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## Aerosol transfer of bladder urothelial and smooth muscle cells onto demucosalized colonic segments for bladder augmentation: *in vivo*, long term, and functional pilot study

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### Summary

**Background**—Bladder augmentation technique has changed over the years and the current practice has significant adverse health effects and long-term sequelae. Previously, we reported a novel cell transfer technology for covering demucosalized colonic segments with bladder urothelium and smooth muscle cells through an aerosol spraying of these cells and a fibrin glue mixture.

**Objective**—To determine the long-term durability and functional characteristics of demucosalized segments of colon repopulated with urothelial cells in the bladder of swine for use in augmentation cystoplasty.

**Study design**—Nine swine were divided into three groups. The first group (control) underwent standard colocystoplasty; the second group underwent colocystoplasty with colonic demucosalization and aerosol application of fibrin glue and urothelial cell mixture; in the third group detrusor cells were added to the mixture described in group two. The animals were kept for 6 months. Absorptive and secretory function was assessed. Bladders were harvested for histological and immunohistochemical evaluation.

**Results**—All animals but one in the experimental groups showed confluent urothelial coverage of the colonic segment in the bladder without any evidence of fibrosis, inflammation, or regrowth of colonic epithelial cells. Ten percent of the instilled water in the bladder was absorbed within an hour in the control group, but none in experimental groups ( $p=0.02$ ). The total urine sediment and protein contents were higher in the control group compared with experimental groups ( $p<0.05$ ).

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Conflict of interest  
None.

**Discussion**—Both study groups developed a uniform urothelial lining. Histologically, the group with smooth muscle had an added layer of submucosal smooth muscle. Six months after bladder augmentation the new lining was durable. We were also able to demonstrate that the reconstituted augmented segments secrete and absorb significantly less than the control colocolostomy group. We used a non-validated simple method to evaluate permeability of the new urothelial lining to water. To determine if the aerosol transfer of bladder cells would have behaved differently in the neurogenic bladder population, this experiment should have been performed in animals with neuropathic bladders.

**Conclusion**—Aerosol spraying of single cell suspension of urothelial and muscular cells with fibrin glue resulted in coverage of the demucosalized intestinal segment with a uniform urothelial layer. This new lining segment was durable without regrowth of colonic mucosa after 6 months. The new reconstituted segment absorbs and secretes significantly less than control colocolostomy.

### Keywords

Aerosol; Bladder augmentation; Demucosalized colonic segment

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### Introduction

Congenital and acquired defects, such as bladder exstrophy, myelomeningocele, spinal cord lesions, and bladder outlet obstruction, are associated with poor bladder storage caused by decreased capacity, abnormal contractility, and poor compliance. This can result in incontinence, infection, vesicoureteric reflux, and renal injury. Reconstruction of this type of bladder using intestinal segments prevents these complications and today is considered the standard of care for managing these end-stage bladders. However, isolation of these segments from the gastrointestinal tract and incorporating them into the urinary tract has been associated with significant long-term sequelae because of the existing secretory and absorptive functions of the intestinal mucosa [1,2]. The secretion of mucus in the urine is associated with infection and urinary stone formation. Exposure of the absorptive surface of the intestinal mucosa to urine leads to metabolic derangements and electrolyte abnormalities. Another rare complication is malignant transformation of the intestinal mucosa, a risk that requires lifelong endoscopic follow-up of these patients [3].

Several natural and synthetic materials have been evaluated for bladder augmentation to avoid the drawbacks of intestinal bladder augmentation. However, ideal materials for bladder augmentation are not yet available. The introduction of tissue engineering technology for bladder substitution has been considered a promising alternative approach [4,5]. However, these tissue-engineered bladders have behaved like grafts, with inadequate delivery of oxygen and nutrients to the transplanted cells and the inefficiency of waste removal. As a result of poor graft take, scarring of these tissue-engineered bladders was frequently observed without improvement in bladder capacity [6]. Use of demucosalized intestinal segments to overcome the absorptive and secretory complications is often associated with shrinkage of the segment and regrowth of the gastrointestinal epithelium [7,8].

