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# ORIGINAL ARTICLE

Prostate Disease

# Re-epithelialization resulted from prostate basal cells in canine prostatic urethra may represent the ideal healing method after two-micron laser resection of the prostate

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The purpose of this study is to characterize the re-epithelialization of wound healing in canine prostatic urethra and to evaluate the effect of this re-epithelialization way after two-micron laser resection of the prostate (TmLRP). TmLRP and partial bladder neck mucosa were performed in 15 healthy adult male crossbred canines. Wound specimens were harvested at 3 days, and 1, 2, 3, and 4 weeks after operation, respectively. The histopathologic characteristics were observed by hematoxylin and eosin staining. The expression of cytokeratin 14 (CK14), CK5, CK18, synaptophysin (Syn), chromogranin A (CgA), uroplakin, transforming growth factor-$\beta_1$ (TGF-$\beta_1$), and TGF-$\beta$ type II receptor in prostatic urethra wound were examined by immunohistochemistry and real-time polymerase chain reaction, respectively. Van Gieson staining was performed to determine the expression of collagen fibers in prostatic urethra and bladder neck would. The results showed that the re-epithelialization of the prostatic urethra resulted from the mobilization of proliferating epithelial cells from residual prostate tissue under the wound. The proliferating cells expressed CK14, CK5, but not CK18, Syn, and CgA and re-epithelialize expressed uroplakin since 3 weeks. There were enhanced TGF-$\beta_1$ and TGF-$\beta$ type II receptor expression in proliferating cells and regenerated cells, which correlated with specific phases of re-epithelialization. Compared with the re-epithelialization of the bladder neck, re-epithelialization of canine prostatic urethra was faster, and the expression of collagen fibers was relatively low. In conclusion, re-epithelialization in canine prostatic urethra resulted from prostate basal cells after TmLRP and this re-epithelialization way may represent the ideal healing method from anatomic repair to functional recovery after injury.

## INTRODUCTION

Benign prostate hyperplasia (BPH) is one of the most common diseases affecting aging males and leads to micturition difficulties in 50% of 50-year-old men with the disease.[1] Approximately, 20% of all BPH patients with symptomatic disease eventually undergo surgery.[2] Transurethral resection of the prostate (TURP) is presently the most common surgical treatment for BPH and has been established as the gold standard surgical procedure.[3] In 2004, the thulium laser was used to treat patients with BPH[4] and two-micron continuous-wave laser is an improvement over the thulium laser.[5,6] Two-micron laser resection of the prostate (TmLRP) for BPH is becoming more accepted owing to the minimal hemorrhage it produces, higher vaporesection speed and superficial coagulation zone.[7–10] Although TmLRP has several advantages over TURP, the thermal injury following tissue penetration undoubtedly becomes a crucial source of necrotic tissue and secondary sloughing during laser therapy, which causes subsequent complications, such as postoperative hemorrhage, urinary

tract infection, and uncomfortable postoperative symptoms, including urinary frequency, urgency, and urodynia. Urothelial prostatic urethra self-healing, also called re-epithelialization, is the fundamental wound healing process following injury and facilitates surgical wound closure to reduce the aforementioned complications. Therefore, understanding of the re-epithelialization process in the prostatic urethra after TmLRP and further identification of suitable methods to accelerate this process are of great value. At present, most available TmLRP reports have focused on the influence of dosimetry on the extent of thermocoagulation or the optimal treatment parameters;[11–13] little is known about the healing response to laser-induced injury and the mechanism involved in the re-epithelialization in human prostatic urethra following TmLRP. In the current study, we carried out a systematic histopathologic examination in a canine prostate model following TmLRP to characterize mechanisms of re-epithelialization in prostatic urethra wounds after laser injury. We also compared re-epithelialization differences in canine prostatic urethra and bladder

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necks wound to evaluate the effect of the re-epithelialization way in the prostatic urethra.

## MATERIALS AND METHODS

### Canines

Fifteen healthy adult male crossbred canines were obtained from Zunyi Medical College. The animal model studies have been obtained approval by Medical Science Ethics Committee of Guizhou provincial people's hospital. The animals were 5–7 years old and weighed 18–22 kg.

### Modeling of two-micron laser resection

All operations were performed using the same two-micron continuous wave Tm:YAG laser system (RevoLix; Lisa Laser Products, Katlenburg, Germany). The laser wavelength was 2.013 μm and the energy was transmitted at 70 W of power output through a flexible 550 μm diameter fiber. After general anesthesia had been achieved with 10% chloral hydrate (0.003 ml g⁻¹), the canine was placed in the supine position on the operating table. The lower abdomen was entered through a medial and longitudinal incision, and the anterior wall of the bladder was freed. A purse suture was performed in the anterior wall the bladder, an incision was made within the purse to allow the placement of a 26F continuous-flow resectoscope, and then the suture was fastened. Under saline irrigation, a resectoscope was placed into the prostatic urethra through the internal urethral orifice. The prostatic laser vaporesection procedure is the same as is applied in patients as described previously.[7,8] During the vaporesection procedure, the fiber was swept continuously in half-moon mode to resect all prostatic urethra and most of the prostatic tissue while avoiding injury to the prostatic capsule.

To compare re-epithelialization differences in canine prostatic urethra and bladder necks wound, every canine also received the resection of partial bladder neck mucosa using two-micron laser at the same time as the prostatic vaporesection a considerable distance from the prostatic urethra wound. After the procedure was successful, the bladder and the abdominal wall were closed. No transurethral catheter was required.

### Histopathogic examination

The animals were randomly divided into five groups, and each group has three canines. The canines were sacrificed, and wound specimens from the prostatic urethra and bladder neck were harvested and fixed in 4% formalin at 3 days and 1, 2, 3, and 4 weeks after laser treatment. Prostate tissue samples were cut in the transverse plane at the level of the mid prostatic urethra to permit inspection of the lesion. After embedding in paraffin, 5 μm slides were examined histologically by hematoxylin and eosin (H and E) staining.

