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ORIGINAL ARTICLE

Male Health

# Tubularized urethral reconstruction using a prevascularized capsular tissue prelaminated with buccal mucosa graft in a rabbit model

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Tubularized graft urethroplasty fails largely because of inadequate graft take. Prefabrication of buccal mucosa lined flap has theoretical indications for constructing neourethra with an independent blood supply. The efficacy of using a tissue expander capsule as an induced vascular bed to prefabricate an axial vascularized buccal mucosa-lined flap for tubularized urethral reconstruction in a rabbit model was tested. The experiments were performed in three stages. First, silicone tissue expanders were inserted into the groin to induce vascularized capsule pouch formation. Next, buccal mucosa grafts were transplanted to the newly formed capsular tissue supplied by the axial vessel for buccal mucosa-lined flap prefabrication. Then, circumferential urethral defects were created and repaired by buccal mucosa graft (Group 1), capsule flap (Group 2) and prefabricated capsule buccal mucosa composite flap (Group 3). With retrograde urethrography, no rabbits in Group 1 maintained a wide urethral caliber. In Group 2, the discontinued epithelial layer regenerated at 1 month, and the constructed neourethra narrowed even though the lumen surface formed intact urothelial cells at 3 months. In Group 3, buccal mucosa formed the lining in the neourethra and kept a wide urethral caliber for 3 months. The capsule may serve as an induced vascular bed for buccal mucosa-lined flap prefabrication. The prefabricated buccal mucosa-lined flap may serve as a neourethra flap for circumferential urethral replacement.

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## INTRODUCTION

Complex urethral problems, especially for patients with long obliterative strictures or urethral defects where part of the existing urethral plate is unsalvageable, represent a particular reconstructive challenge in urology.<sup>1–3</sup> For these patients, standard onlay or inlay techniques are not always possible, as these techniques require the graft or flap to be anastomosed to the healthy edges of an existing urethral plate.<sup>4–6</sup> Previously tubularized tissue grafts have been tried for 1-stage tube urethroplasty,<sup>7</sup> but free grafts require a bed for vascularization, and appropriate tissue is often lacking because of extensive fibrosis in these patients. From the perspective of clinicians, it is desirable to construct a vascularized tissue flap with an axial vascular pedicle for circumferential urethral replacement.

In plastic surgery, the flap prefabrication technique provides an effective measure for vascularized tissue construction, and the feasibility of using an axial vascular bundle as a vascular carrier for oral mucosa lined flap prefabrication has been investigated.<sup>8–10</sup> Since oral mucosa is the ideal lining for the urethra, the constructed axial oral mucosa-lined flap may be theoretically regarded as an ideal neourethral

flap for circumferential urethral replacement. However, these studies relied solely on revascularization of preexisting tissues rather than induction of newly vascularized tissues, and a prefabricated oral mucosa-lined flap has never been reported in urethral reconstruction.

Previous studies have clarified that the axial-type capsular flap induced by a tissue expander or silicone block has adequate vascularity and that the induced vascularized capsule can be used as a robust vascular bed for composite flap prefabrication.<sup>11–15</sup> Encouraged by successful results with autologous cells transplanted onto the prefabricated capsule pouch and skin grafting on an induced capsular tissue,<sup>11–15</sup> we began to observe morphological and histological variation in buccal mucosa grafts (BMGs) after transplantation into the capsule pouch to determine whether the axial vascularized capsule can be used as an induced vascular bed for buccal mucosa-lined flap prefabrication for tubularized urethral reconstruction.

## MATERIALS AND METHODS

### Animals

For the three stages of the experiment, we used New Zealand white male rabbits (Stage I:  $n = 2$ , Stage II:  $n = 4$ , Stage III:  $n = 24$ ) weighing

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between 2.5 kg and 3.0 kg and housed at the animal facilities at Shanghai Jiao Tong University (SJTU) School of Medicine (Shanghai, China). All rabbits were given general anesthesia with 20–30 mg kg<sup>-1</sup> of intravenous injection of sodium pentobarbital, and all surgical procedures were performed under sterile conditions. The whole animal experimental protocol was approved by the SJTU School of Medicine Animal Care and Use Committee.