To solve the issues of shrinkage and regrowth of gastrointestinal epithelium in demucosalized segments, we have substituted the intestinal mucosa with autologous bladder urothelium and muscle. We have reported previously a novel cell transfer technology for covering demucosalized colonic segments with bladder urothelium and smooth muscle cells through aerosol spraying of these cells and a fibrin glue mixture [9,10]. These segments were used for immediate bladder augmentation. After a pilot study for the feasibility of this novel technology [9], a 6-week controlled experiment demonstrated that the colonic segments used for bladder augmentation had confluent epithelial coverage without fibrosis and regrowth of intestinal mucosa [10]. However, the long-term durability of this lining and the absorptive and secretory function of the resurfaced segment remained unknown.

Therefore, in this study, we aimed to determine whether this new urothelial lining is impermeable, non- or less secretory, and will persist over a long period of time (6 months) without regrowth of colonic epithelium.

## Subjects and methods

### Experimental design

The study included nine Yucatan pigs weighing 20 kg each. The animals were divided into three equal groups and underwent one of the following procedures: group 1 (control group), classic colocolostomy; group 2, demucosalized colocolostomy with aerosol application of fibrin glue and single cell suspension of urothelial cells; group 3, demucosalized colocolostomy with aerosol application of fibrin glue and single cell suspension of urothelial and smooth muscle cells. The animals were kept for 6 months, after which they underwent endpoint surgery.

### Methods and procedures

**Preoperative preparation and anesthetic considerations**—The experimental protocol was reviewed and approved by the university animal research committee. Animal handling and all procedures were conducted following the institutional animal care and use committee guidelines and with the university's veterinary supervision.

**Animal bowel preparation**—The animals were fed Ensure (Abbott Laboratories, IL, USA) for 1 day, transitioned to a clear liquid diet for another 1 day, and then fasted overnight before the surgery.

**Anesthesia and prophylactic antibiotics**—After intramuscular injection of telazol and xylazine, the animals were intubated and maintained on isoflurane anesthesia throughout the surgery. Prophylactic metronidazole (10 mg/kg) was given via intravenous injection during surgery. In addition, enrofloxacin was given intramuscularly just before surgery and every 12 hours as a prophylactic antibiotic for 48 hours thereafter.

## Surgical procedure

**In all groups**—The peritoneal cavity was entered through a midline abdominal incision followed by removal of a 2×2-cm segment of bladder for preparation of urothelial and smooth muscle suspension (see *Urothelial cell preparation*).

**Group 1**—A 10-cm segment of the sigmoid colon was mobilized and then mesenteric exclusion was performed of the blood supply of the sigmoid segment. Continuity of the colon was restored using interrupted 3-0 polyglycate sutures. The isolated segment was opened along the anti-mesenteric border and reconfigured into a U-shaped patch, which was used for bladder augmentation. The colonic segment was anastomosed to the bladder with a single layer of continuous 3-0 polyglycate suture.

**Groups 2 and 3**—A 16F Foley catheter was inserted through the anus and the balloon of the Foley catheter was milked into the middle of the sigmoid colon. A 10-cm segment of the sigmoid colon was mobilized and the lumen was occluded using two vessel loops brought through holes in the sigmoid mesentery. The isolated sigmoid segment was filled with sterile water through the Foley catheter to facilitate colonic dissection of the seromuscular layer off the mucosal layer of the sigmoid as described by Lima [11]. The seromuscular layer was incised along the antimesenteric border followed by elevation of this layer off the mucosa along the entire circumference from both sides. The 10-cm isolated seromuscular segment was transferred medially with its mesentery. The redundant mucosa was replaced into the lumen and the incised edges of the colon were reapproximated using interrupted 3-0 polyglycate sutures.

In group 2, the isolated demucosalized segment was sprayed with fibrin glue and urothelial cells, while in group 3, the surface was sprayed with fibrin glue and a mixture of urothelial cells and smooth muscle cells. The bladder was opened in a clam fashion and the constructs were then used for bladder augmentation with a single layer of continuous 3-0 polyglycate sutures between the bladder and the colonic segment. The abdominal incision and skin were closed. We did not use any catheters or drains in the postoperative period based on the experience gained in our previous short-term experiments [9,10]. These studies confirmed that it was safe to perform the procedure without using catheters as these animals had normal bladders without storage or voiding issues.