### Immunohistochemistry staining

Immunohistochemical staining was performed as described earlier.[14] Briefly, the sections were treated with blocking buffer (Dako Denmark A/S, Glostrup, Denmark) for 30 min at room temperature (RT) and thereafter incubated with anti-cytokeratin 14 (CK14) antibody (1:300 dilution in Tris–NaCl buffer; Abcam, Cambridge, UK), anti-CK5 antibody (1:250 dilution in Tris–NaCl buffer; Epitomics Inc., Burlingame, CA, USA), anti-CK18 antibody (1:500 dilution in Tris–NaCl buffer; Epitomics Inc., Burlingame, CA, USA), anti-synaptophysin (Syn) antibody (1:250 dilution in Tris–NaCl buffer; Epitomics Inc., Burlingame, CA, USA), anti-chromogranin A (CgA) antibody (1:200 dilution in Tris–NaCl buffer; Epitomics Inc., Burlingame, CA, USA), anti-uroplakin antibody (1:300 dilution in Tris–NaCl buffer; Abcam, Cambridge, UK), anti-transforming

growth factor-$\beta_1$ (TGF-$\beta_1$) antibody (1:200 dilution in Tris–NaCl buffer; Abcam, Cambridge, UK), and anti-TGF-β type II receptor antibody (1:50 dilution in Tris–NaCl buffer; Abcam, Cambridge, UK) overnight at 4°C. Following a thorough rinse in Tris–NaCl buffer, the sections were incubated with secondary antibodies; example, biotinylated goat anti-mouse IgG or biotinylated goat anti-rabbit IgG (diluted 1:200 in Tris–NaCl buffer) for 60 min at RT. Sections were subsequently incubated with avidin-biotinylated enzyme complex and DAB and then dehydrated with increasing concentrations of ethanol, cleared with xylene, and mounted in permount. Negative controls for these immunohistochemical procedures were incubated with nonimmune serum instead of the primary antibodies, which resulted in no detectable staining.

The optical densities (ODs) of CK14, CK5, uroplakin, TGF-$\beta_1$ and TGF-β type II receptor-like immunoreactivity (IR) in prostatic urethra wound from 3 day to 4 weeks after TmLRP were measured by using a CM2000B Biomedical Image Analysis System (Beihang University, China). The OD of CK14, CK5 and uroplakin-like IR was analyzed by microdensitometry in proliferating prostatic epithelial cells and regenerated epithelial cells at the cavity surface, respectively. The OD of TGF-$\beta_1$ and TGF-β type II receptor-like IR was analyzed by microdensitometry in residual prostatic epithelial cells far away from the wound, proliferating prostatic epithelial cells under the wound, and regenerated epithelial cells at the cavity surface, respectively. Five random fields of interest were measured, and the OD measurements were averaged.

### Real-time polymerase chain reaction

The mRNA levels of CK14, CK5, CK18, and Syn in the regions of proliferating prostatic epithelial cells and unproliferating residual prostatic epithelial cells far away from the wound at the prostatic urethra wound from 3 days to 4 weeks after TmLRP were detected by real-time polymerase chain reaction (PCR). Real-time PCR was performed as described earlier.[14] In brief, we selected the regions of proliferating prostatic epithelial cells and unproliferating residual prostatic epithelial cells far away from the wound according to H and E staining from 3 days to 4 weeks, respectively. Total RNA was extracted from these two regions with the RecoverAll Total Nucleic Acid Isolation Optimized for formalin-fixed paraffin-embedded samples kit (Ambion Inc., Austin, Texas, USA). The RNA preparations were treated with three units of RQ RNase-free DNase (Promega) for 10 min at 37°C in order to remove any traces of genomic DNA present. The purity and quantity of RNA were determined with UV spectrophotometer with A260/A280 ratio > 1.9. Total RNA was reverse transcribed with Moloney murine leukemia virus reverse transcriptase and Oligo-dT primers. The forward and reverse primers for selected genes were designed using Primer Express software (Biosystems, Foster City, CA, USA) and listed in **Table 1**. The real-time quantitative reverse transcription-PCR was performed by using the ABI PRISM 7300 Sequence Detection System (Applied Biosystems) and analyzed with GeneAmp 7300 SDS software, in which the SYBR green Master Mix (Applied Biosystems, Foster City, CA, USA) was used. The transcript levels were estimated by using the formula 2-ΔCT, where ΔCT represents the difference in cycle time (CT) values between target and housekeeping assays. The relative differences in expression between these two regions were expressed using CT values, in which the difference in CT between the genes of interest was first normalized with β-actin, and then calculated as relative increases by setting the regions of unproliferating residual prostatic epithelial cells as 100% in comparison.

## Van Gieson staining

Van Gieson staining was performed according to the manufacturer's instructions (HT25; Sigma-Aldrich, St. Louis, MI, USA). Briefly, tissue was deparaffinized with xylene and rehydrated with a graded series of ethanol. The sections were placed in Weigert's working hematoxylin for 10 min. After washing in distilled water, the sections were stained 3 min in Van Gieson's Solution. Sections were rinse in 95% alcohol, dehydrated, cleared, and coverslipped. Collagen fiber, smooth muscle, red blood corpuscle, and nuclei stained red, yellow, yellow, and blue, respectively. The expression of collagen fiber was analyzed by semi-quantitative analysis of Van Gieson stain indicates no red (collagen fiber); ± indicates minimal red (collagen fiber), barely visible at low magnification, clearly visible at high magnification; + indicates minimal red (collagen fiber) with linear distribution clearly visible at low magnification; ++ indicates a large amount of red (collagen fiber) with wispy or small plexiform distribution; +++ indicates large flake red distribution (collagen fiber).

## Statistical analysis

The means were compared by the two-tailed Student's *t*-test using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA) in order to determine if there were statistically differences.

**Table 1: PCR primers used in the study**

| mRNA | Sequences | Length (bp) |
|---|---|---|
| CK14 | 5'-GCTGACGACTTCCGTACCAA-3' | 189 |
| | 3'-AGCGTTCATTTCCTCCTCGT-5' | |
| CK5 | 5'-GTTCTTTGAGGCGGAGCTGT-3' | 238 |
| | 3'-TTGCTTGGTGTTGCGGAGAT-5' | |
| CK18 | 5'-CGCGTCAAGTATGAGACGGA-3' | 177 |
| | 3'-ACCCTTTACTTCCTCCTCGTG-5' | |
| Syn | 5'-TCGTGTTCAAGGAGACAGGC-3' | 300 |
| | 3'-GGCTGCATTGACCAGACTACA-3' | |
| β-actin | 5'-CAGCCTTCCTTCCTGAGCAT-3' | 137 |
| | 3'-CCAGGGTACATGGTGGTTCC-5' | |

PCR: polymerase chain reaction; Syn: synaptophysin; CK14: cytokeratin 14
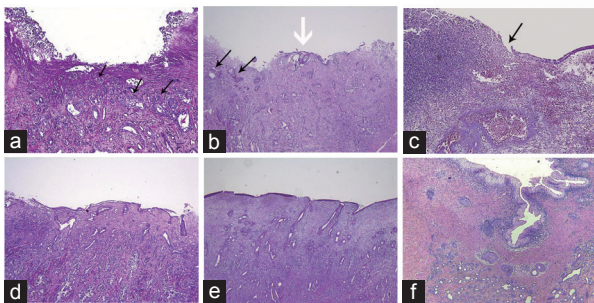


