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ORIGINAL ARTICLE

Prostate Disease

A novel mouse model simulating transurethral laser vaporization prostatectomy

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Benign prostatic hyperplasia (BPH) is a common disease in elderly men, and transurethral laser prostatectomy (TULP) has been widely used in the clinic to remove bladder outlet obstruction caused by BPH. Previous animal models for wound repair after prostatectomy have many limitations, and there have been no previous reports of a mouse model of TULP. Therefore, this study aimed to establish a novel mouse model of TULP. Twelve healthy adult Kunming (KM) mice received transurethral laser vaporization prostatectomy with a 200-μm thulium laser. The mice were sacrificed, and wound specimens from the prostatic urethra and bladder neck were harvested at 1 day, 3 days, 5 days, and 7 days after surgery. Hematoxylin-eosin (HE) and immunohistochemistry were applied to confirm the establishment of the mouse TULP model. One day after the surgery, urothelium expressing uroplakin (UPK) was absent in the urethral wound site, and a large number of necrotic tissues were found in the wound site. There was no UPK-positive urothelium in the wound 3 days after surgery. At 5 days after surgery, monolayer urothelium expressing UPK was found in the wound site, indicating that the re-epithelization of the wound had been completed. On the 7th day after surgery, there were multiple layers of urothelium with UPK expression, indicating that the repair was completed. It is feasible to establish a mouse TULP model by using a microcystoscope system and a 200-μm thulium laser.

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common disease in elderly men. The incidence of BPH in males over 60 years of age is approximately 50%–60%.[1] Approximately 10% of BPH patients require surgery to remove bladder outlet obstruction.[2] Prostatectomy has been performed for more than 100 years and mainly includes open prostatectomy, transurethral prostatectomy, transurethral laser prostatectomy (TULP), and other minimally invasive surgeries.[3] With the improvement in surgical equipment and the development of minimally invasive surgery, transurethral laser vaporization prostatectomy has been widely used in the clinic. However, there is still controversy about the mechanism of prostatic urethra repair after TULP.[4–7] Previous studies on the mechanism of wound repair after prostatectomy have mostly used canines as animal models, but this model has many limitations, and there have been no previous reports of a mouse model of TULP. The aim of our study was to establish a new mouse model for research on wound repair after TULP.

MATERIALS AND METHODS

Animals

Twelve healthy adult Kunming (KM) mice from the Animal Center of Guizhou Medical University (Guiyang, China) were selected. The animal model was examined and approved by the Ethics Committee of Guizhou Provincial People's Hospital (ethical approval No. 2018025). The mice were 16–18 months old and weighed 55–60 g.

Surgical procedure

Mice were anesthetized by intraperitoneal injection of 35 mg kg^{-1} pentobarbital sodium at 1% concentration. The screen, thulium laser (1940 nm thulium laser, Raykeen, Shanghai, China), homemade pneumatic microcystoscope holder, microcystoscope, and work sheath (PD-D-1083, PolyDiagnost, Berlin, Germany) were connected for preparation. The mice were anesthetized and fixed in the supine position. A lower abdominal transverse incision was made to enter the abdominal cavity and find the bladder. The bladder was lifted, incised, and inserted into the 1300 μm microcystoscope work sheath with suture fixation (**Figure 1**). After the bladder and urethra were filled with normal saline, an obvious bladder neck was observed. After the microcystoscope was pushed further through the bladder neck, the prostate urethra was found (**Figure 2**). The laser fiber was inserted into the urethra of the prostate, and the power was adjusted to 5 W to vaporize the urothelium of the prostatic urethra. After the wound was constructed, the work sheath was pulled out, and the bladder and abdominal cavity were closed without an indwelling catheter.

Hematoxylin-eosin (HE) and immunohistochemistry

The mice were randomly divided into four groups, and each group had three mice. The mice were sacrificed, and wound specimens from the prostatic urethra and bladder neck were harvested and fixed in 4% formalin at 1 day, 3 days, 5 days, and 7 days after surgery. The tissue samples were embedded in paraffin and sectioned continuously at