### Stage 1: vascularized capsule induction

Following general anesthesia induction, two 15 ml spherical, custom-made silicone tissue expanders were placed superficial to the superficial circumflex iliac (SCI) vessels (branches of the femoral artery and vein) underneath the bilateral inguinal skin (**Figure 1a**). After wound closure, the expanders were filled with 3 ml of saline intraoperatively and on days 10, 13, 16 and 19 postoperatively to achieve the final volume of 15 ml (**Figure 1b**). One week after full inflation, the animals were reanesthetized, and the tissue expanders were removed (**Figure 1c** and **1d**). To examine blood perfusion into the capsular tissue via SCI vessels, the femoral artery was cannulated (24-gauge catheter) and subsequently flushed with warm heparin solution (100 IU ml<sup>-1</sup>), followed by injection of 20 ml heparinized Indian ink solution. The capsular tissue containing axial vessels was transected and fixed for histologic examination.

### Stage 2: buccal mucosa lined flap prefabrication

After removal of the tissue expander, normal saline (3 ml) was infiltrated into the inferior surface of the cheek. Subsequently, the BMG with intact basement membrane and partial submucosal tissue of 1.5 cm × 0.8 cm was peeled from the cheek (**Figure 1e**). The harvested BMG was then placed in front of the SCI artery and vein and stretched and sutured on the capsule vascularized by the SCI artery and vein (**Figure 1f**). To help mucosa attach to the capsule and facilitate mucosal

spreading, the water-filled tissue expander was placed back into the capsule pouch. Finally, skin incisions were closed with 5-0 nylon interrupted sutures. One month after prelamination, the mucosal grafts were scrutinized macroscopically, and the surface area of the mucosa was traced without any tension. It was translated into square millimeters and compared with the original size of the mucosal graft. Indian ink was used to examine blood perfusion in the prefabricated capsule buccal mucosa composite flap.

### Stage 3: transposition of mucosal prelaminated flap for tubularized urethral reconstruction

In this stage, 24 rabbits were divided randomly into 3 groups of 8 each. In Group 1, 1.5 cm × 0.8 cm BMG was used for tubularized urethroplasty. In Group 2, a capsule induction technique was used for tubularized urethroplasty with the capsule flap. In Group 3, a 1.5 cm × 0.8 cm BMG was transplanted into the capsule pouch, and urethral defects were repaired with the prefabricated capsule buccal mucosa composite flap.

Urethral defects were created according to the previously described methods.<sup>16,17</sup> Briefly, the penis was freed by incising the fold between the ventral penile portion and the anus, facilitating access to the urethra. After exposing the urethra, the penile portion of it was completely separated from the corpus cavernosum, exposing the tunica albuginea (**Figure 1i**). The urethra, approximately 2.0 cm from the external urethral orifice, was entirely sectioned transversally, maintaining a distance of 1.5 cm. In Group 1, tubularized penile urethral replacement was performed with the tubularized BMG according to the previously described methods.<sup>17</sup> In Group 2 and Group 3 animals, a subcutaneous tunnel connecting the groin and the ventral penile region was created surgically. Subsequently, the capsule flap and the prefabricated capsule buccal mucosa composite flap (1 month after prelamination) were raised, and the pedicled flaps were subcutaneously tunneled without tension toward the ventral penile region (**Figure 1j**). The flaps were trimmed and tubularized over an 8F urethral sound to construct a neourethra (**Figure 1k**). The constructed neourethra was then aligned and anastomosed with the two urethral ends (**Figure 1l** and **1m**). Urethral repair was performed with 6-0 PDS sutures, and the corpora and skin were closed in layers. A urethral catheter was left in place and secured with sutures to provide bladder drainage for 14 days. Prophylactic penicillin G (2 g day<sup>-1</sup>) was given intravenously for 5 days after the surgery.

### Postoperative evaluation

Four animals in each group were humanely euthanized at 1 month and 3 months postimplantation after the urethral caliber was assessed with retrograde urethrograms. The rabbit penis was then excised at its base, and the entire penis in each group was subsequently sent for histological study with hematoxylin and eosin (H&E), Masson trichrome and immunohistochemical staining.

