Asian Journal of Andrology (2020) 22, 459–464
www.asiaandro.com; www.ajandrology.com

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ORIGINAL ARTICLE

Male Health

Tissue expander capsule as an induced vascular bed to prefabricate an axial vascularized buccal mucosa-lined flap for tubularized posterior urethral reconstruction: preliminary results in an animal model

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Surgical repair of complex posterior urethral disruptions remains one of the most challenging problems in urology. The efficacy of using a tissue expander capsule as an induced vascular bed to prefabricate axial vascularized buccal mucosa-lined flaps for tubularized posterior 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 into the newly formed capsular tissue supplied by axial vessels for buccal mucosa-lined flap prefabrication. Then, circumferential posterior urethral defects were created and repaired with the buccal mucosa graft (Group 1), the capsule flap (Group 2), and the prefabricated capsule buccal mucosa composite flap (Group 3). After surgery, notable contracture of the tubularized buccal mucosa graft was observed in the neourethra, and none of the rabbits in Group 1 maintained a wide urethral caliber. In Group 2, the retrieved neourethra showed little evidence of epithelial lining during the study period, and the lumen caliber was narrowed at the 3-month evaluation. In Group 3, the buccal mucosa formed the lining in the neourethra and maintained 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 posterior urethral replacement.

Asian Journal of Andrology (2020) **22**, 459–464; doi: 10.4103/aja.aja_133_19; published online: 10 January 2020

Keywords: buccal mucosa; flap; prefabrication; urethra; vascularization

INTRODUCTION

In contrast to anterior urethral strictures, most posterior urethral disruptions are associated with pelvic fractures and result in circumferential urethral defects.[1,2] For patients with long complex posterior urethral disruptions, the surgical solutions are often compounded by the deep location, surrounding fibrosis and limited urethral length.[1,2] In these situations, traditional surgical techniques are not sufficient to guarantee tension-free anastomosis, and substitution urethroplasty with free tissue grafts is not feasible.[2–4] From the perspective of reconstructive surgeons, it is desirable to use vascularized tissue flaps with their own vascular supply to replace the urethral defects to help shorten the urethral gap between the urethral stumps.[4]

In our previous study, we proved that the capsule may serve as an induced vascular bed for buccal mucosa (BM)-lined flap prefabrication and that the prefabricated BM-lined tube can be used for tubularized penile urethral replacement.[5] To determine whether the prefabricated BM-lined flap can be used for posterior urethral replacement, we created a posterior urethral defect model in rabbits and transplanted the prefabricated BM-lined flap near the pelvic floor for tubularized posterior urethroplasty. To our knowledge, this is the first study of reconstructing the entire segment of devastated urethra in such a manner and may serve as a preliminary research for the evaluation of a tubular BM-lined flap for posterior urethral defect urethroplasty.

MATERIALS AND METHODS

Animals

Thirty New Zealand white male rabbits were used for the experiments, and the experiments were performed in three stages (Stage I: $n = 2$, Stage II: $n = 4$, and Stage III: $n = 24$). In Stage I, skin tissue expanders (volume: 15 ml, Shanghai Winner Plastic Surgery Products Co.,

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Received: 24 July 2019; Accepted: 09 September 2019

Ltd., Shanghai, China) were used to induce vascularized capsule formation. In Stage II, a buccal mucosa graft (BMG) was transplanted into the vascularized capsule for BM-lined flap prefabrication. In Stage III, posterior urethral defects were created and repaired by the prefabricated BM-lined flap. The animal protocol was approved by the Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine, Shanghai, China. All rabbits were anesthetized with an intravenous injection of 20–30 mg kg^{-1} sodium pentobarbital (P3761, Sigma-Aldrich, St. Louis, MO, USA), and all surgical procedures were performed under sterile conditions.