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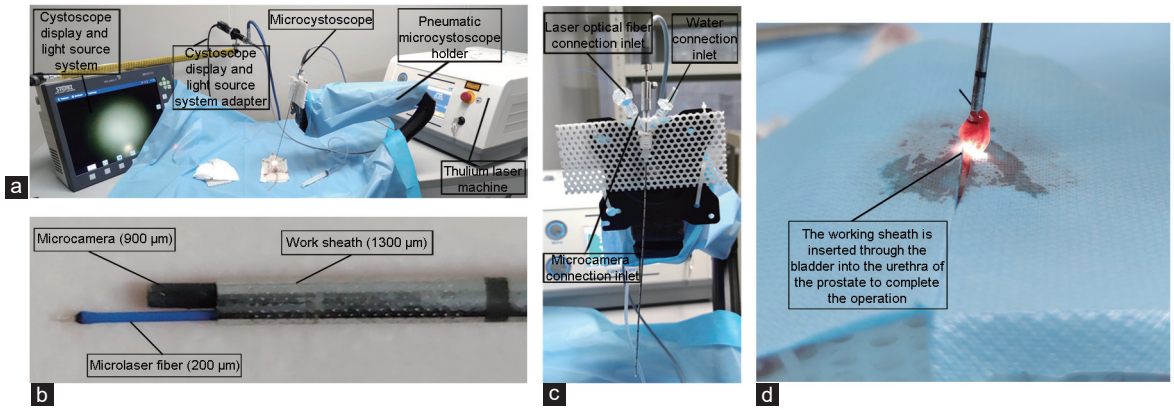

Figure 1: Surgical instruments for establishment of the mouse TULP model. Display of (**a**) the assembly of the surgical instruments, (**b**) work sheath, and (**c**) microcystoscope, laser optical fiber, and water connection. (**d**) The working sheath was inserted through the bladder into the urethra of the prostate to complete the operation. TULP: transurethral laser prostatectomy.

5-μm thickness to observe the establishment and repair of the wound by HE and immunohistochemical staining.

The sections were incubated with blocking buffer (Dako Denmark A/S, Glostrup, Denmark) at room temperature for 30 min. Then, uroplakin (UPK) antibody (1:200, Abcam, Cambridge, UK)-labeled urothelium was incubated overnight at 4°C. After the sections were washed with Tris-NaCl buffer (Thermo Fisher Scientific, Waltham, MA, USA), they were incubated with anti-rabbit antibody (Santa Cruz Biotechnology, Dallas, TX, USA) for 60 min. Immunohistochemically stained sections were observed under a CX-21 microscope (Olympus, Tokyo, Japan).

RESULTS

HE changes in the prostatic urethra of the mice after construction of the TULP model

After a thulium laser was used to vaporize the prostatic urethra of the mice, the urethra turned white and vaporized immediately under cystoscopy, and the wound was smooth without obvious bleeding. The HE changes in each group are shown in **Table 1**. One day after the surgery, the tissue structure of the wound site was disordered, and it was difficult to distinguish the specific tissue structure, but a large amount of coagulative necrotic tissue and inflammatory exudates were observed. Residual prostatic duct epithelium was observed under the wound, a large number of acute and chronic inflammatory cells were infiltrated, small blood vessels showed slight proliferation, and the wound was not covered by regenerative epithelium (**Figure 3a**). Three days after surgery, the tissue structure of the wound became clearer than that at one day, and the structure of the urethral lumen could be clearly distinguished. Coagulation necrosis and inflammatory exudate attachment were found at the wound site, and the inflammatory cell number was reduced. Intercellular bridges were found between the wound-proliferating cells, indicating squamous cell metaplasia. Most wounds were not covered with regenerated epithelium (**Figure 3b**). Five days after surgery, the wound was covered by a single layer of regenerative epithelium with no obvious polarity (**Figure 3c**). Seven days after surgery, most of the wounds were covered by regenerative epithelium, which thickened to 4–5 layers. Umbrella cells were observed on the surface, and the cells showed polarity (**Figure 3d**).

Expression of UPK in the prostatic urethra of the mice after construction of the TULP model

The UPK expression observed in each group is shown in **Table 1**. One day after the surgery, the urothelium expressing UPK was absent in the urethral wound site, and a large number of necrotic tissues were found in the wound site. There was no UPK-positive urothelium in the wound 3 days after surgery. At 5 days after surgery, monolayer urothelium expressing UPK was observed in the wound site, indicating that the re-epithelization of the wound had been completed. On the 7th day after surgery, there were multiple layers of urothelium with UPK expression, indicating that the repair was completed (**Figure 3e–3h**).