**Figure 1:** Histopathology changes on re-epithelialization of wound healing in canine prostatic urethra after two-micron laser resection of the prostate. (**a**) At 3 days, prominent proliferation of the epithelium from acinar and ductal prostatic epithelium was observed under coagulation necrosis (arrows) (×200). (**b**) At 1 week, prominent proliferation of the epithelium with distinct squamous metaplasia originating from the underlying prostatic acinar and ductal tissue (black arrows), the proliferating epithelium extended to the lumen of the cavity with focal re-epithelization of the surface (white arrows) (×100). (**c**) At 1 week, adjacent to the transitional epithelium of the bladder neck margin, inflammatory exudate was noted (arrow) (×200). (**d**) At 2 weeks, marked proliferation of the epithelial glandular elements, with notable squamous metaplasia, extended to the surface of the cavity with more regenerative epithelium (×100). (**e**) At 3 weeks, re-epithelialization was basically complete (×100). (**f**) At 4 weeks, the regenerative epithelium lining the central cavity appeared normal with a distinct layer of umbrella cells (×200).

# RESULTS

## Histopathology changes in re-epithelialization in canine prostatic urethra after two-micron laser resection of the prostate

Three days after TmLRP, cavitation, a zone of coagulation necrosis and acute inflammatory exudate on the wound surface was evident. There was no cell regeneration at the cavity surface. Below the coagulation necrosis, proliferating epithelial cells from residual acinar and ductal prostatic epithelium under the wound were seen (**Figure 1a**). At 1 week after laser irradiation, histology also showed large areas of cavitation, coagulation necrosis, and acute inflammatory exudate on the wound surface. At the periphery of the coagulation necrosis, there was prominent proliferation of the epithelium with distinct squamous metaplasia originating from the underlying prostatic epithelium. The proliferating cells had large nuclei, prominent nucleoli, and increased chromatin. This germinating epithelium extended in some areas to the lumen of the cavity with focal re-epithelialization of the cavity by a thin one to two cell thick epitheliums (**Figure 1b**). It is worth noting that we couldn't observe epithelial regeneration commencing from the edges of the wound at the bladder neck. Adjacent to the transitional epithelium at the edges of the bladder neck was inflammatory exudate (**Figure 1c**). At 2–3 weeks after surgery, the wound response gradually changed into the regenerative phase. At 2 weeks, we observed marked proliferation of the epithelial glandular elements with notable squamous metaplasia extending to the surface of the cavity with increased regenerative epithelium (**Figure 1d**). This regenerative epithelium was three to four layers thick, but had no polarity. Adjacent to the transitional epithelium at the edges of the bladder neck there was also inflammatory exudate that was not continuous with the regenerative epithelium, suggesting that re-epithelialization of the prostatic urethra did not result from the cells from the wound edges of the bladder neck. At 3 weeks after TmLRP, chronic inflammatory cell infiltration was gradually replaced by granulation tissue and re-epithelialization was essentially complete in the canine prostate model (**Figure 1e**). The histology changes at 4 weeks included a gradual decrease in squamous metaplasia and the granulation tissue was replaced by well-organized underlying connective tissue oriented parallel to the cavity surface. The transitional epithelium lining the central cavity appeared normal with a distinct layer of umbrella cells (**Figure 1f**).
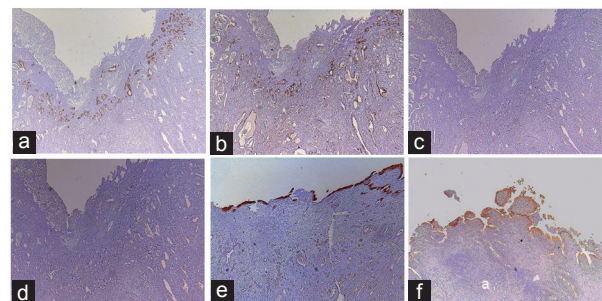


**Figure 2:** The expression of cytokeratin 14 (CK14), CK5, CK18, synaptophysin (Syn), and uroplakin in canine prostatic urethra wound after two-micron laser resection of the prostate by immunohistochemical staining. (**a**) At 3 days, the proliferating prostate cells expressed CK14 (×100). (**b**) At 3 days, the proliferating prostate cells expressed CK5 (×100). (**c**) At 3 days, the proliferating prostate cells did not express CK18 (×100). (**d**) At 3 days, the proliferating prostate cells did not express Syn (×100). (**e**) At 3 weeks, the regenerated epithelial cells expressed uroplakin (×100). (**f**) At 4 weeks, the regenerated epithelial cells expressed uroplakin (×100).

*Expression of cytokeratin 14, cytokeratin 5, cytokeratin 18, synaptophysin, chromogranin A and uroplakin at the prostatic urethra wound after two-micron laser resection of the prostate*

From 3 days to 4 weeks after TmLRP, the immunohistochemical staining displayed that the prostatic secretory cell in inner layers were positivity expression for CK18, prostatic basal cell in outer layers were positivity expression for CK14, CK5, and minority neuroendocrine cell were positivity expression for Syn and CgA at the residual acinar and ductal prostatic epithelium far away from the prostatic urethra wound. The proliferating prostatic epithelial cells under the coagulation necrosis showed positivity expression for CK14, CK5 (**Figure 2a** and **2b**), and negativity expression for CK18 (**Figure 2c**), Syn (**Figure 2d**), and CgA at 3 days after laser irradiation. The same immunohistochemical staining pattern of proliferating prostatic epithelial cells were also observed from 1 to 4 weeks after TmLRP. The regenerated epithelial cells at the cavity surface also expressed CK14, CK5, and began to express uroplakin protein at 3 and 4 weeks (**Figure 2e** and **2f**). The expression intensity of CK14, CK5, and uroplakin in proliferating prostatic epithelial cells and regenerated epithelial cells at the cavity surface were no significant changes, and the OD of CK14, CK5, and uroplakin-like IR had no statistical differences at the different timepoints. The OD of CK14, CK5 and uroplakin-like IR in proliferating prostatic epithelial cells and regenerated epithelial cells at the cavity surface from 3 day to 4 weeks after TmLRP were shown in **Table 2**.

We also measured the mRNA levels of CK14, CK5, CK18 and Syn in the region of proliferating prostatic epithelial cells and unproliferating residual prostatic epithelial cells far away from the wound from 3 days to 4 weeks after TmLRP. When compared with the region of unproliferating residual prostatic epithelial cells far away from the wound, the relative mRNA quantification of CK14, CK5 in the region of proliferating prostatic epithelial cells were increased (**Figure 3a** and **3b**), the relative mRNA quantification of CK18, Syn were not marked change from 3 days to 4 weeks after TmLRP (**Figure 3c** and **3d**).