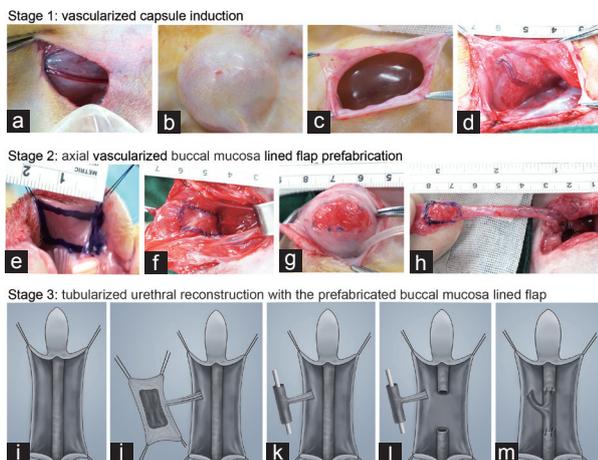
### Statistical analysis

All data were presented as the means ± standard deviation (s.d.). The surface area of the mucosa before and after grafting was compared using a paired *t*-test, and one sample *t*-tests were used to compare urethral patency after surgery. *P* < 0.05 was considered significant.

## RESULTS

### Characteristics of the capsule and the BMG

Visual inspection revealed that a highly vascularized capsule in the shape of a hollow viscus with a smooth surface was induced by the



**Figure 1:** Animal experiment. (a) tissue expander was placed close to the separated SCI vessels; (b) groin exploration 1 week after the expander was full inflated with saline solution; (c) the induced capsule pouch was opened by forceps; (d) gross appearance of the induced vascularized capsule with axial vessels; (e) harvesting BMG; (f) transplanting BMG to the capsule pouch; (g) BMG showed a complete survival on the vascularized capsule; (h) BM lined flap pedicled on SCI vessels was raised and ready for urethral reconstruction; (i) the penile urethra was separated from the corpus cavernosum; (j) BM lined flap was subcutaneously tunneled toward the ventral penile region; (k) BM lined flap was tubularized over an urethral sound to construct a neourethra; (l) a penile urethra defect was created; (m) urethral defects were repaired with the constructed neourethra flap. BMG: buccal mucosa graft; SCI: superficial circumflex iliac vessels.

tissue expansion process. The SCI vessels were located in the center of the capsule and remained pulsatile (**Figure 1d**). Indian ink injection studies showed many new blood vessels originating from the axial vessels and extending to the periphery of the capsule. Pathologic studies showed evidence of neovascularization, and abundantly impregnated vascular structures near the SCI vessels were observed with parallelly developed collagen fibers (**Figure 2a**). The vascular network within the capsule was filled with black ink.

The harvested BMG has an ivory-white appearance with a smooth surface at the time of transplantation. Histological examination confirmed that the graft contained intact oral mucosa epithelia (typical stratified squamous epithelia) and partial submucosal tissue (**Figure 2b**). A high capillary density was observed at the submucosal layer. The BMG attached to the vascularized capsule well (**Figure 1f**).

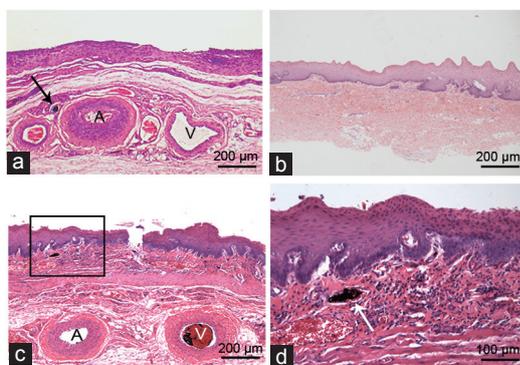
### Gross view and histopathologic evaluation of the prefabricated composite flap

All rabbits survived until their scheduled time points. The oral donor site completely healed at 1 month. After 1 month of incubation, capillary vessel extensions into the overlying mucosa were evident, and fibrous tissue in the capsule without buccal mucosa lining became denser, which easily distinguished the mucosa from the adjacent native capsular tissue with remarkable boundaries (**Figure 1g**). The axial vascular bundle remained pulsatile, and the prefabricated capsule buccal mucosa composite flap could be raised from the abdominal wall (**Figure 1h**). H&E and Masson's trichrome staining revealed that the mucosa still showed the characteristic features of oral mucosa with papillae and stratified squamous epithelial structure. Indian ink filled vessels, and the axial vascular bundle was evident in the submucosa layers of the flap (**Figure 2c** and **2d**).