Stage I: vascularized capsule induction

Following general anesthesia induction, skin incisions were made in the bilateral inguinal region, and the superficial circumflex iliac (SCI) vessels (branches of the femoral artery and vein) were separated and isolated from the surrounding inguinal fat. A spherical custom-made silicone tissue expander (15 ml) was placed superficial to the SCI vessels underneath the inguinal skin (**Figure 1a**). After wound closure, the expander was filled with 3 ml of saline intraoperatively and was repeatedly inflated on days 10, 13, 16, and 19 postoperatively to achieve a 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 verify the blood perfusion into the capsular tissue via the axial artery, the homolateral femoral artery was cannulated (24-gauge catheter) and subsequently flushed with 100 IU ml^{-1} heparin solution (12 500 IU, Chengdu Hepatunn Pharmaceutical Co., Ltd., Chengdu, China), followed by an injection of 20 ml heparinized Indian ink solution (100 ml, Beijing Leagene Biotech Co., Ltd., Beijing, China). The capsular tissue containing axial vessels was then resected and fixed for histologic analyses (n = 2).

Stage II: BM-lined flap prefabrication

After the 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 was stretched and sutured on the capsule vascularized by the SCI artery and vein (**Figure 1f**). To help mucosa attach to the capsule and to facilitate mucosal spreading, the water-filled tissue expander was placed back into the capsule pouch. Finally, the 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. The surface area was translated into square millimeters and compared with the original size of the mucosal graft (n = 4).

Stage III: tubularized posterior urethral reconstruction with the prefabricated BM-lined flap

In this stage, 24 rabbits were divided randomly into three groups (n=8 for each). In Group 1, a BMG of 1.5 cm × 0.8 cm in size

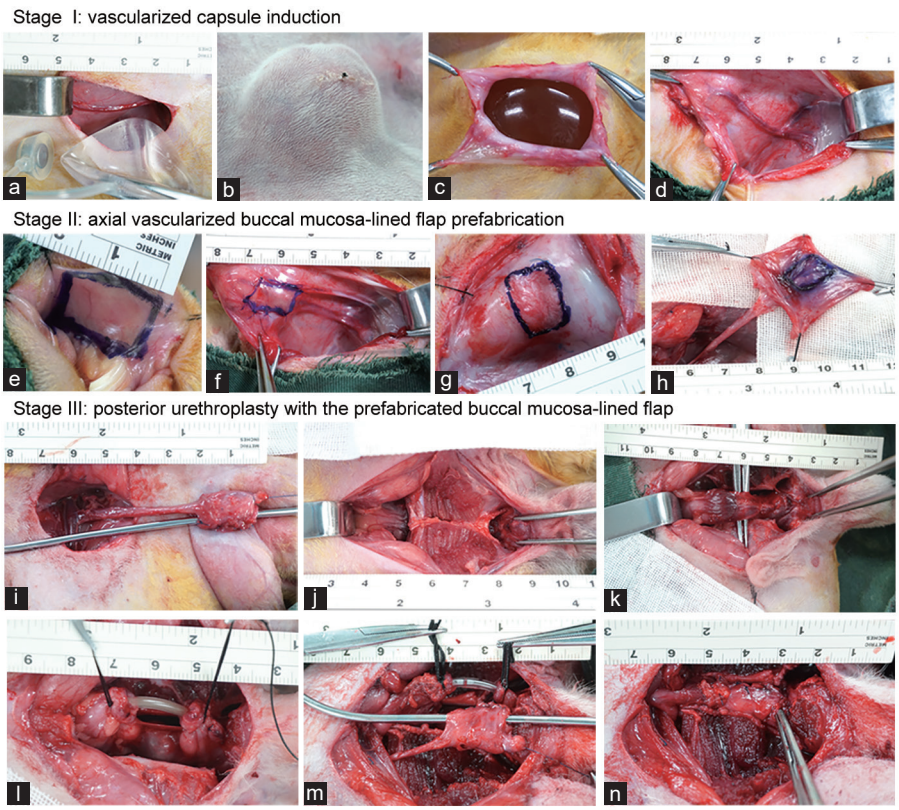

Figure 1: Animal experiment. (**a**) Tissue expander was placed close to the separated SCI vessels; (**b**) groin exploration 1 week after the expander was full inflated with saline solution; (**c**) the induced capsule pouch was opened by forceps; (**d**) gross appearance of the induced vascularized 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 was raised with axial vessels; (**i**) BM-lined flap was tubularized over an urethral sound to construct a neourethra; (**j**) the symphysis pubis was exposed; (**k**) the symphysis pubis was cut apart and the posterior urethra was separated; (**l**) a posterior urethra defect was created; (**m**) the constructed neourethra flap was tunneled and ready for posterior urethral replacement; (**n**) posterior urethral defects were repaired with the constructed neourethra flap. BM: buccal mucosa; BMG: buccal mucosa graft; SCI: superficial circumflex iliac vessels.

was used for tubularized posterior urethroplasty. In Group 2, a capsule induction technique was used for tubularized posterior urethroplasty with the capsule flap. In Group 3, a BMG of 1.5 cm × 0.8 cm in size was transplanted into the capsule pouch, and posterior urethral defects were repaired with the prefabricated capsule BM composite flap.