Pain was managed with buprenorphine every 8 hours for the first 48 hours and then buprenorphine was given as needed. Postoperative care also included administration of antibiotics for 48 hours. Feeding resumed slowly until the animal initiated a bowel movement.

## Urothelial cell preparation

Urothelial cell preparation was performed, as previously described [9] simultaneously with the colonic demucosalization procedure. A full thickness 2×2-cm segment of bladder was harvested. The urothelial lining was separated from the remainder of the segment and each was placed in a separate tube. The tissue samples were minced into smaller pieces and then digested with 10 mg of collagenase IV in 5 ml of keratinocyte serum-free media for 1.5

hours. The resultant urothelial and muscle single cell suspensions were individually centrifuged at 600 g for 3 minutes. The medium was discarded, and 5 ml of autologous porcine serum was added to the cell suspension.

The cell suspension was aerosolized and sprayed on to the demucosalized sigmoid segment concomitantly with 5 ml of fibrin glue using a tri-nozzle air compressor sprayer with separate compartments for the cells and the fibrin glue. The mixture was left to adhere on the demucosalized surface for 10 minutes.

## Endpoint surgery

**Secretion test**—After administration of anesthesia, 50 ml of urine was collected and centrifuged. The solid precipitate was weighed and expressed as mg/ml of urine. The total protein content in the urine for the different animals was measured using the Lowry protein assay kit following the instructions provided by the manufacturer.

**Absorption test**—After ligation and division of both ureters, the bladder was drained and then filled with 300 ml of distilled water. The residual volume of distilled water in the bladder was measured after 1 hour to evaluate the absorptive capacity of the augmented segment.

**Histological studies**—The augmented segments were harvested and fixed in 10% buffered formalin for 48 hours followed by embedding in paraffin. Serial sections of the construct, representing peripheral and central areas of each segment, were stained with hematoxylin and eosin and examined by a single pathologist. Masson trichrome was used to stain for muscle and collagen, and periodic acid Schiff (PAS) was used to detect polysaccharides in mucosal cells. Immunohistological staining with mouse monoclonal anti-cytokeratin peptide 7 (clone LDS-68) and rabbit polyclonal anti-urolaklin III was performed in groups 2 and 3 to reveal terminal urothelial differentiation of the sprayed colonic segments.

## Results

### Histological studies

Hematoxylin and eosin stained sections showed evidence of normal colon mucosa in group 1. All of the animals in groups 2 and 3 did not show any evidence of colonic mucosa regrowth (Figures 1 and 2, respectively). All animals in group 2, and all but one in group 3, showed normal appearing multilayered urothelial-like epithelium with a spontaneously segregated underlying layer of bladder smooth muscle cells resting on colonic submucosa with minimal fibrosis, as well as evidence of underlying neo-vascularization. A continuous colonic submucosal layer was seen separating the colonic muscle layers from the newly formed detrusor muscle layer. PAS staining of the constructs showed no evidence of colonic mucosa regrowth, there was minimal fibrosis with mild inflammatory cell infiltrates in Masson trichrome-stained sections. One animal in group 3 showed an extensive scar with sloughed urothelium, fibroblasts, and massive collagen deposition (Figure 2).

## Immunohistochemistry

To demonstrate that the newly formed hybrid constructed epithelium was indeed true urothelium, immunohistochemical analyses for various urothelium-specific differentiation markers were performed. There was a uniform positive staining for cytokeratin 7 (Figure 3) in most epithelial cells of reconstructed segments. Monoclonal antibodies against uroplakin III, a transmembrane protein, which constitutes a specific terminal differentiation product of urothelial cells showed a strong continuous linear outline of the superficial cell membrane of the umbrella cells.

## Absorptive studies

There was no reduction in the volume of instilled water in the experimental groups after 1 hour of observation, compared with the control group in which an average of 10% of the instilled water was absorbed ( $273 \pm 6$  ml) ( $p=0.027$ ).