As shown by immunohistochemistry, the original free BMG expressed AE1/AE3 (the specific marker for epithelial cells), CK 14 (the specific marker of oral mucosa primarily localized to the basal cells), P63 (a marker for stem and progenitor cells) and Ki67 (a marker for proliferating cells). After 1 month of incubation, consistent with the results of the gross view and histology, the mucosa retained the phenotype of native oral mucosa with positive expression of AE1/AE3, CK 14, P63 and Ki67 (**Figure 3**).

### Analysis of urethroplasty

The capsule and the prefabricated capsule buccal mucosa composite flap were long enough for penile urethral reconstruction (**Figure 4**). At 1 month and 3 months postoperatively, retrograde contrast urethrography was performed to analyze the urethroplasty and to measure the caliber



**Figure 2:** Representative histology of the capsule (a), buccal mucosa graft (b), and (c) the prefabricated capsule buccal mucosa composite flap; (d) Indian ink perfused vessels were observed in the submucosa layer of the composite flap. A: artery; V: vein; Arrows: India ink perfused vessels.

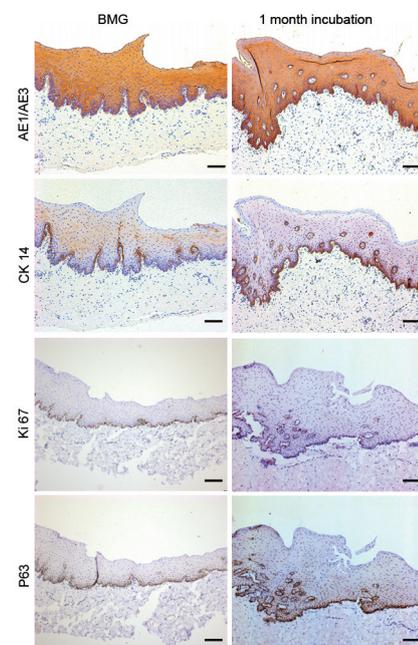
of the neourethra. Of all 24 investigated rabbits, 9 had a wide urethral caliber, including 3 in Group 2 and 6 in Group 3. In the remaining 15 rabbits, partial narrowing of the urethra was observed 10 times and was associated with a fistula 6 times. Isolated fistulae were observed 4 times, and 1 rabbit showed complete stenosis. The rabbits in Group 3 had the fewest complications, and none of the rabbits in Group 1 had a normal urethrogram (**Figure 5a** and **5b**).

The neourethral patency was evaluated 1 month and 3 months after surgery. The narrowest lumen calibers of the neourethra were measured on urethrograms and were analyzed as a percentage of the urethra caliber of control rabbits not submitted to any surgery (100%,  $n = 4$ ). Comparing the neourethra caliber to a control urethra, the neourethral patency in Group 1 evaluated 1 month ( $33.75\% \pm 33.51\%$ ) and 3 months ( $23.75\% \pm 30.92\%$ ) after surgery was significantly different from the theoretical mean of 100% ( $P < 0.05$ ). Regarding the capsule flap, a wide urethral caliber was found at 1 month ( $81.25\% \pm 19.31\%$ ). However, the lumen caliber was narrowed ( $57.50\% \pm 23.27\%$ ) at the 3-month evaluation ( $P < 0.05$ ). In contrast, the prefabricated capsule buccal mucosa composite flap kept a wide urethral caliber compared with the normal urethra (**Figure 5c**).

### Histopathologic evaluation of the neourethra

Considering the sparse quality images of rabbit urethra, we first evaluated the normal structure of the rabbit urethra. In control rabbits, the native urethral lumen was star-shaped and lined by typical urothelial cells, under which a thin lamina propria was present and surrounded by longitudinal smooth muscle bundles. Around the longitudinal smooth muscle bundles, several lacunar vascular structures were visualized in the encompassing connective tissue layer (**Figure 6**).