*Expression of growth factors transforming growth factor-$\beta_1$ and transforming growth factor-$\beta$ type II receptor at prostatic urethra wound after two-micron laser resection of the prostate*

The weak positive expression of TGF-$\beta_1$ and TGF-$\beta$ type II receptor was seen in residual prostatic epithelial cells far away from the wound from 3 days to 4 weeks. TGF-$\beta_1$ protein also expressed in particular to the muscle cells. The TGF-$\beta_1$ like-IR in these proliferating prostatic cells under the wound was strikingly increased as compared to the residual prostatic cells far away from the wound at 3 days and lasted till 1, 2 and 3 weeks after TmLRP, respectively. At 4 weeks, when the regenerated epithelium started to show differentiation with superficial umbrella cells, the TGF-$\beta_1$ like-IR in proliferating prostatic epithelial cells returned to weak positive as in residual prostatic epithelium far away from the wound. In addition, the

TGF-$\beta_1$ like-IR in regenerated epithelial cells at the cavity surface also was increased at 1 and 2 week and maximally at 3 and 4 weeks when compared to the residual prostatic epithelial cells (**Figure 4a–4c**). TGF-$\beta$ type II receptor was expressed by the same cells, and its expression pattern followed that of TGF-$\beta_1$ (**Figure 4d–4f**). The OD of TGF-$\beta_1$ and TGF-$\beta$ type II receptor in residual prostatic epithelial cells far away from the wound, proliferating prostatic epithelial cells under the wound and regenerated epithelial cells at the cavity surface at the prostatic urethra wound from 3 days to 4 weeks after TmLRP were shown in **Table 3**.

*Histopathology changes in the re-epithelialization in canine bladder neck after two-micron laser resection of partial bladder neck mucosa*

Three days after laser resection of the canine bladder neck epithelium, there was much coagulation necrosis and inflammatory exudates attached to the wound surface. At 1 week, there was largely granulation tissue under the necrotic tissue and no cell regeneration at the cavity surface (**Figure 5a**). At 2 weeks, there were distinct proliferating
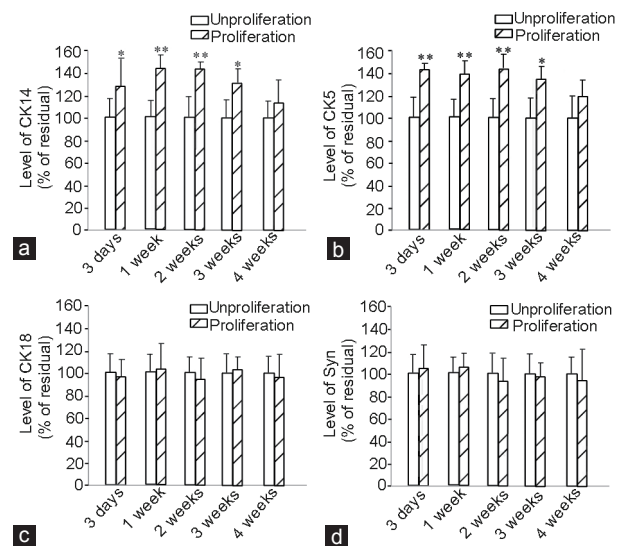


**Figure 3:** Cytokeratin 14 (CK14), CK5, CK18 and synaptophysin at mRNA level in the region of proliferating prostatic epithelial cells and unproliferating residual prostatic epithelial cells far away from the wound from 3 days to 4 weeks after two-micron laser resection of the prostate detected by real-time polymerase chain reaction. (**a**) The mRNA level of CK14. (**b**) The mRNA level of CK5. (**c**) The mRNA level of CK18. (**d**) The mRNA level of Syn. Values in the bar graphs are means ± s.d. of 3 canines. *$P < 0.05$ and **$P < 0.01$ in comparison to the region of unproliferating residual prostatic epithelial cells far away from the wound, as determined by an analysis of the two-tailed Student's *t*-test using SPSS15.0 software. Unproliferation means in the region of unproliferating residual prostatic epithelial cells far away from the wound; proliferation means in the region of proliferating prostatic epithelial cells.

**Table 2: Levels (OD) of CK14, CK5 and uroplakin-like IR in proliferating prostatic epithelial cells and regenerated epithelial cells at the cavity surface at the prostatic urethra wound from 3 days to 4 weeks after TmLRP**

| | CK14 | | CK5 | | Uroplakin | |
|---|---|---|---|---|---|---|
| | *Proliferating prostatic epithelial cells* | *Regenerated epithelial cells* | *Proliferating prostatic epithelial cells* | *Regenerated epithelial cells* | *Proliferating prostatic epithelial cells* | *Regenerated epithelial cells* |
| 3 days | 0.26±0.04 | 0.25±0.05 | 0.27±0.04 | 0.26±0.05 | - | - |
| 1 week | 0.25±0.05 | 0.25±0.07 | 0.26±0.07 | 0.26±0.04 | - | - |
| 2 weeks | 0.26±0.06 | 0.27±0.03 | 0.26±0.04 | 0.25±0.05 | - | - |
| 3 weeks | 0.24±0.03 | 0.26±0.04 | 0.25±0.04 | 0.26±0.05 | - | 0.28±0.04 |
| 4 weeks | 0.25±0.06 | 0.26±0.05 | 0.26±0.05 | 0.25±0.06 | - | 0.29±0.06 |

Data are presented as mean±s.d. s.d.: standard deviation; OD: optical density; CK14: cytokeratin 14; IR: immunoreactivity; TmLRP: two-micron laser resection of the prostate
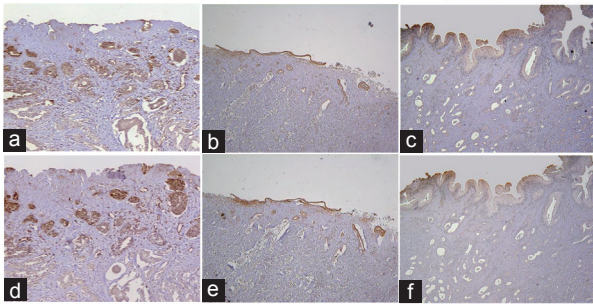
**Figure 4:** The expression of transforming growth factor-$\beta_1$ (TGF-$\beta_1$) and TGF-$\beta$ type II receptor in canine prostatic urethra wound after two-micron laser resection of the prostate by immunohistochemical staining. (**a**) An increased TGF-$\beta_1$ expression in the proliferating prostatic epithelial cells under the wound was shown at 3 days (×200). (**b**) At 2 weeks, an increased TGF-$\beta_1$ expression in the proliferating prostatic epithelial cells and regenerated epithelial cells were shown (×100). (**c**) At 4 weeks, TGF-$\beta_1$ expression were strikingly increase in regenerated epithelial cells but returned to weak positive in proliferating prostatic epithelial cells (×100). (**d**) An increased TGF-$\beta$ type II receptor expression in the proliferating prostatic epithelial cells under the wound was shown at 3 days (×200). (**e**) At 2 weeks, an increased TGF-$\beta$ type II receptor expression in the proliferating prostatic epithelial cells and regenerated epithelium cells were shown (×100). (**f**) At 4 weeks, TGF-$\beta$ type II receptor expression were strikingly increase in regenerated epithelial cells but returned to weak positive in proliferating prostatic epithelial cells (×100).
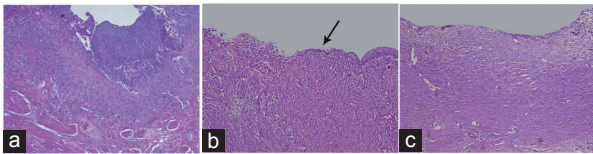