## Sediment and protein content

Total urine sediments and total protein contents were significantly lower in groups 2 and 3 when compared with those in the control group (Table 1). The control group had mean total urine sediment of 3.52 mg/ml ( $SD \pm 0.38$ ). Group 2 had mean total urine sediment of 1.52 mg/ml ( $SD \pm 0.74$ ) and group 3 had a mean of 1.39 mg/ml ( $SD \pm 0.017$ ). Statistical significance ( $p < 0.01$ ) was found for both groups 2 and 3 when compared with the total sediment level in the control group. Urine protein levels were also significantly different ( $p < 0.01$ ) between the control group and groups 2 and 3. The control group had a mean urine protein concentration of 0.82 mg/ml ( $SD \pm 0.13$ ), group 2 had a mean of 0.02 mg/ml ( $SD \pm 0.02$ ), and group 3 had a mean of 0.08 mg/ml ( $SD \pm 0.05$ ).

## Discussion

This study demonstrates the long-term durability of bladder augmentation by a novel technology using demucosalized sigmoid colon that was repopulated with bladder urothelial and smooth muscle cells. These cells were prepared from the animal's own bladder tissue and mixed with fibrin glue for spraying.

Previously, Hafez *et al.* showed that 4 weeks after augmentation with aerosol sprayed demucosalized colon, a histological intact urothelial layer is apparent with a randomly aligned, but distinctly segregated layer of smooth muscle cells [9]. This concept was further tested in a 6-week controlled study, which showed that demucosalized colocolocystoplasty without the cellular component of the spray was associated with severe fibrosis and contracture; however, when the demucosalized segment was repopulated using the aerosol spraying technology with either bladder urothelial cells or a mixture of bladder urothelial and smooth muscle cells, a layer of neo-urothelium that rested directly on the colonic submucosa was generated without fibrosis or shrinkage [10]. Furthermore, the mixed urothelial and smooth muscle cells in the suspension demonstrated a striking ability to organize spontaneously into a luminal epithelial and subepithelial muscle layer [10].

The rationale for the using both urothelial and smooth muscle cell suspension came from Baskin's previous work [12], which showed that mesenchymal-epithelial interactions are necessary for the development of the bladder smooth muscle, and the mechanism responsible for this interaction involves locally diffusible growth factors. In our study we found that both groups developed a robust and uniform urothelial lining. Histologically, the group with smooth muscle had an added layer of submucosal smooth muscle. The addition of the detrusor smooth muscle did not make the experimental procedure more complex; however, we are unable to demonstrate any objective benefit from this additional layer in our study.

We used a non-validated simple method to evaluate permeability of the new urothelial lining to water. By occluding both ureters, we ensured that no additional volume entered the bladder and so the change in the volume of instilled water could be used as a surrogate for absorption. We had performed an extensive literature search and failed to find a validated method to evaluate the absorption ability of augmented bladders in pigs. More research is needed to develop a precise and validated method.

Before translating this technology to clinical application in human subjects there were additional questions that needed to be answered. What is the long-term durability of this lining? Will these newly reconstructed urothelial cells continue to multiply and renew? Or will intestinal mucosal cells regrow and repopulate the original segment? Furthermore, functional properties of the augmented segment needed to be explored. More specifically, is the new urothelial lining impermeable? Lastly, will this technique effectively prevent mucus secretion by the reconstructed augmented segment? Our long-term study was designed to try to answer these questions. We showed that 6 months after bladder augmentation the new lining was durable. There was no evidence of colonic epithelial regrowth, the urothelial cells continued to renew and to form a consistent uniform urothelial layer. We were also able to demonstrate that the reconstituted augmented segments secrete and absorb significantly less than the control colocoloplasty group.

We had one failed procedure in which the sprayed cells did not repopulate the demucosalized segment and an extensive inflammatory and fibrotic reaction was seen. We believe that this severe fibrosis might be attributed to the failure of the urothelial cells to seed on the demucosalized bed, therefore resulting in exposure of the demucosalized segment to urine. We do not have a definitive explanation for this one failed experiment.