In cross-sections, a centered and star-shaped lumen was distinguishable. In Group 1 animals, portions of the tubularized BMG survived within the urethral lumen at 1 month. Occasionally, in samples harvested after 3 months, the buccal mucosa lining was absent, and the urethral lumen was narrowed and was composed of



**Figure 3:** Immunohistochemical staining revealed the original buccal mucosa and the new mucosa in the capsule retained positive expression of AE1/AE3, CK 14, P63 and Ki67. Scale bars = 100 μm.



**Figure 4:** Three months after urethroplasty, the axial vessel remained pulsatile and the urethral defect was nicely reconstructed by the prefabricated capsule buccal mucosa composite flap.

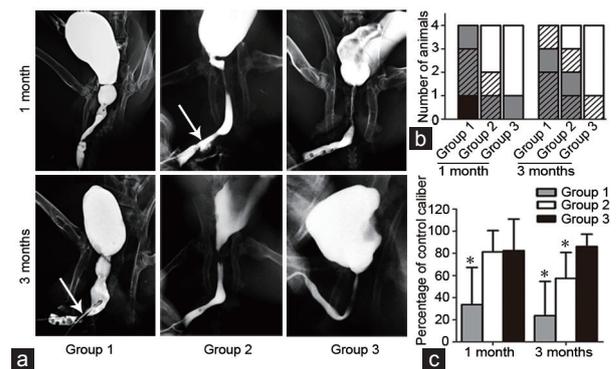
only minimal urothelial cells. In Group 2 animals, a discontinued epidermal layer developed on the luminal surface of the neourethra 1 month after surgery, and the cellular layer continued to increase over time. At 3 months, the luminal surface formed an intact and multilayer urothelium. However, the urethral caliber narrowed and the smooth muscle content decreased at this time. Masson's trichrome staining confirmed that progressive infiltration of fibrosis was observed in Group 2 animals but not in Group 3 animals. In Group 3 animals, a wide urethral caliber was maintained, and the neourethra was covered by a typical squamous epithelium (**Figure 6**). The typical papillae morphology of the buccal mucosa was retained in the neourethra, and the basilar layers of the neourethra were positive for P63 and Ki67. Furthermore, neovascularization was evident, and the smooth muscle content beneath the new buccal mucosa area continued to increase over time in the neourethra.

## DISCUSSION

In the past decade, BMG has been considered an ideal graft material for substitution urethroplasty. However, it could only be used as a patch to replace approximately half the strictured circumferential urethral wall, and the full circumferential urethral replacements often fail because of inadequate graft take, as the free graft is not circumferentially surrounded by vascularized tissue.<sup>18–20</sup> The present study demonstrates that BMG can survive and grow in the prefabricated capsule pouch and that the prefabricated capsule buccal mucosa composite flap can be used for tubularized urethral reconstruction. Theoretically, the pedicled buccal mucosa-lined flap is superior to the penile and preputial skin flaps because buccal mucosa lining offers the advantages of being accustomed to a wet environment, hairless, resilient to infection and the skin diseases such as lichen sclerosus. Furthermore, the axial vessels provide adequate and independent blood supply for the constructed neourethra, and therefore, the viability of the neourethra is more secure.

### *Advantages of the capsule induction technique for buccal mucosa-lined flap prefabrication*

The capsule induction technique provides several essential advantages for buccal mucosa-lined flap prefabrication. The prefabricated capsule pouch has adequate vascularity and provides an aseptic, moist, protected environment for the grafted tissue. Furthermore, the mucosa has a dense submucosa with a dense capillary network