**Figure 5:** Histopathology changes in the re-epithelialization in canine bladder neck after two-micron laser resection of partial bladder neck mucosa. (**a**) At 1 week, there was largely granulation tissue under the necrotic tissue and no cell regeneration at the cavity surface (×200). (**b**) At 2 weeks, re-epithelialization of the bladder neck wound was incipient and resulted from the migration of proliferating epithelial cells from the edges of the wound at the bladder neck (arrows) (×200). (**c**) At 4 weeks, re-epithelialization was not complete in bladder neck wounds (×200).
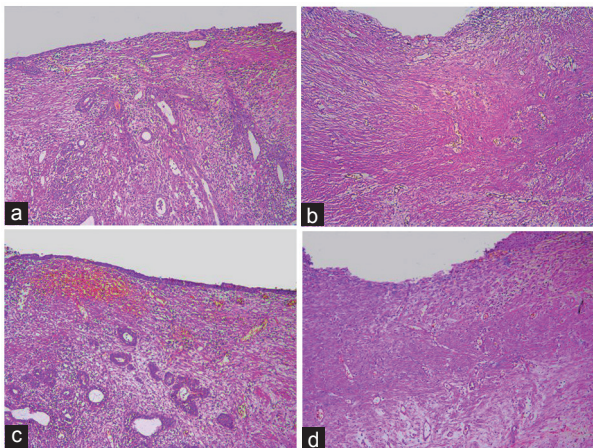


**Figure 6:** The expression of collagen fibers in prostatic urethra and bladder neck wounds by Van Gieson staining. (**a**) At 3 weeks, the expression of collagen fibers were minimal in prostatic urethra wounds (×200). (**b**) At 3 weeks, the expression of collagen fibers were a large amount in bladder neck wounds (×200). (**c**) At 4 weeks, the expression of collagen fibers were limited in prostatic urethra wounds (×200). (**d**) At 4 weeks, there was large expression of collagen fibers in bladder neck wounds (×200).

epithelial cells in the edges of the wound at the bladder neck and epithelial regeneration commenced from the edges of the bladder neck wound (**Figure 5b**). At 3 weeks, below the wound surface, granulation tissue was gradually replaced by fibroblasts. Re-epithelialization of the bladder neck by a thin, two to three cell thick epithelium resulted from the migration of proliferating epithelial cells from the edges of the wound at the bladder neck. At 4 weeks, histology showed granulation tissue gradually becoming fibrotic and there was a large degree collagen deposition in bladder neck wounds; however, re-epithelialization was not complete by 4 weeks in bladder neck wounds (**Figure 5c**).

### *The expression of collagen fibers in prostatic urethra and bladder neck wounds at 3 and 4 weeks by Van Gieson staining*
Semi-quantitative analysis of Van Gieson staining showed that the expression of collagen fibers were (+) in prostatic urethra wounds at 3 and 4 weeks (**Figure 6a** and **6c**) and the expression of collagen fibers were (++ to +++) and (+++) in bladder neck wounds at 3 and 4 weeks (**Figure 6b** and **6d**), respectively.

## DISCUSSION
Wound healing is a complex, multi-step process. A critical and important feature of a healed wound is the restoration of an intact epidermal barrier through wound re-epithelialization. Re-epithelialization can be conceptually viewed as the result of three overlapping cell functions: proliferation, migration, and differentiation.[15] The traditional concept that prevails among urologists is that re-epithelialization of the prostatic urethra results from migration and differentiation of proliferating epithelial cells from the edges of the wound at the bladder neck after injury analogous to skin wound repair. However, results from Pow-Sang *et al.*[16] and Orihuela *et al.*[17] showed that re-epithelialization of the prostatic urethra results from residual prostatic epithelium under the wound in canine prostate models and in BPH patients following injury. Our previous studies[18,19] also showed prostate tissues might be the important organizations for re-epithelialization of prostatic urethra after TmLRP in canine prostate model. We observed a marked proliferation of the prostatic epithelial glandular elements from the underlying prostatic epithelium of glands and ductal prostatic epithelium under the wound in canine prostatic urethra after laser irradiation from 3 days to 4 weeks. This phenomenon indicates that prostatic epithelial cells have an excellent regenerative faculty after injury and may play an important role in wound healing following laser injury. At 1 week, the germinating prostatic epithelium extended in some areas to the lumen of the cavity with focal re-epithelialization. Then, at 3 weeks, re-epithelialization was essentially completed, and the regenerated epithelium was gradually replaced by transitional epithelium with a distinct layer of umbrella cells. We were unable to observe epithelial regeneration commencing from the edges of the wound at the bladder neck. The re-epithelialize was discontinuous with the transitional epithelium at the edges of the bladder neck and adjacent to the transitional epithelium at the edges of the bladder neck we saw inflammatory exudate from 1 week to 2 weeks. These histopathologic results suggest that the re-epithelialization of prostatic urethra results from the migration and differentiation of proliferating epithelium from the residual acinar and ductal prostatic epithelium under the wound, and not from the transitional epithelium at the edges of the bladder neck, in accordance with the Pow-Sang *et al.*[16] In this healing process, a prominent phenomenon in this re-epithelialization process were the proliferation of prostatic epithelial cells with notable squamous metaplasia from 1 to 3 weeks. Moreover, the marked proliferation of the epithelial glandular elements with notable squamous metaplasia extended to the surface of the cavity with regenerative epithelium.