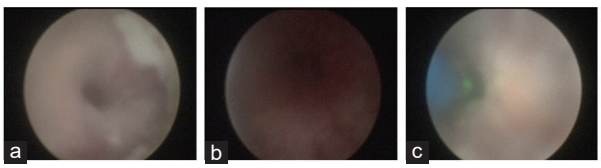

Figure 2: The process of establishing a mouse TULP model under a microcystoscope (because the microcystoscope diameter is small, the image resolution is lower than that of a normal cystoscope). (**a**) After the work sheath was inserted, the bladder neck was first identified. (**b**) The microcystoscope was inserted along the bladder neck into the prostatic urethra, and the prostatic urethra is ruddier than the bladder tissue. The distal end is the turning of the mouse urethra, and the surgical area is from the bladder neck to the turning site of distal urethra. (**c**) The prostatic urethra was cauterized with the laser fiber to construct the wound until the ruddy urethra became white. TULP: transurethral laser prostatectomy.

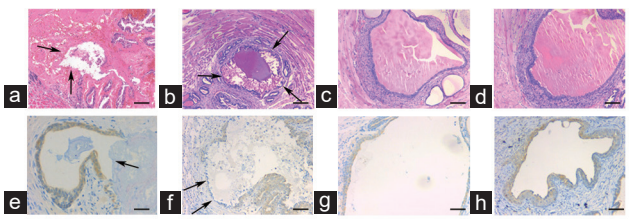

Figure 3: HE staining and UPK immunohistochemical staining of wounds after surgery. HE changes in the prostatic urethra of the mice after constructing the TULP model at (**a**) 1 day, (**b**) 3 days, (**c**) 5 days, and (**d**) 7 days. Expression of UPK in the prostatic urethra of the mice after construction of the TULP model (**e**) 1 day, (**f**) 3 days, (**g**) 5 days, and (**h**) 7 days. The arrow indicates the site of wound. Scale bars = 300 μm. TULP: transurethral laser prostatectomy; UPK: uroplakin; HE: hematoxylin-eosin.

DISCUSSION

Wound repair after prostatectomy, including TULP, is a complex pathophysiological process; thus, the establishment of animal models is an important way to study the re-epithelization of wounds after prostatectomy. The destruction of the urinary tract epithelium will

Table 1: Hematoxylin-eosin changes and uroplakin expression observed in each group

Days after surgery	*HE changes*	*UPK-positive urothelial cells*
1 day	The tissue structure of the wound was disordered, and a large number of coagulative necrotic tissue and inflammatory exudates were observed. Residual prostatic duct epithelium was observed under the wound	No regenerative UPK-positive urothelial cells were found in the wound
3 days	The tissue structure of the wound became clearer than that at day 1, and the structure of the urethral lumen could be clearly distinguished. Coagulation necrosis and inflammatory exudate attachment were found on the wound surface, and the inflammatory cell number was reduced	Most wounds were not covered by regenerative UPK-positive urothelial cells
5 days	The wound surface was covered by a single layer of regenerative epithelium, and the polarity was not obvious	There were single-layer urothelial cells expressing UPK in the wound site
7 days	Most of the wounds were covered by regenerative epithelium, which thickened to 4–5 layers. Umbrella cells could be observed on the surface, and the cells showed polarity	There were multiple layers of urothelial cells expressing UPK

UPK: uroplakin; HE: hematoxylin-eosin

lead to local inflammation, and under the stimulation of urine, sensory neurons in deep tissue will be excited, leading to the occurrence of frequent urination, urgency, and pain. This repair process of wound urothelium is called re-epithelialization or urothelium regeneration. It is important to restore the normal anatomy and barrier function of the urethra after surgery, and this process can reduce postoperative complications and improve the quality of life of patients.[8] Because canines have BPH similar to humans and have a large body size, human conventional cystoscopy can be used for prostatectomy; thus, a prostatectomy model in canines is considered to be a good model for studying this issue.[8] Our previous study was mainly based on the canine model of prostatectomy. However, the canine surgery model also has limitations that cannot be ignored. First, there are few specific antibodies for canine research, which makes it impossible for us to research various issues of interest. Second, as an experimental animal, canines are much less flexible and economical than small animals such as mice. Finally, and most importantly, the "gold standard" for wound repair and cell differentiation research – lineage tracing after gene editing – cannot be performed in canine species. To solve these problems, we designed and constructed a novel model of TULP in mice.