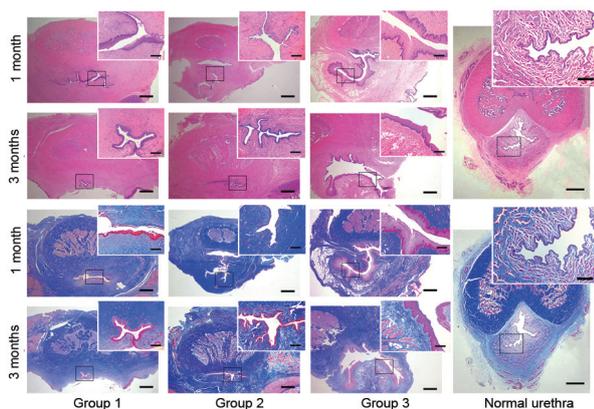


**Figure 5:** Rabbit urethrogram findings at evaluation. (a) Comparison of urethrograms in each group 1 month and 3 months after urethroplasty. (b) Complications revealed by urethrogram: complete stenosis (black), urethral narrowing (<66.7% control; gray), fistula (hatching), no complication (white). (c) The neourethral patency at evaluation. Narrowest lumen calibers of the neourethra were measured on urethrograms at 1 month and 3 months and were analyzed as percentage of the urethra caliber of control rabbits not submitted to any surgery ( $n = 4$ ). Statistical significance (one sample  $t$ -test towards the theoretical mean of 100%) is shown as  $*P < 0.05$ . Arrow: the fistula site of the urethra.

combined with the highly vascularized capsule flap constitute ideal prerequisites for mucosa lined flap prefabrication. Using this method, revascularization with definitive incorporation occurs within a week of implantation, a period known from skin graft transplantations to be enough for graft take.<sup>11</sup> The axial pattern vessel was expected to play a modifying role for inosculation of microvascular networks within the BMG and the capsule owing to the impregnated vascular structures. Additionally, the functional support of the BMG may lead to sustained maintenance of the underlying vascularized tissue and the prefabricated capsule buccal mucosa composite flap is stable enough to be used as a free flap.

### *Vascularization and epithelial covering are vital for urethral reconstruction*

Historically, pedicled flaps and free tissue grafts have been used for substitution urethroplasty. However, the majority of cases using tubularized BMG for penile stricture disease have had poor outcomes, and the most common complications were graft shrinkage and stricture formation.<sup>20</sup> The poor results in Group 1 animals further confirmed this finding. In our experience, it is hard for the tubed graft to achieve early inosculation of the neovasculature with the surrounding penile tissues, and reconstruction without sufficient revascularization during the initial period may lead to graft shrinkage and even graft failure. Consequently, there is a higher rate of stricture and fistulae in Group 1 animals. In Group 2 animals, the pedicled capsule flap has its own axial vessels and discontinued epithelial cells formed on the surface of the vascularized neourethra at 1 month. Without intact urothelial lining, the unprotected capsular tissue is in direct contact with urine, which promotes destructive events, including excessive scarring, wound breakdown, stricture and fistula formation. In Group 3, prefabrication of a buccal mucosa-lined flap as the neourethra has the advantage of using axial vessels to provide independent vascular supply to resist contraction while maintaining a stable buccal mucosa lining as a successful urine barrier. Thus, infiltration of smooth muscle cells to the subepithelial layer after several weeks was viewed, and the importance of constructing a pedicled neourethra flap with epithelium lining was verified.



**Figure 6:** Overviews of cross-sections of rabbit penises. In Group 1, part of the tubularized buccal mucosa graft survived in the neourethra at 1 month. At 3 months, there was no evidence of buccal mucosa lining in the neourethra, and the urethral lumen became worse and was composed of only urothelium cells. In Group 2, a discontinued epidermal layer developed on the lumen surface of the neourethra at 1 month, and the cellular layer continued to increase over time. At 3 months, the constructed neourethra narrowed even though the lumen surface formed intact and multilayer urothelium. In Group 3, the neourethra lumen was lined with typical squamous epithelial layers, and the constructed neourethra kept a wide urethral caliber for 3 months. Scale bars on the gross images and in the expanded area were 1.0 mm and 200  $\mu$ m, respectively.