To determine if the aerosol transfer of bladder cells would have behaved differently in the neurogenic bladder population, this experiment should have been performed in animals with neuropathic bladders. However, long-term maintenance of an animal model with neurogenic dysfunction is very challenging. Although we do not anticipate that the dissociated urothelium and smooth muscle cells on a colonic matrix would behave differently, this question will obviously remain unanswered until such time as this technology is ready to be applied and tested in a few pilot human cases.

One of the advantages of this technology is the ability to obtain a cell suspension in a single operative setting without the need for *ex vivo* cell culture and expansion. In this study, the



bladder dissociation was processed in our urology basic science laboratory. As the ultimate goal of this technology is to translate this work to pediatric and adult patients who require bladder augmentation or substitution, we are currently working on a method to perform the cellular dissociation by the surgical team in the operation room. We hope to be able to simplify the procedure and make it widely available for every standard operating room without the need for sophisticated tissue culture laboratory facilities.

## Conclusion

Our long-term animal study demonstrated that aerosol spraying of single cell suspension of urothelial and muscular cells with fibrin glue resulted in coverage of the demucosalized intestinal segment with a uniform urothelial layer. This new lining segment was durable without regrowth of colonic mucosa after 6 months. The new reconstituted segment absorbs and secretes significantly less than control colocolostoplasty.

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## References

1. McDougal WS. Metabolic complications of urinary intestinal diversion. *J Urol.* 1992; 147:1199–208. [PubMed: 1569649]
2. Gros DA, Dodson JL, Lopatin UA, Gearhart JP, Silver RI, Docimo SG. Decreased linear growth associated with intestinal bladder augmentation in children with bladder exstrophy. *J Urol.* 2000; 164:917–20. [PubMed: 10958756]
3. Husmann DA, Rathbun SR. Long-term follow up of enteric bladder augmentations: the risk for malignancy. *J Pediatr Urol.* 2008; 4:381–5. [PubMed: 18653384]
4. Reddy PP, Barrieras DJ, Wilson G, Bagli DJ, McLorie GA, Khoury AE, et al. Regeneration of functional bladder substitutes using large segment acellular matrix allografts in a porcine model. *J Urol.* 2000; 164:936–41. [PubMed: 10958712]
5. Atala A. Bladder regeneration by tissue engineering. *BJU Int.* 2001; 88:765–70. [PubMed: 11890251]
6. Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet.* 2006; 367(9518):1241–6. [PubMed: 16631879]
7. Dewan PA, Close CE, Byard RW, Ashwood PJ, Mitchell ME. Enteric mucosal regrowth after bladder augmentation using demucosalized gut segments. *J Urol.* 1997; 158(3 pt 2):1141–6. [PubMed: 9258158]
8. Jednak R, Schimke CM, Barroso U JR, Barthold JS, Gonzalez R. Further experience with seromuscular colocolostoplasty lined with urothelium. *J Urol.* 2000; 164:2045–9. [PubMed: 11061922]
9. Hafez AT, Bagli DJ, Bahoric A, Aitken K, Smith CR, Herz D, Khoury AE. Aerosol transfer of bladder urothelial and smooth muscle cells onto demucosalized colonic segments: a pilot study. *J Urol.* 2003; 169:2316–9. [PubMed: 12771789]
10. Hafez AT, Afshar K, Bägli DJ, Bahoric A, Aitken K, Smith CR, Khoury AE. Aerosol transfer of bladder urothelial and smooth muscle cells onto demucosalized colonic segments for porcine bladder augmentation in vivo: a 6-week experimental study. *J Urol.* 2005; 174(4 pt 2):1663–7. [PubMed: 16148677]