**Table 3: Levels (OD) of TGF-$\beta_1$ and TGF-$\beta$ type II receptors-like IR in residual prostatic epithelial cells far away from the wound, proliferating prostatic epithelial cells under the wound and regenerated epithelial cells at the cavity surface at the prostatic urethra wound from 3 days to 4 weeks after TmLRP**

| | TGF-$\beta_1$ | | | TGF-$\beta$ type II receptor | | |
|---|---|---|---|---|---|---|
| | Residual prostatic epithelial cells | Proliferating prostatic epithelial cells | Regenerated epithelial cells | Residual prostatic epithelial cells | Proliferating prostatic epithelial cells | Regenerated epithelial cells |
| 3 days | 0.10±0.04 | 0.23±0.05* | - | 0.09±0.04 | 0.24±0.02* | - |
| 1 week | 0.10±0.05 | 0.25±0.06* | 0.20±0.03* | 0.10±0.02 | 0.25±0.05* | 0.20±0.05* |
| 2 weeks | 0.11±0.02 | 0.24±0.04* | 0.20±0.04* | 0.09±0.04 | 0.23±0.06* | 0.19±0.04* |
| 3 weeks | 0.10±0.03 | 0.25±0.03** | 0.26±0.03** | 0.11±0.05 | 0.24±0.04* | 0.27±0.03** |
| 4 weeks | 0.09±0.04 | 0.11±0.04 | 0.25±0.02** | 0.11±0.04 | 0.12±0.03 | 0.26±0.04** |

Data are presented as mean±s.d. **$P<0.01$ and *$P<0.05$ as compared to residual prostatic epithelial cells. s.d.: standard deviation; OD: optical densities; TGF-$\beta_1$: transforming growth factor beta 1; IR: immunoreactivity; TmLRP: two-micron laser resection of the prostate

Squamous metaplasia has been described to develop following conditions that result in the proliferation of the prostatic glandular epithelium, such as ischemia, infarction, or after estrogen hormonal therapy or laser treatment.[20] We presume that squamous metaplasia also is a distinct feature during the re-epithelialization process of wound healing in canine prostatic urethra following thermal injury and may be present for a prolonged period of time before it is replaced by normal transitional epithelium.

Prostatic epithelium is composed of three types of cells: secretory, basal, and neuroendocrine cells. The different prostatic epithelial cells can be distinguished based on their phenotypic characteristics. For instance, secretory cells express androgen receptor (AR), prostate-specific antigen (PSA), and a low molecular weight CK, such as CK8 or CK18. However, basal cells are nonsecretory cells that express P63 and a high molecular weight CK, such as CK34βE12, CK14, and CK5, but not AR, PSA, CK8, and CK18. Neuroendocrine cells express Syn and CgA. Our previous studies[18,19] have shown that proliferating prostatic cells expressed CK34βE12, p63, but not PSA, which suggested these proliferating prostatic cells may be prostatic basal cell. To further identify if prostatic basal cell is involved in this re-epithelialization process, we investigated the expression of CK14, CK5, CK18, Syn, and CgA protein in proliferating prostatic cells by immunohistochemical staining in this study. The immunohistochemical staining displayed that the proliferating prostatic epithelial cells expressed prostatic basal cell markers CK14, CK5, but not prostatic secretory cell or neuroendocrine cells markers CK18, Syn, and CgA from 3 day to 4 weeks after TmLRP. These results demonstrated that the proliferating prostate cells were likely prostate basal cells. The regenerated epithelial cells began to express uroplakin protein at 3 and 4 weeks which suggested the regenerated epithelium was replaced by transitional epithelium since 3 weeks. We also measured the mRNA levels of CK14, CK5, CK18 and Syn in the regions of proliferating prostatic epithelial cells and unproliferating residual prostatic epithelial cells far away from the wound from 3 days to 4 weeks after TmLRP. As compared with the region of unproliferating residual prostatic epithelial cells, the relative mRNA quantification of CK14, CK5 in these regions of proliferating prostatic epithelial cells were increased, the relative mRNA quantification of CK18, Syn were not marked change from 3 days to 4 weeks. These results confirmed that there were more prostate basal cells in these regions of proliferating prostatic cells. In other words, these results also supported these proliferating prostatic cells are likely prostate basal cells.

Numerous studies have shown that prostate basal cells have a strong proliferation capacity, and also have glandular epithelium and transitional epithelial differentiation capacity.[21–23] In addition, there may be prostate stem cells in the prostate basal cell layer, which maintain prostate development, maturation, and function following differentiation.[24–26] In this study prostatic basal cells also were shown a prominent regenerative faculty in a canine prostate model after TmLRP. We hypothesize that tissue damage induced by laser thermal injury may result in microenvironmental changes that are capable of stimulating the proliferation, migration, and differentiation of prostatic basal cells into other lineages to finish the re-epithelialization process.

A variety of biologically active substances plays an important role in the regulation of wound healing events, such as cell growth factors. Cell growth factors orchestrate a complex and dynamic balance of signals that induce cellular proliferation, migration, and differentiation, underscoring the complex integration of signals in the wound healing environment. TGF-$\beta_1$ is an important cell growth factor and plays a key role in wound healing of the skin epithelium. The ability of TGF-$\beta_1$ to directly accelerate wound healing has been shown in rat incisional wounds.[27,28] Exogenous application of TGF-$\beta_1$ to skin wounds also enhanced epithelial regeneration *in vivo*.[29,30] Upon injury to skin, TGF-$\beta_1$ are released by both mesenchymal cells (e.g., blood platelets, endothelial cells, and activated, infiltrating macrophages) and epithelial cells, especially in the epithelial cells next to the site of injury,[31,32] which suggest that epithelia play a central role, not only as a key cell type in repair, but also as the source of growth factors during wound healing. In the analogy to the skin epidermis, it may be hypothesized that TGF-$\beta_1$ may be derived from epithelial cells during re-epithelialization process in the prostatic urethra after injury. In this current study, we investigated the spatio-temporal changes of TGF-$\beta_1$ and TGF-$\beta$ type II receptor. Our study demonstrated that a low level of TGF-$\beta_1$ and TGF-$\beta$ type II receptor protein were noted in unproliferating acinar and ductal prostatic epithelium far away from the wound, whereas during wound healing of proliferation and migration phases the proliferating prostate basal cells were stained more intensively compared with unproliferating prostatic epithelium. At 3 and 4 weeks, regenerated epithelial cells at the cavity surface stained for TGF-$\beta_1$ and receptor, coinciding with differentiation of the superficial cells into umbrella cells. These dates indicated that proliferating prostate basal cells and regenerated epithelial cells were one of the resources of TGF-$\beta_1$ and urothelial regeneration in canine prostatic urethra wound after TmLRP can be achieved not only by paracrine, but also by autocrine acting, prostate basal cells-derived growth factors. These dates also support the idea that re-epithelialization in canine prostatic urethra after TmLRP may be brought about by migration and differentiation of proliferating prostate basal cells under the wound.