To obtain adequate exposure of the posterior urethra, a transpubic approach was utilized for posterior urethroplasty. Briefly, a 6-cm midline incision across the symphysis was made, and the attachments of the rectus abdominis muscle and the corpus cavernosum were cleared off of the outer surface of the pubis using a periosteal elevator approximately 0.8 cm from each side of the symphysis pubis (**Figure 1j**). Subsequently, the symphysis pubis was cut apart with scissors and was forced apart with the hands, maintaining a distance of 1.5 cm. Then, the urethra behind the symphysis pubis was separated, and a 1.5-cm long sample of tubular urethral tissue was completely removed (**Figure 1k** and **1l**). In Group 1, tubularized substitution urethroplasty was performed with the tubularized BMG. In Group 2 and Group 3 animals, the capsule flap and the prefabricated capsule BM composite flap (1 month after prelamination) were raised (**Figure 1h**), and a tunnel connecting the groin and the pelvis was created. Subsequently, the flaps were trimmed and tubularized over an 8F urethral sound to construct a neourethra (**Figure 1i**). Then, the neourethra flap was tunneled without tension toward the posterior urethral defect region and was aligned and anastomosed with the two urethral ends (**Figure 1m**, **1n** and **2**). Urethral repair was performed with 6-0 PDS sutures (ETHDZ432H, Johnson and Johnson Medical Devices Ltd., Shanghai, China), and the symphysis pubis was pulled together with 0 silk thread passing through the obturator foramen. An 8F urethral catheter was placed, and the wound was closed in layers. The catheter was left in order to provide bladder drainage for 14 days. Prophylactic cefuroxime sodium (0.5 g day^{-1}; Yue Kang Pharmaceutical Ltd., Zhuhai, China) was given intravenously for 5 days after the surgery.

Postoperative evaluation

Four animals in each group were humanely euthanized 1 month and 3 months postoperatively after the urethral caliber was assessed with retrograde urethrograms. The entire posterior urethra of the rabbits was excised and sent for histological study with hematoxylin and eosin (H and E), Masson's trichrome, and immunohistochemical staining.

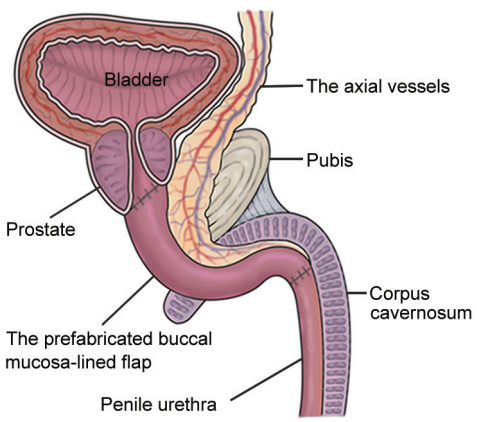

Figure 2: Diagrammatic appearance of the prefabricated axial vascularized buccal mucosa-lined flap after the procedure shown in **Figure 1**.

RESULTS

Characteristics of the capsule and the BMG

A macroscopic vascularized capsular tissue in the shape of a hollow viscus with a smooth surface was induced by the tissue expansion process. The SCI vessels were located in the center of the capsule (**Figure 1d**). As axial vessels, the SCI vessels remained pulsatile, and numerous small vessels were observed originating from the axial vessels and extending to the periphery of the capsule. Indian ink injection studies revealed that the ink first flowed through the femoral artery and SCI artery and then diffused into the capsular tissue. Cross sections of the ink-perfused capsule indicated that the vascular network within the capsule was filled with black ink (**Figure 3a**).