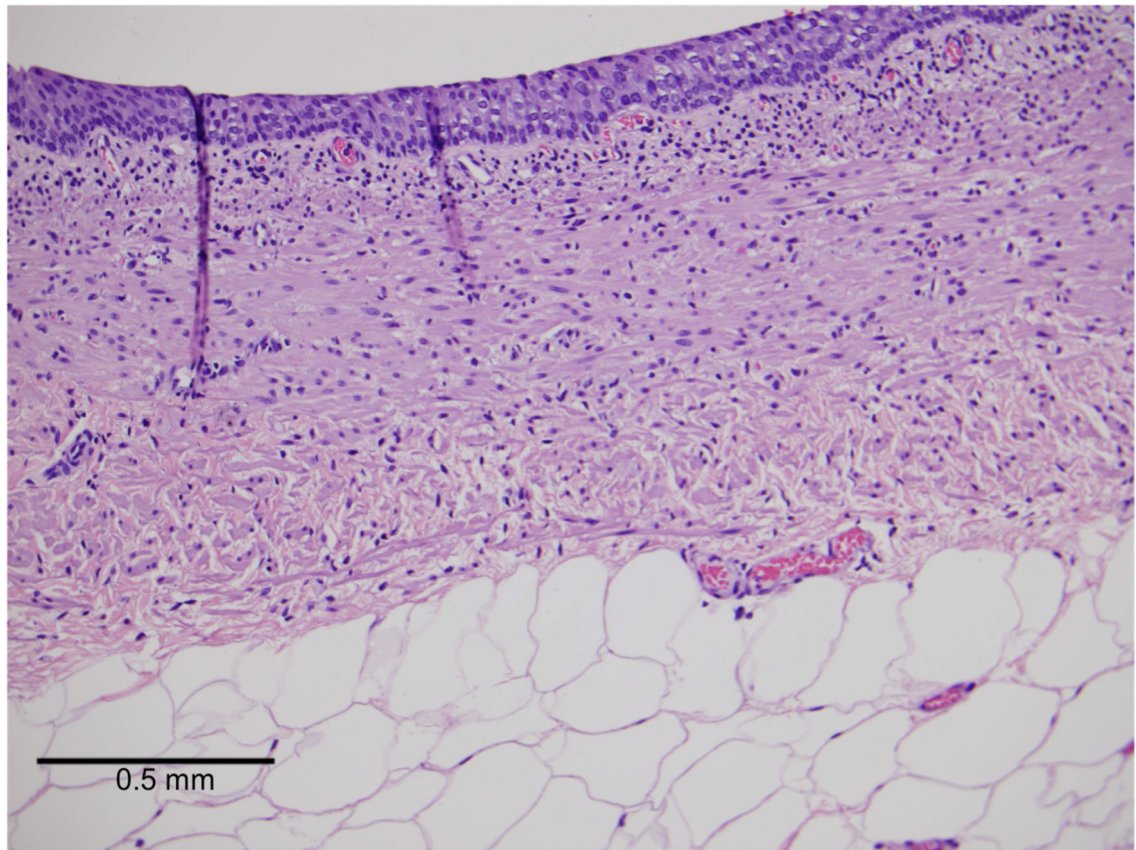
11. Lima SV, Araújo LA, Vilar FO, Mota D, Maciel A. Experience with demucosalized ileum for bladder augmentation. *BJU Int.* 2001; 88:762–4. [PubMed: 11890250]
12. Baskin LS, Hayward SW, Young P, Cunha GR. Role of mesenchymal-epithelial interactions in normal bladder development. *J Urol.* 1996; 156:1820–7. [PubMed: 8863624]