It is not clear why re-epithelialization resulted from residual prostate basal cells under the prostatic urethra wound, but not from the edges of the wound at the bladder neck, a clinical concept that has traditionally prevailed among urologists. To further evaluate

the effect of the re-epithelialization from prostate basal cells, we also observed the histopathological re-epithelialization changes in the bladder neck wound after two-micron laser resection of the bladder neck epithelium and compared the differences between the two re-epithelialization methods in the same animals. Our study showed that the re-epithelialization of the canine bladder neck resulted from the mobilization of proliferating epithelial cells from the edges of the wound at the bladder neck analogous to skin wound repair. Re-epithelialization of the bladder neck is incipient at 2 weeks, and complete re-epithelialization was not observed following 4 weeks of healing. Compared with re-epithelialization of the bladder neck, re-epithelialization of canine prostatic urethra from prostate basal cells is achieved over a short time interval. Such an early onset and rapid epithelialization of the prostatic urethra wound results in the quick restoration of the functional integrity of these epithelia and reduces the incidence of complications.

Another noteworthy finding is that the healing response of the prostatic urethra is relatively free of contractures compared with the bladder neck. Van Gieson staining results showed that the amount of collagen fibers were extraordinarily decreased in prostatic urethra wound from 3 and 4 weeks after injury compared with the bladder neck wound. These findings imply that the regenerative processes of the prostatic urethra following thermal injury are successfully accomplished free of contractures. Bladder neck contracture (BNC) as a complication of prostatectomy that has been noted for many years.[33] The reported incidence of BNC varies from 0.14% to 20%.[34–35] BNCs are apparently related to extensive resection or excessive fulguration of the bladder neck and are seen more frequently when TURP is carried out in small prostates in which the obstructing element is predominantly fibromuscular.[36] In contrast, development of contractures or strictures in the prostatic urethra is an extremely rare event following laser radiation or TURP in clinical observation; i.e., patients with BPH treated by laser or TURP are usually free of intraprostatic urethral strictures. It is not readily apparent what prevents the prostatic urethra from developing contractures after thermal injury or TURP. One possible explanation is that the rapid epithelialization of the prostatic urethra results in only minor granulation tissue and limited fibrosis. Another potential explanation is that prostate glandular epithelial elements might provide protection against contractures in the prostatic urethra. In the wound healing process of third-degree skin burns, granulation tissue gradually becomes fibrotic, resulting in greatly scar tissue. However, in second-degree skin burns in which dermal appendages have not been lost, there is limited fibrous and scar tissue. It is generally accepted that some tissues in the dermis have inherent characteristics that prevent the development of fibrous and scar tissue in wound healing, such as skin attachment tissue. We presume that prostate epithelial cells, which are similar to skin attachment tissue, may secrete certain substances that degrade excess extracellular matrix components and reduce the generation of collagen fibers, a theory that should be further investigated in detail. In addition, we observed the decreased TGF-$\beta_1$ and TGF-$\beta$ type II receptor expression in proliferating prostatic epithelial cells at 4 weeks when re-epithelialization was basically complete. TGF-$\beta_1$, at its optimal dose, is known to accelerate the re-epithelialization process. However, TGF-$\beta_1$ has been shown to be a potent inducer of granulation tissue formation and directly stimulate extracellular matrix synthesis (i.e. procollagen type I) through the mediation of TGF-$\beta$/Smads signaling pathway in cultured fibroblasts.[37,38] The decreased TGF-$\beta_1$ and TGF-$\beta$ type II receptor expression in proliferating prostatic epithelial cells at 4 weeks also results in diminish granulation tissue

and extracellular matrix synthesis after successful re-epithelialization.

In summary, we observed that the re-epithelialization of the prostatic urethra resulted from the migration and differentiation of proliferating prostate basal cells from residual prostatic epithelium under the wound after TmLRP in a canine prostate model. Compared with the regenerative process of the bladder neck, the regenerative process of the canine prostatic urethra is relatively fast and free of contractures, which may be the ideal wound healing method in prostatic urethra from anatomic repair to functional recovery after injury. Despite the fact that human prostate tissue differs from canine tissue as the former has a larger stromal component, the findings of this study may provide insight into the re-epithelialization process of the prostate following laser thermal injury. Studies aimed at identifying contributing factors to improve the epithelization process of the prostatic urethra might yield innovative approaches for the treatment of BPH.

## AUTHOR CONTRIBUTIONS
YC and PH carried out the histopathologic examination, immunohistochemistry staining, real-time PCR and analyzes. GHL, XSY, JXH and HS carried out the modeling of two-micron laser resection. LL carried out Van Gieson staining and performed the statistical analysis. ZLS and SJX conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

## COMPETING INTERESTS
All authors declare no competing interests.

## ACKNOWLEDGMENTS

## REFERENCES
1   Sulser T, Jochum W, Heitz PU, Hauri D. Histomorphological changes after neodymium: YAG laser-coagulation of the human prostate with the side focus fiber. Effect of power setting and time. *Urol Res* 1997; 25 Suppl 1: S7–12.
2   Jønler M, Bruskewitz RC. Prostatic histology in secondary transurethral resection of the prostate. *J Urol* 1995; 154: 119–22.
3   Madersbacher S, Alivizatos G, Nordling J, Sanz CR, Emberton M, *et al*. EAU 2004 guidelines on assessment, therapy and follow-up of men with lower urinary tract symptoms suggestive of benign prostatic obstruction (BPH guidelines). *Eur Urol* 2004; 46: 547–54.
4   Fried NM, Murray KE. High-power thulium fiber laser ablation of urinary tissues at 1.94 microm. *J Endourol* 2005; 19: 25–31.
5   Bach T, Wendt-Nordahl G, Michel MS, Herrmann TR, Gross AJ. Feasibility and efficacy of thulium:YAG laser enucleation (VapoEnucleation) of the prostate. *World J Urol* 2009; 27: 541–5.
6   Herrmann TR, Bach T, Imkamp F, Georgiou A, Burchardt M, *et al*. Thulium laser enucleation of the prostate (ThuLEP): transurethral anatomical prostatectomy with laser support. Introduction of a novel technique for the treatment of benign prostatic obstruction. *World J Urol* 2010; 28: 45–51.
7   Xia SJ, Zhang YN, Lu J, Sun XW, Zhang J, *et al*. [Thulium laser resection of prostate-tangerine technique in treatment of benign prostate hyperplasia]. *Zhonghua Yi Xue Za Zhi* 2005; 85: 3225–8. [Article in Chinese].
8   Xia SJ. Two-micron (thulium) laser resection of the prostate-tangerine technique: a new method for BPH treatment. *Asian J Androl* 2009; 11: 277–81.
9   Xu Y, Sun D, Wei Z, Hong B, Yang Y. Clinical study on the application of a 2-μm continuous wave laser in transurethral vaporesection of the prostate. *Exp Ther Med* 2013; 5: 1097–100.
10  Luo GH, Xia SJ, Sun ZL. *In vitro* comparison of the vaporesection of human benign prostatic hyperplasia using 70- and 120-W 2-μm lasers. *Asian J Androl* 2011; 13: 636–9.
11  Orihuela E, Motamedi M, Cammack T, Torres JH, Pow-Sang M, *et al*. Comparison of thermocoagulation effects of low power, slow heating versus high power, rapid heating Nd:YAG laser regimens in a canine prostate model. *J Urol* 1995; 153: 196–200.