The harvested BMG showed an ivory-white appearance with a smooth surface at the time of transplantation and attached well to the vascularized capsule (**Figure 1f**). Histological examination confirmed that the graft contained intact oral mucosa epithelium (typical stratified squamous epithelia) and partial submucosal tissue (**Figure 3b**). A high capillary density was observed at the submucosal layer (**Figure 3b**).

Gross view and histopathologic evaluation of the prefabricated composite flap

The oral donor site completely healed 1 month postoperatively. After 1 month of incubation, capillary vessel extension into the overlying buccal mucosa was evident, and the mucosal surface became redder than the capsule, which easily distinguished the mucosa from the adjacent native capsular tissue with remarkable boundaries (**Figure 1g**). H and 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 were evident in the submucosal layers of the flap (**Figure 3c** and **3d**).

As shown by immunohistochemistry, the original free BMG expressed AE1/AE3 (the specific marker for epithelial cells), Ki67 (a marker for proliferating cells), and P63 (a marker for stem and progenitor cells). After 1 month of incubation, the mucosa retained the phenotype of native oral mucosa, with positive expression of AE1/AE3, Ki67, and P63.

Analysis of posterior urethroplasty

The capsule and the prefabricated capsule buccal mucosa composite flap were long enough for posterior urethral reconstruction (**Figure 4**). Retrograde urethrograms were performed for the assessment of urethral

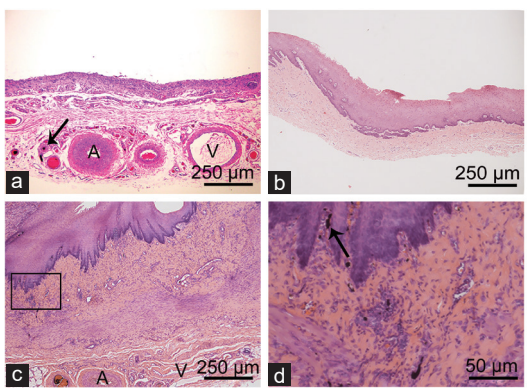

Figure 3: Representative histology of (**a**) the capsule, (**b**) buccal mucosa graft, and (**c**) the prefabricated capsule buccal mucosa composite flap; (**d**) Indian ink-perfused vessels were observed in the submucosa layer of the composite flap. A: artery; V: vein; arrows: Indian ink-perfused vessels.

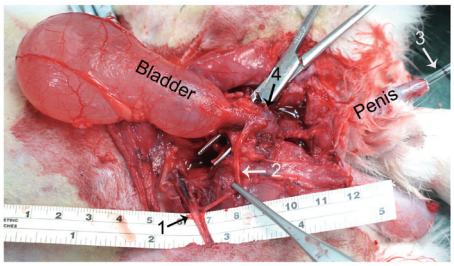

Figure 4: Three months after urethroplasty, the axial vessels remained pulsatile, and the posterior urethral defect was nicely reconstructed by the prefabricated capsule buccal mucosa composite flap. 1: femoral artery and vein; 2: superficial circumflex iliac vessels; 3: urethral catheter; 4: reconstructed neourethra.

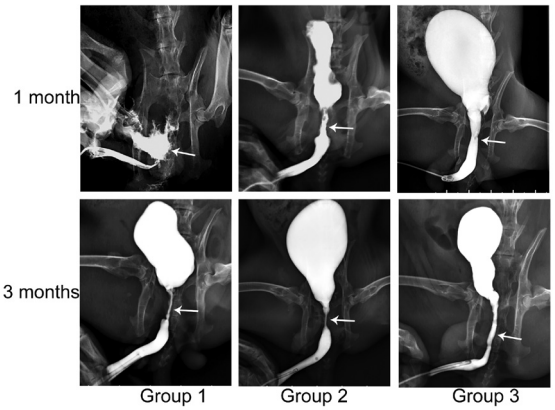

Figure 5: Comparison of urethrograms in each group 1 month and 3 months after urethroplasty. The arrow indicates the site of the reconstructed neourethra.

caliber and identification of postoperative complications, including fistulas and strictures. In Group 1, urethral fistulas developed in three rabbits (3/8, 37.5%), and the rabbits died from sepsis within 1 month. Retrograde urethrography revealed strictures in the remaining five rabbits (5/8, 62.5%) in Group 1. All 16 rabbits in Group 2 and Group 3 survived until sacrifice. In Group 2 animals, mild strictures of the urethra were observed in three rabbits (3/8, 37.5%) 1 month postimplantation, and remarkable urethral strictures were observed in five rabbits (5/8, 62.5%) at 3 months postimplantation. In Group 3, a mild stricture was observed in 1 rabbit (1/8, 12.5%) at 1 month, and the remaining rabbits maintained a wide urethral caliber at each time point (**Figure 5**).

