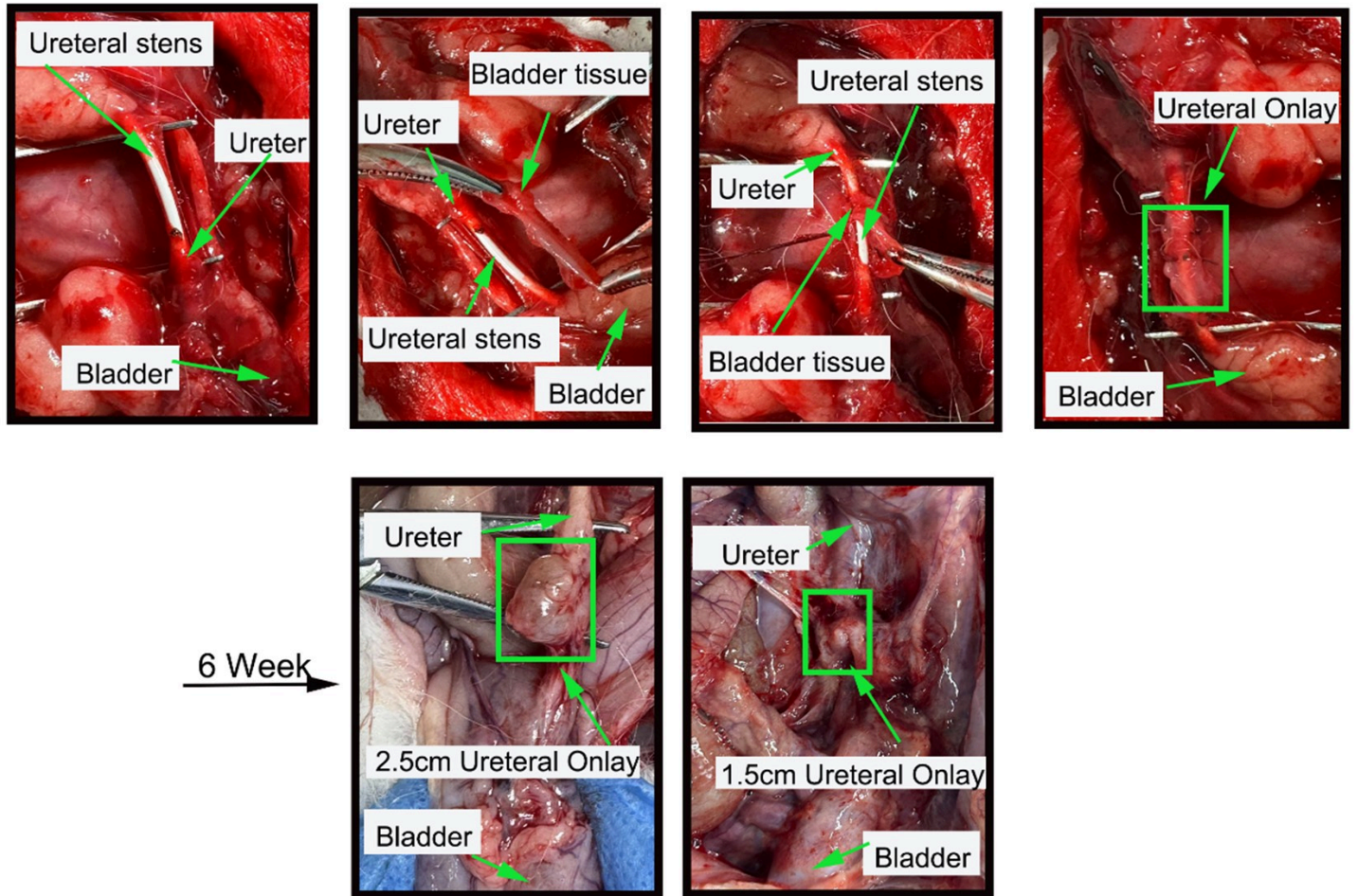
## Intraoperative



**Figure 1**

Tubularized reconstruction can recured ureter stricture. First, a ureteral stent was implanted from the ureterovesical junction. Next, full-thickness bladder tissue segments measuring 1.5 cm and 2.5 cm in length were excised and an end-to-end anastomosis was performed.