As a common animal model platform, mice are widely used in skin wound repair research.[9] The main reason is that the surgery in mice is easy to perform, and the animals breed rapidly and are economical. In addition, age, sex, and genetic background can be highly standardized in mice. More importantly, the gene editing technology used to study repair and regeneration can be easily implemented in mice.[10] Mice also have certain disadvantages, such as small size, a short lifespan, and genetic background differences from humans. However, no model can completely simulate the process of human wound repair. There have been no previous reports of mouse TULP models, mainly because of the small size of the bladder, urethra, and prostate, and there are no suitable transurethral surgical instruments for the construction of surgical models. However, with the development of minimally invasive endoscopic technology, microendoscopic systems have been widely used in the clinic, allowing construction of a mouse TULP model. Therefore, considering the advantages and disadvantages of mice and our future research direction, we believe that the construction of a TULP model in mice is feasible and important for scientific research.

The wound after prostatectomy is essentially the destruction of urinary tract epithelium and the exposure of the prostatic duct or acinus.[8] Because the cell types of the prostate duct and prostate acinus are the same, as long as there is damage to the urinary tract epithelium and the prostate duct or prostate acinus is exposed, the model is consistent with the characteristics of the wound after prostatectomy. The most important anatomical difference of the mouse and human prostate is that the mouse prostate is lobulated and is divided into the ventral lobe, lateral lobe, and dorsal lobe. Unlike human or canine prostate, which has a dense capsule, mouse prostate still has a catheter opening to the urethra;[11] therefore, after we used a laser to cauterize the prostatic urethra, we found that the prostate urethra was completely damaged in pathological section observation, and the duct of each lobe of the prostate had been exposed. These ducts contain prostatic epithelial cells and basal cells as well as other key cells for re-epithelization of wounds. Therefore, we believe that although there are some differences in prostate anatomy between mice and humans, the nature of the wound is the same after similar types of trauma, that is, the destruction of urinary tract epithelium and the exposure of prostate duct or acinus. By establishing our model, we confirmed the damage to the urethral urothelium and the exposure of the prostatic duct in mice by endoscopic observation and postoperative pathological sectioning. We also observed that the re-epithelization of the wound was preliminarily completed in the mice approximately 5 days after surgery, which confirmed the establishment of our mouse TULP model. We established a transurethral resection of the prostate (TURP) model in canines; the time to re-epithelization after surgery was approximately one week, and the repair time of the mouse was much shorter. This discrepancy may be because the prostatic urethra of mouses is relatively small, and the area of wound to be repaired is much smaller than that of canines, so the repair time will be much shorter.[12] Notably, there are differences between the canine model and the mouse model, and the canine prostatic urethra and prostate are more similar to those of humans than the mouse structures, which is the primary reason that most of the researchers use the canine model for studying wound repair after prostatectomy. We established this mouse model not because this model is more similar to human surgery than the canine model or because we ignored the difference between these models, but because we wanted to develop a new platform for future research such as lineage tracing after gene editing to confirm the specific repair process of the wound.

In the construction of the mouse TULP model, we established the following points:

1. The prostatic urethra of mouse is not as obvious as that of humans, and because the shape of prostate hyperplasia pressing into the urethra is not obvious, it is not as easy to locate the mouse prostatic urethra as it is in humans; however, after many attempts, we believe that the bladder neck and urethral turning site can be used as the anatomic location marks of the surgery, and the prostatic duct opening in the urethra will be exposed when the wound model of the prostate is constructed between these two points.
2. The prostatic urethra of the mouse is very small; if laser vaporization is excessive, the prostatic urethra can be cut through, leading to irrigation fluid entering the subcapsular stroma of

the prostate. This condition is not conducive to the repair of the wound, and it will also lead to increased mortality caused by postoperative infection. Therefore, the laser power should be controlled below 5 W to avoid direct contact of the optical fiber on the urethra for vaporization. If the subcapsular edema of the prostate is observed, the modeling will fail.

3. This process must be carefully performed, and the flushing water pressure should not be too high. Although the fluid flushed through the working sheath can be discharged along the urethra, excessive water pressure may lead to the occurrence of TURP syndrome in mice.

Therefore, the surgical model can be established without excessive flushing under clear visual conditions.

CONCLUSION

It is feasible to establish a mouse TULP model and investigate wound healing by using a microcystoscope system and a 200-μm thulium laser.

AUTHOR CONTRIBUTIONS

HZ, GHL, and SJX conceived and designed the experiment. HZ and YT performed the experiments. HZ, LYA, GHL, and BY analyzed the data. HZ and GHL wrote the paper. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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