Histopathologic evaluation of the retrieved urethra

Considering the sparse number of quality images of the posterior urethra of the rabbits, we first evaluated the normal structure of the posterior urethra of the 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 Group 1 animals, the urethral lumen was narrowed, and portions of the tubularized BMG survived within the urethral lumen at 1 month. At 3 months, excessive contracture of the tubularized BMG was observed. In Group 2 animals, there were no evident epidermal cells present on the luminal surface of the neourethra 1 month postimplantation. At 3 months, the urethral caliber was narrowed, although some discontinued epidermal layers developed on the luminal surface of the neourethra. In Group 3 animals, a wide urethral caliber was maintained, and the neourethra was covered by intact and stratified squamous epithelium (**Figure 6**). The typical papilla morphology of the buccal mucosa was retained in the neourethra, and the basilar layers of the neourethra were positive for P63 and Ki67 (**Supplementary Figure 1**). Furthermore, neovascularization was still evident beneath the new buccal mucosa area in the neourethra.

DISCUSSION

Surgery for complex posterior urethral disruptions is compounded by problems of access, limited urethral length, and surrounding fibrosis.[1] Impaired vascularity, persistent inflammation due to perineal fistula/periurethral abscess, and a lacerated bladder neck further compound the problem and create enormous difficulties during reconstruction. Undoubtedly, the golden triad for a successful outcome is the complete excision of the fibrous segment, the lateral fixation of the healthy mucosa of the urethral ends, and the creation of tension-free anastomosis. However, the standard transperineal bulbomembranous anastomosis urethroplasty cannot achieve tension-free anastomosis in some patients with long-segment posterior urethral disruptions, and free tissue grafts do not take on a poor vascular bed.[6] In these situations, several approaches have been described in the literature.

Substitution urethroplasty using a pedicled preputial tube to bridge the long gap between the anterior and posterior urethra has been described by Hunter *et al*.[7] The main advantage of the technique is the feasibility of creating a long urethral segment in one stage. The disadvantages are the frequent unavailability of the required tissue (*i.e*., history of circumcision and prior use of penile skin) and the common formation of large diverticula when preputial flaps are used.[8]

Enterourethroplasty technique using an intestinal flap to bridge urethral defects in 11 patients with otherwise unsalvageable urethral defects has been described by Mundy and Andrich.[4] The main advantage of the procedure is a one-stage repair that immediately produces a tubular urethra. The drawbacks of the procedure are the need for an abdominal and a perineal approach and the need for enteroenteric reanastomoses with their predictable potential complications. The resulting colonic or duodenal urethra is then theoretically prone to diverticula formation and mucus production.

An alternative innovative three-stage approach was described by Wu *et al*.[3] The first stage allows the replacement of a long posterior urethral gap using the penile urethra as a tubular flap for pendulous-prostatic anastomosis. In the second stage, the penile urethra is transected at the coronary sulcus, and the penis is restraightened. The third stage of surgery involves the reconstruction of the anterior urethra 6 months later. The drawbacks of this procedure are the need for numerous operations and the eventual need for pendulous urethral tubularization with risks of fistula formation and poor cosmetic appearance. Furthermore, after transection at the sulcus, doubt remains about the possibility of revascularization of the penile urethral stump by means of the penile-prostatic anastomosis by the uncertain bulbar arteries or periurethral tissues.