## Intraoperative



**Figure 2**

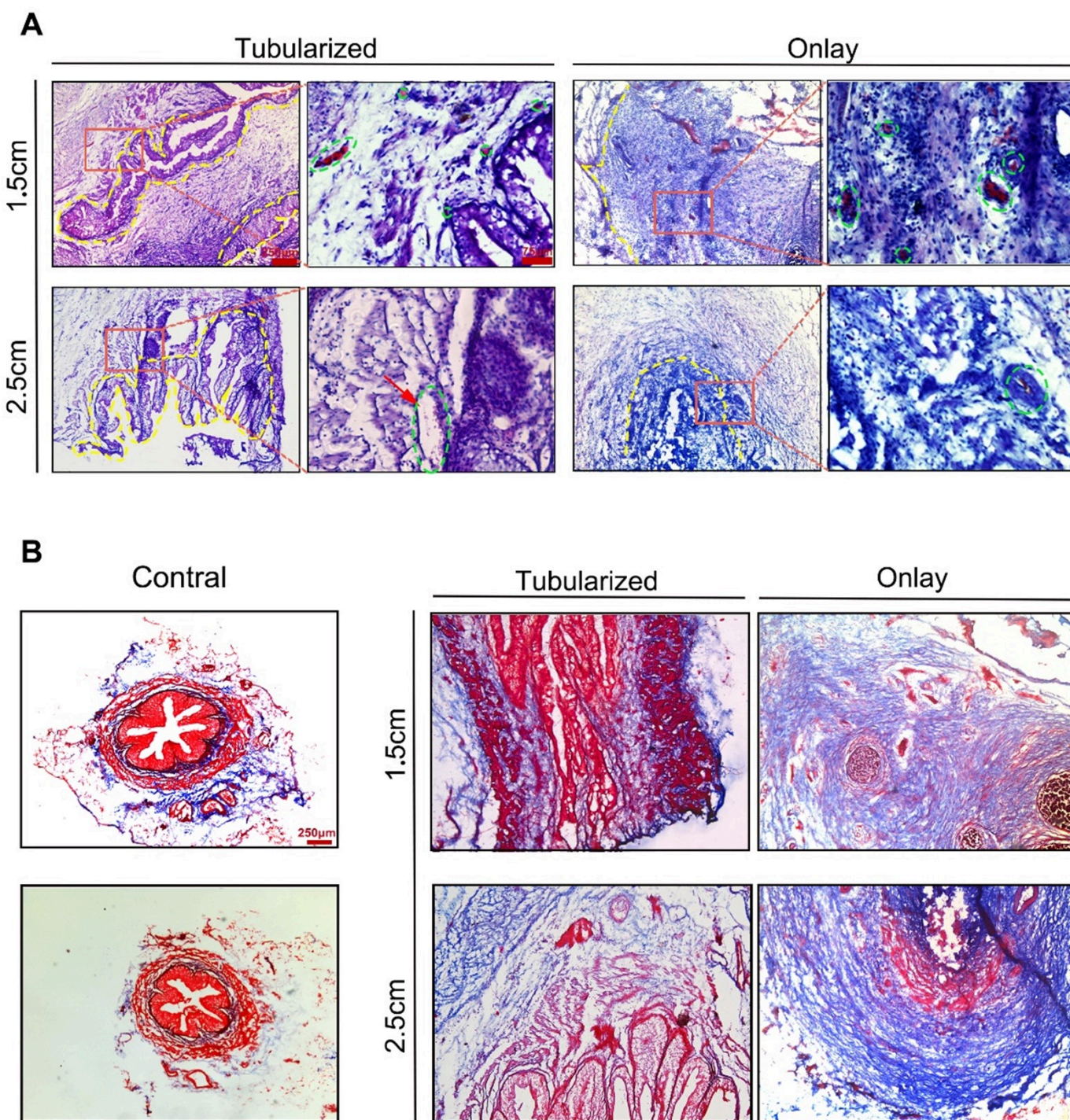
Onlay reconstruction can recured ureter stricture. First, a ureteral stent was implanted. Next, the ureteral stricture area was incised, and full-thickness bladder tissue segments measuring 1.5 cm and 2.5 cm in length were excised for suturing. The ureteral reconstruction was then evaluated six weeks later.





**Figure 3**

No urinary leakage was observed on the postoperative day 5. (A). urethrography in Tubularized reconstruction; (B). urethrography in Onlay reconstruction.



**Figure 4**

Pathological staining did not reveal severe tissue damage, but varying degrees of fibrosis were observed. (A). HE staining was used to examine pathological changes in the tubularized and onlay reconstruction areas. (The coiled tube is a longitudinal section, and the patch is a transverse section.); (B). Masson staining was used to observe fibrosis.