Tissue-engineered autologous bladders for patients needing cystoplasty

Anthony Atala, Stuart B Bauer, Shay Soker, James J Yoo, Alan B Retik

Summary

Background Patients with end-stage bladder disease can be treated with cystoplasty using gastrointestinal segments. The presence of such segments in the urinary tract has been associated with many complications. We explored an alternative approach using autologous engineered bladder tissues for reconstruction.

Methods Seven patients with myelomeningocele, aged 4–19 years, with high-pressure or poorly compliant bladders, were identified as candidates for cystoplasty. A bladder biopsy was obtained from each patient. Urothelial and muscle cells were grown in culture, and seeded on a biodegradable bladder-shaped scaffold made of collagen, or a composite of collagen and polyglycolic acid. About 7 weeks after the biopsy, the autologous engineered bladder constructs were used for reconstruction and implanted either with or without an omental wrap. Serial urodynamics, cystograms, ultrasounds, bladder biopsies, and serum analyses were done.

Results Follow-up range was 22–61 months (mean 46 months). Post-operatively, the mean bladder leak point pressure decrease at capacity, and the volume and compliance increase was greatest in the composite engineered bladders with an omental wrap (56%, 1·58-fold, and 2·79-fold, respectively). Bowel function returned promptly after surgery. No metabolic consequences were noted, urinary calculi did not form, mucus production was normal, and renal function was preserved. The engineered bladder biopsies showed an adequate structural architecture and phenotype.

Conclusions Engineered bladder tissues, created with autologous cells seeded on collagen-polyglycolic acid scaffolds, and wrapped in omentum after implantation, can be used in patients who need cystoplasty.

Lancet 2006; 367: 1241–46

Published Online
April 4, 2006
DOI:10.1016/S0140-6736(06)68438-9

See Comment page 1215

Department of Urology and Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA (A Atala MD, S Soker PhD, J J Yoo MD); and Department of Urology, Children's Hospital Boston and Harvard Medical School, Boston, MA, USA (S B Bauer MD, A B Retik MD)

Correspondence to:
Dr Anthony Atala
aatala@wfubmc.edu

Introduction

A range of injuries can lead to damage or loss of the bladder, necessitating eventual replacement or repair of the organ. Children with congenital anomalies such as bladder exstrophy, myelomeningocele, or posterior urethral valves, can develop high-pressure and hypertonic low-compliant bladders.[1–3] These patients often need cystoplasty when drug treatment fails.

Gastrointestinal segments are frequently used as donor tissues for cystoplasty. However, when such tissues are incorporated into the urinary tract, several complications can ensue, such as metabolic disturbances, urolithiasis, increased mucous production, and malignant disease.[4,5]

Because of the these problems, many investigators over the past 100 years have attempted to use alternative methods, materials, and tissues for replacement or repair of the bladder. The first application of a free tissue graft for bladder replacement was reported by Neuhof in 1917, when fascia was used to augment bladders in dogs.[6] Since that first report, several other materials have been used for free grafts experimentally and clinically, including skin, bladder submucosa, omentum, dura, peritoneum, placenta, seromuscular grafts, and small intestinal submucosa.[7–12] Synthetic materials that have been tried in experimental and clinical settings include polyvinyl sponge, tetrafluoroethylene (Teflon), gelatin sponge, collagen matrices, vicryl matrices, resin-sprayed paper, and silicone.[13–18] These attempts have usually failed because of mechanical, structural, functional, or biocompatibility problems. Usually, permanent synthetic materials succumb to mechanical failure, and urinary stone formation and use of degradable materials lead to fibroblast deposition, scarring, graft contracture, and a reduced reservoir volume over time. Evidently, bladder tissue, with its elastic properties and urothelial permeability, cannot be easily replaced. Therefore, the use of bowel tissue remains the gold standard more than a century after it was proposed, despite associated problems, since no better alternative is available. Anastomoses between two sets of urological tissue are preferable functionally, but the limited amount of autologous urological tissues for reconstruction generally precludes this option. Engineering bladder tissue with selective cell transplantation might provide a means to create functional new tissues.[19]