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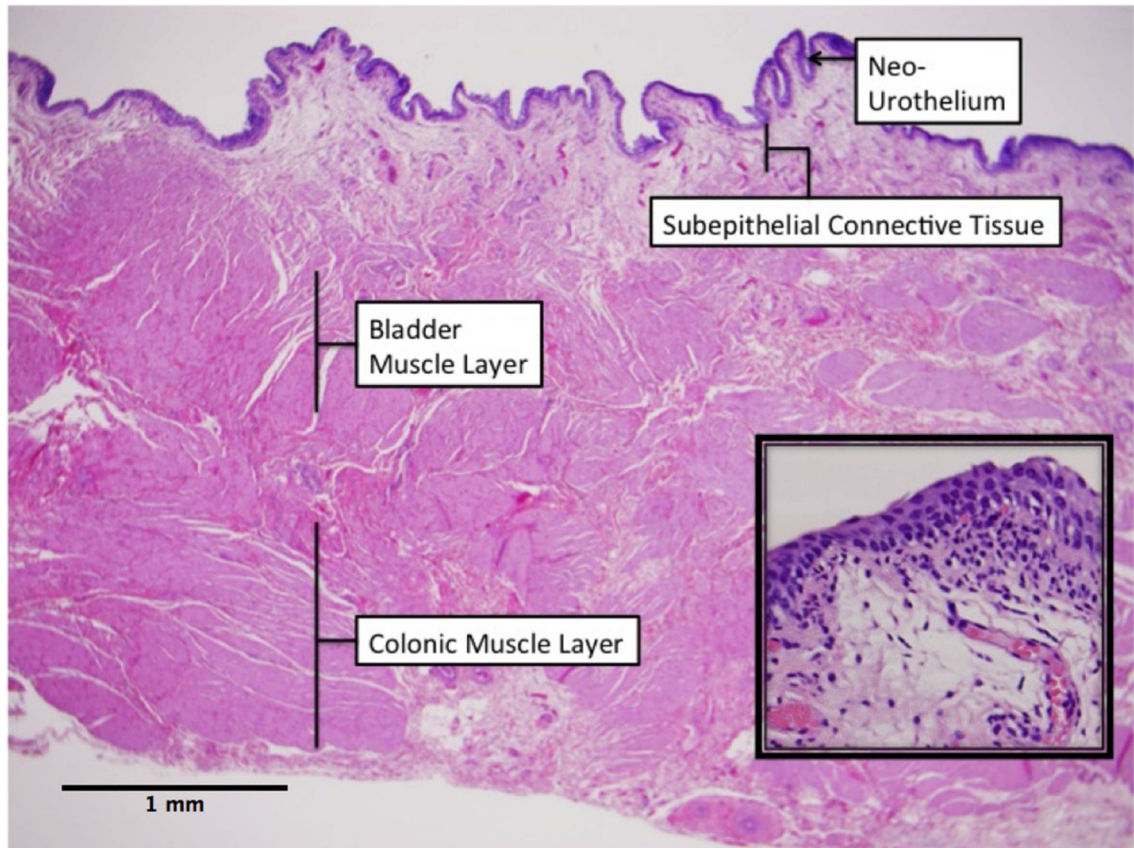
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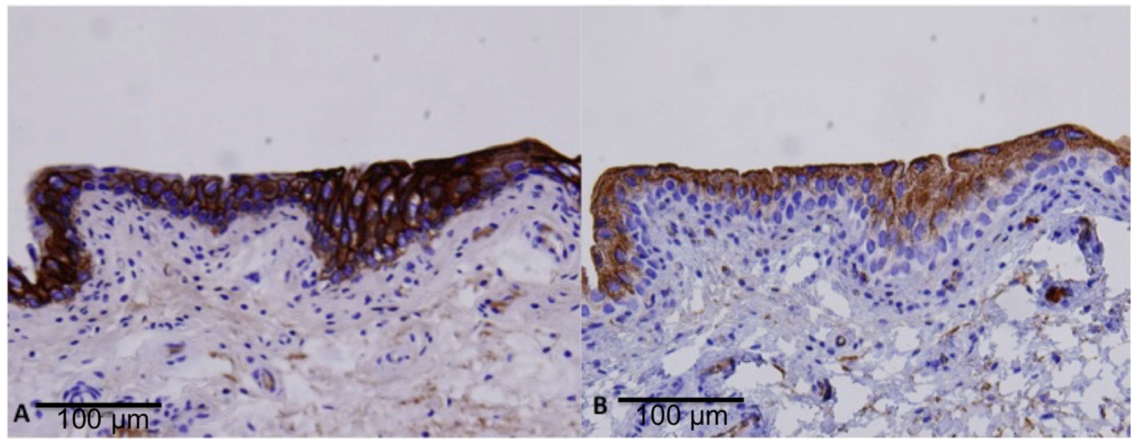


**Figure 1.** Hematoxylin and eosin-stained sections of the reconstructed segment of urothelial cells (group 2) show normal appearing, multilayered urothelial-like epithelium with no evidence of colonic mucosa regrowth or suburothelial fibrosis. 20× magnification