Tissue engineering has been considered a new alternative source for complex urethral repair.[9–11] In recent studies, it has been demonstrated that off-the-shelf tubular constructs are effective for tubularized urethral replacement. However, the indications for the use of acellular matrices in tubularized grafts may be limited by the size of the defect (<0.5 cm).[9,10] In contrast, the inclusion of cells has been proven to be

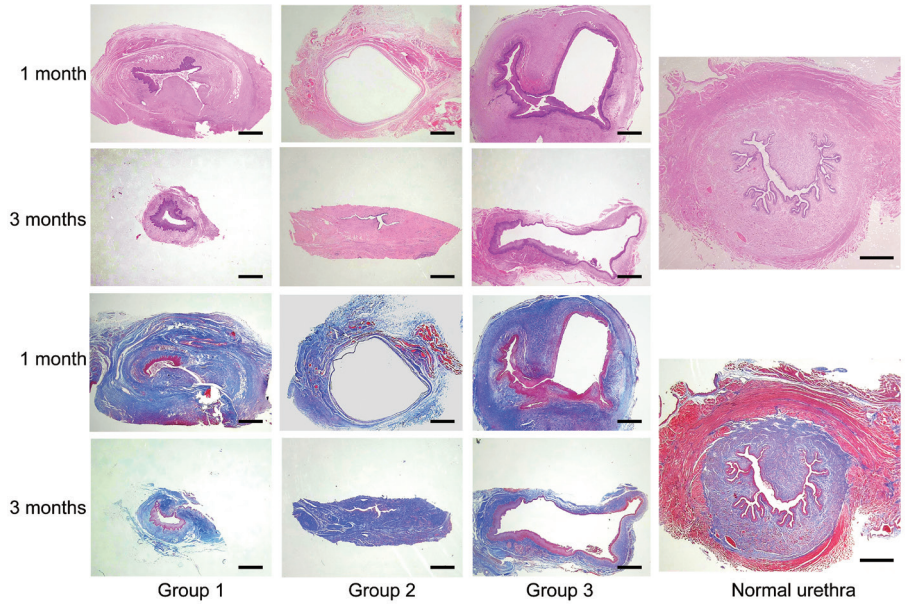

Figure 6: Overviews of cross sections of rabbit posterior urethras. In Group 1, part of the tubularized buccal mucosa graft survived in the neourethra at 1 month. At 3 months, excessive contracture of the tubularized BMG was observed. In Group 2 animals, there was no evidence of epidermal lining on the luminal surface of the neourethra 1 month postimplantation. At 3 months, the urethral caliber was narrowed, although some discontinued epidermal layers developed on the luminal surface of the neourethra. In Group 3, the neourethra lumen was lined with typical squamous epithelial layers, and the constructed neourethra maintained a wide urethral caliber for 3 months. Scale bars = 1.0 mm.

effective for long urethral defects, and using cell-seeded scaffolds may reduce the probability of encountering side effects.[10,11] Remarkably, however, the inclusion of cells is perceived as too problematic, and the costs of cellular implants are higher than those of off-the-shelf acellular implants since two procedures are needed (cell harvesting in urine or biopsy and urethroplasty), and *in vitro* cell expansion may be needed.[12,13] Tissue engineering techniques for urethral reconstruction remain challenging in terms of clinical translation.

The development of the technique presented in this study relied on the modification of several previously published reports of prelamination and prefabrication techniques. The technique of using a prefabricated buccal mucosa-gracilis composite flap for the repair of a devastated urethra has been described by Nikolavsky.[14] The gracilis muscle provides a vascular bed for the BMG and reduces the risk of developing a ventral diverticulum or a fistula. The main disadvantages of the procedure are represented by the drawbacks of sacrificing the gracilis muscle.[15,16] In the present study, we used vascularized capsular tissue as an induced vascular bed for buccal mucosa-lined flap prefabrication for tubularized posterior urethral reconstruction. The axial vessels provided an adequate and independent blood supply for the constructed neourethra, and the buccal mucosa lining offers a successful urine barrier, which guarantees a successful long-term outcome.

The capsule provides an aseptic, moist, protected environment for the buccal mucosa and that the mucosa has a dense submucosa with a dense capillary network, in combination with the highly vascularized capsule flap, constitutes ideal prerequisites for mucosa-lined flap prefabrication. The prefabricated capsule buccal mucosa composite flap bears the major advantage that the construct is directly reperfused after implantation at its final destination, avoiding the use of allogeneic grafting material, cell culture, hair-bearing skin, and the gracilis muscle or the recruitment of gastrointestinal segments.