#### **Potential application of the flap prefabrication technique for urethral tissue engineering**

Reconstructive surgery using cell-seeded scaffolds has been studied as an alternative approach for urethral tissue engineering.<sup>21–23</sup> However, all of these cell-seeded scaffolds were used as a free graft for urethral reconstruction. Due to the lack of independent vascularization, the engineered tubularized constructs tend to stricture over time, and clinical studies have proved tubularized urethral reconstruction with free grafts have a high proportion of failure (sometimes over 50%).<sup>18,22,24</sup> Previously, classic vascularization strategies have focused on the stimulation of vascular ingrowth into tissue constructs, which can be achieved by incorporation of growth factors or endothelial cells.<sup>25–27</sup> However, these so-called angiogenic approaches face the problem that the average growth rate of newly developing microvessels is only approximately  $5 \mu\text{m h}^{-1}$ .<sup>28,29</sup> Thus, the complete vascularization of large implants after transplantation needs a prolonged time period that is associated with major tissue loss due to hypoxic conditions. In plastic surgery, the flap prefabrication technique allows free tissue grafts to be transferred as pedicled flaps and has the major advantage that the tissue construct is directly reperfused after implantation at its final destination.<sup>29,30</sup> The success of the prefabricated buccal mucosa-lined flap for tubularized urethral reconstruction further confirmed that the flap prefabrication technique may have potential application for urethral tissue engineering.

#### **Potential clinical transition for complicated urethral strictures**

The procedure used in this study is technically feasible and has an attractive theoretical transition to clinical usage. Applications may include placement of the tissue expander in the vicinity of large vessels (for example, the descending branch of the lateral circumflex femoral vessel) with gradual filling with time.<sup>31,32</sup> After the axial pattern of vascularized capsular tissue formation, BMG was harvested and transplanted into the capsule for maturation. The expander may provide adequate pressure for the BMG to survive and grow in the prefabricated capsule pouch. After maturation of the composite flap, we hypothesized

that tubularized urethral reconstruction can be performed via a technique similar to what we describe through a subcutaneous tunnel to the urethra. This concept may have potential for reconstruction of complicated urethral strictures, especially for those patients with complex posterior urethral disruptions who have undergone failed previous surgical treatments. For these patients, posterior urethroplasty is more difficult because the new defects may be longer and the surgical solutions are limited by the poor tissue conditions compromised by wide scars. In these situations, the corporeal body separation, inferior pubectomy, and urethral rerouting are not enough to guarantee a new tension-free, end-to-end anastomosis<sup>2</sup> and free tissue grafts do not take on scarred tissues. In these situations, it is ideal to use a vascularized tissue flap with an axial vascular pedicle for circumferential urethral replacement. Our study may provide a new referable method for complex posterior urethral disruptions, and we will evaluate the possibility of the prefabricated buccal mucosa-lined flap for circumferential posterior urethral replacement in our next series of studies.

#### **Factors that may limit its clinical transition**

It must be stressed that the urethral defect models were established in a normal healthy urethral model in this study. This model cannot fully simulate the exact clinical situation of the urethral stricture, which is characterized by a fibrotic urethra bed. Furthermore, long segments of buccal mucosa are needed for replacement of long circumferential urethral segments. Harvesting a longer BMG in a dog or a porcine model may be more convincing. Additionally, the penile urethral defects model is not the ideal model for the evaluation of the prefabricated buccal mucosa lined flap for tubularized urethral reconstruction because the majority of penile urethral strictures are not faced with circumferential urethral defects, and the vast majority of patients can be treated with onlays or inlays as well as two-staged urethroplasties. In contrast, most posterior urethral disease is caused by pelvic fracture urethral injuries and are faced with circumferential urethral defects. Surgeries for patients with long-segment posterior urethral disruptions are limited, and two-stage BMG substitution urethroplasty is not feasible in these cases. The present study may provide a referable method for these patients, and additional studies will be needed to determine if posterior urethral defects can be repaired in a similar manner.

#### **CONCLUSIONS**

The vascularized capsule provides adequate nutrition for the BMG to survive and grow and can be used as a vascular bed for buccal mucosa lined flap prefabrication. The prefabricated capsule buccal mucosa composite flap has independent vascularity and is effective for tubularized urethral reconstruction. The technique of prefabricating a vascularized matrix lined with buccal mucosa may provide a method for future reconstruction of genitourinary systems.

#### **AUTHOR CONTRIBUTIONS**

HLG carried out the project and drafted the manuscript. ZMJ participated in project design and coordination and helped to draft the manuscript. LW, XQB and YCH participated in project design and coordination. JMZ, HX and XJY helped to collect the data and performed the statistical analysis. FC conceived of the study and supervised the project. All authors read and approved the final manuscript.

#### **COMPETING INTERESTS**

All authors declare no competing interests.



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