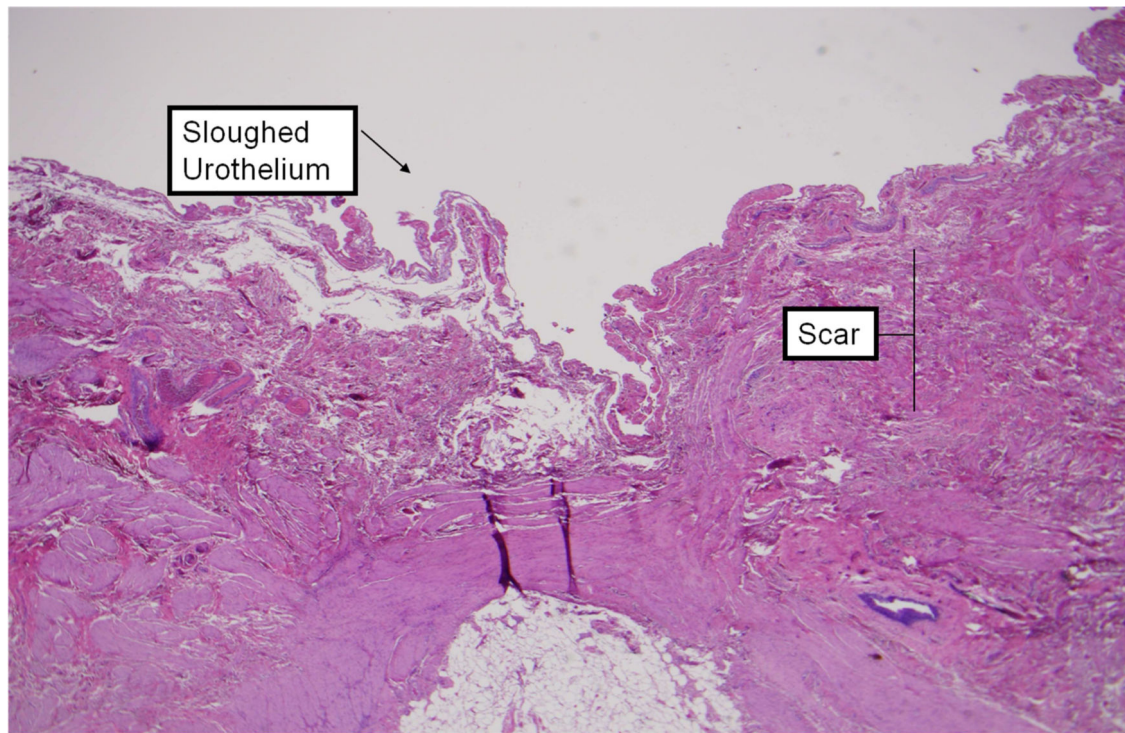
**Figure 2.** Hematoxylin and eosin-stained sections of the reconstructed segment of both urothelial and smooth muscle cells (group 3) show normal appearing, multilayered urothelial-like epithelium with no evidence of colonic mucosa regrowth or suburothelial fibrosis. Light microscopy, 2× magnification, inset 20× magnification





**Figure 3.**

(A) Cytokeratin 7 expression is uniformly positive in all layers of neo-urothelium of demucosalized colostoplasty reconstructed segment with sprayed urothelial and smooth muscle cells (group 3). Immunohistochemistry, light microscopy, 20× magnification. (B) Uroplakin III expression by superficial umbrella cell layer of neo-urothelium of demucosalized colostoplasty reconstructed segment with sprayed urothelial and smooth muscle cells (group 3). Immunohistochemistry, light microscopy, 20× magnification.



**Figure.**  
One animal in group three showed an extensive scar with sloughed urothelium, fibroblasts, and massive collagen deposition.

**Table 1**

Urine total sediment and protein concentration in the different study groups.

Group	Urine total sediment, mg/ml (mean $\pm$ SD)	Urine protein, mg/ml (mean $\pm$ SD)
Control	3.52 $\pm$ 0.38	0.82 $\pm$ 0.13
Urothelial only	1.52 $\pm$ 0.74 <sup>a</sup>	0.02 $\pm$ 0.02 <sup>a</sup>
Urothelial and muscle	1.39 $\pm$ 0.017 <sup>a</sup>	0.08 $\pm$ 0.05 <sup>a</sup>

<sup>a</sup>  $p < 0.01$  when compared with the control group.

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