Prefabrication of buccal mucosa-lined flaps is technically feasible and has an attractive transition for clinical usage. Applications may include the placement of a skin tissue expander in the vicinity of large vessels near the pelvic floor (for example, the descending branch of the lateral circumflex femoral vessel) to induce vascularized capsule formation.[17,18] Then, the BMG can be 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 hypothesize that tubularized posterior urethral reconstruction can be accomplished via a technique similar to what we describe through a subcutaneous tunnel to the posterior urethra. This concept may have potential for the reconstruction of complicated urethral strictures, especially in patients with complex posterior urethral disruptions who have undergone failed previous surgical treatments.

Although a majority of urethral defects can be resolved through transperineal urethroplasty, there are certain limitations to its use. The repair of a concomitant rectourethral fistula, an open bladder neck and a periurethral cavity requires wider exposure of the pelvis, bladder neck repair, debridement, and omentoplasty.[19,20] In addition, the success rate of perineal urethroplasty is reportedly low in prepubescent boys and in patients undergoing repeat urethroplasty.[21] Considering the limited perineal space and in order to obtain good exposure of the posterior urethra, we adopted transpubic urethroplasty in rabbits. This technique not only provides wide and excellent exposure for urethral anastomosis but also allows the synchronous repair of urethrorectal fistulas and bladder neck incompetence as well as the excision of periurethral cavities.[22] It is now believed to be a safe procedure because complications related to pubic resection are infrequently seen. Furthermore, the transpubic route facilitates the use of a pedicled omental graft to obliterate the perianastomotic dead space, absorb inflammatory debris, and prevent fibrosis.[23,24]

There are some limitations to this study. First, 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

posterior urethral disruptions, which are characterized by a fibrotic urethral bed. Second, the number of experimental animals that were evaluated per time point was small, and the results only represent short-term success. A larger sample size with a longer follow-up period would be more convincing to evaluate the patency rates of the composite constructs. Furthermore, the urethras of Group 3 animals did not resemble those of the control animals. There was no presence of a star-shaped urethra, and the wide urethra may not be desired in patients. The fabrication of a bionic urethra that contains smooth muscle bundles and an epithelial lining may be more applicable for urethral reconstruction. Finally, a long segment of buccal mucosa is needed for the replacement of long posterior urethral disruptions. Harvesting a longer BMG in a dog or in a porcine model may be more convincing.

CONCLUSIONS

The present study demonstrates that a BMG can survive and grow in a prefabricated capsule pouch and that the prefabricated capsule BM composite flap can be used for tubularized posterior urethral reconstruction. The axial vascular bundle provides an adequate vascular supply for the flap to resist contraction after transplantation. Theoretically, the prefabricated BM-lined flap has an independent vascularity and can also survive under poor tissue conditions. Furthermore, redundant vascularized capsular tissue can be used to obliterate perianastomotic dead space, absorb inflammatory debris, and prevent fibrosis. The method is safe, extroperitoneal, and relatively easy to perform, and the prefabricated flap is thin and flexible and may be a new choice for complex posterior urethral disruptions.

AUTHOR CONTRIBUTIONS

HLG carried out the project and drafted the manuscript. LW participated in the project design and coordination and helped to draft the manuscript. ZMJ, XQB, and YCH participated in the project design and coordination. JMZ, HX, and XJY helped 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 declared no competing interests.

ACKNOWLEDGMENTS

This study was supported by grants from the Ph.D. Candidate Innovation Fund of Shanghai Jiao Tong University School of Medicine (No. BXJ201741) and the National Natural Science Foundation of China (No.81470911, No.81870459).

Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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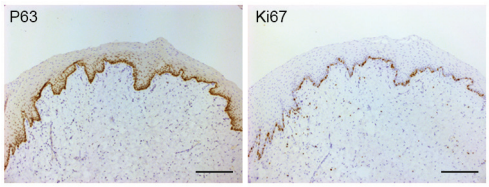

Supplementary Figure 1: Immunohistochemical staining of the buccal mucosa-lined neourethra with P63 and Ki67. Scale bars = 200 μm.