12  Fried NM. New laser treatment approaches for benign prostatic hyperplasia. *Curr Urol Rep* 2007; 8: 47–52.

13  Fried NM. High-power laser vaporization of the canine prostate using a 110 W Thulium fiber laser at 1.91 microm. *Lasers Surg Med* 2005; 36: 52–6.

14  Cao Y, Xiao Y, Ravid R, Guan ZZ. Changed clathrin regulatory proteins in the brains of Alzheimer's disease patients and animal models. *J Alzheimers Dis* 2010; 22: 329–42.

15  SivamaniK RK, Garcia MS, Isseroff RR. Wound re-epithelialization: modulating keratinocyte migration in wound healing. *Front Biosci* 2007; 12: 2849–68.

16  Pow-Sang M, Orihuela E, Motamedi M, Torres J, Adesokan A, *et al*. Healing response of the canine prostate to Nd:YAG laser radiation. *Prostate* 1996; 28: 287–94.

17  Orihuela E, Pow-Sang M, Motamedi M, Cowan DF, Warren MM. Mechanism of healing of the human prostatic urethra following thermal injury. *Urology* 1996; 48: 600–8.

18  Luo GH, Luo L, Yang XS, Liu J, Sun ZL, *et al*. [New understanding of dog's prostatic urethral re-epithelial underwent two-micron laser prostatic vaporesection]. *Zhonghua Yi Xue Za Zhi* 2013; 93: 261–4. [Article in Chinese].

19  Cao Y, Luo GH, Luo L, Yang XS, Liu J, *et al*. Histopathologic changes of repair in trauma in canine prostatic urethra after two-micron laser resection of the prostate. *J Clin Exp Pathol* 2013; 29: 1084–8.

20  Costello AJ, Bolton DM, Ellis D, Crowe H. Histopathological changes in human prostatic adenoma following neodymium:YAG laser ablation therapy. *J Urol* 1994; 152: 1526–9.

21  Thorson P, Swanson PE, Vollmer RT, Humphrey PA. Basal cell hyperplasia in the peripheral zone of the prostate. *Mod Pathol* 2003; 16: 598–606.

22  Kwon OJ, Zhang L, Ittmann MM, Xin L. Prostatic inflammation enhances basal-to-luminal differentiation and accelerates initiation of prostate cancer with a basal cell origin. *Proc Natl Acad Sci U S A* 2014; 111: E592–600.

23  Goldstein AS, Huang J, Guo C, Garraway IP, Witte ON. Identification of a cell of origin for human prostate cancer. *Science* 2010; 329: 568–71.

24  Barclay WW, Axanova LS, Chen W, Romero L, Maund SL, *et al*. Characterization of adult prostatic progenitor/stem cells exhibiting self-renewal and multilineage differentiation. *Stem Cells* 2008; 26: 600–10.

25  Goldstein AS, Lawson DA, Cheng D, Sun W, Garraway IP, *et al*. Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics. *Proc Natl Acad Sci U S A* 2008; 105: 20882–7.

26  Leong KG, Wang BE, Johnson L, Gao WQ. Generation of a prostate from a single adult stem cell. *Nature* 2008; 456: 804–8.

27  Mustoe TA, Pierce GF, Thomason A, Gramates P, Sporn MB, *et al*. Accelerated healing of incisional wounds in rats induced by transforming growth factor-beta. *Science* 1987; 237: 1333–6.

28  Finnson KW, McLean S, Di Guglielmo GM, Philip A. Dynamics of transforming growth factor beta signaling in wound healing and scarring. *Adv Wound Care* (*New Rochelle*) 2013; 2: 195–214.

29  Lynch SE, Colvin RB, Antoniades HN. Growth factors in wound healing. Single and synergistic effects on partial thickness porcine skin wounds. *J Clin Invest* 1989; 84: 640–6.

30  Mustoe TA, Pierce GF, Morishima C, Deuel TF. Growth factor-induced acceleration of tissue repair through direct and inductive activities in a rabbit dermal ulcer model. *J Clin Invest* 1991; 87: 694–703.

31  Kane CJ, Hebda PA, Mansbridge JN, Hanawalt PC. Direct evidence for spatial and temporal regulation of transforming growth factor beta 1 expression during cutaneous wound healing. *J Cell Physiol* 1991; 148: 157–73.

32  Levine JH, Moses HL, Gold LI, Nanney LB. Spatial and temporal patterns of immunoreactive transforming growth factor beta 1, beta 2, and beta 3 during excisional wound repair. *Am J Pathol* 1993; 143: 368–80.

33  Wettlaufer JN, Kronmiller P. The management of post-prostatectomy vesical neck contracture. *J Urol* 1976; 116: 482–3.

34  Bruskewitz RC, Larsen EH, Madsen PO, Dørflinger T. 3-year followup of urinary symptoms after transurethral resection of the prostate. *J Urol* 1986; 136: 613–5.

35  Edwards LE, Bucknall TE, Pittam MR, Richardson DR, Stanek J. Transurethral resection of the prostate and bladder neck incision: a review of 700 cases. *Br J Urol* 1985; 57: 168–71.

36  Lee YH, Chiu AW, Huang JK. Comprehensive study of bladder neck contracture after transurethral resection of prostate. *Urology* 2005; 65: 498–503.

37  Van Obberghen-Schilling E, Roche NS, Flanders KC, Sporn MB, Roberts AB. Transforming growth factor beta 1 positively regulates its own expression in normal and transformed cells. *J Biol Chem* 1988; 263: 7741–6.

38  Keski-Oja J, Raghow R, Sawdey M, Loskutoff DJ, Postlethwaite AE, *et al*. Regulation of mRNAs for type-1 plasminogen activator inhibitor, fibronectin, and type I procollagen by transforming growth factor-beta. Divergent responses in lung fibroblasts and carcinoma cells. *J Biol Chem* 1988; 263: 3111–5.