Cell-based approaches to engineer bladder tissue have been reported,[20–31] and bioengineering has allowed creation of functional neo-bladder tissues in several animal models.[28,32–35] In dogs, successful transplantation of autologous bladders with bioengineering techniques has been reported.[36,37] Our approach in the dog experiments used autologous cells, thus avoiding rejection, wherein a biopsy of tissue is obtained from the host, the cells are dissociated and expanded in vitro, attached to a biodegradable matrix, and reimplanted into the same host. After implantation, histological evidence showed that the engineered bladders continued to develop until they appeared normal anatomically and functionally. Another study[21] showed that engineered muscle formed from cells of normal and diseased bladders showed similar phenotypic and functional properties, suggesting that cells from unhealthy bladders

have the potential to be engineered into normal tissues. Encouraged by these results, we aimed to engineer human bladder tissues by seeding autologous cells on matrices, and to implant these constructs in patients with end-stage bladder disease requiring cystoplasty.

Methods

Study design and patients

The study was approved by the institutional review board and oral informed consent was obtained from the patients' legal guardians. All bladder tissue processing was done at a cell-processing facility approved by the US Food and Drug Administration. Our clinical experience was gathered over 3 years, with up to 5 years of follow-up. The planned slow accrual of patients and long-term follow-up allowed us to modify the protocol whenever improvements were needed. Based on our initial preclinical studies, a collagen matrix derived from decellularised bladder submucosa was used for cell seeding in three patients.[36] Additional animal studies showed that full omental coverage of the implant improved tissue vascularisation, and that collagen-polyglycolic acid (PGA) composite matrices performed better in the long-term.[37] Our subsequent preclinical findings in animals, as well as the findings of limited decreases in bladder compliance with the cell-seeded collagen matrix engineered bladders without the omental wrap once the clinical experience was in progress, prompted us to modify the protocol twice. A cell-seeded collagen matrix bladder with a full omental wrap was implanted in one patient. The last three patients in the series were implanted with cell-seeded composite collagen-PGA bladders wrapped with omentum. Two additional patients, one who had a collagen engineered implant and one who had a composite engineered implant, were lost to follow-up because of personal and social reasons, hence, their consent, as required by the Health Insurance Portability and Accountability Act (which became a requirement after completion of the implantation procedures), could not be obtained for data disclosure, and their results are not included in this manuscript.

All seven patients for whom data were available (three male and four female, mean age 11 years, range 4–19 years) had a poorly compliant bladder due to a myelomeningocele, had frequent urinary leakage as often as every 30 min despite maximum pharmacotherapeutic intervention and frequent intermittent catheterisation, had not responded to medical treatment, and were judged to be candidates for cystoplasty.

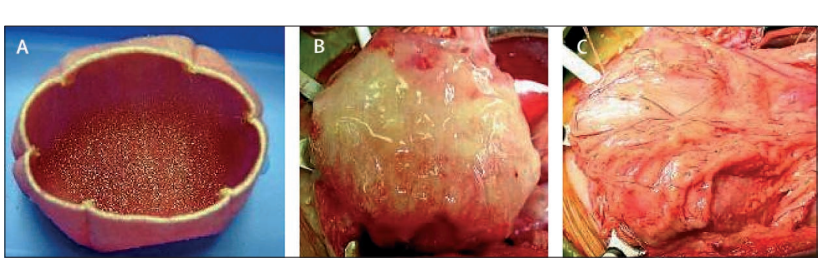

Figure 1: **Construction of engineered bladder**
Scaffold seeded with cells (A) and engineered bladder anastamosed to native bladder with running 4–0 polyglycolic sutures (B). Implant covered with fibrin glue and omentum (C).

Procedures

All patients underwent a urodynamic study, a cystogram, a genitourinary ultrasound, and analyses of serum and urine. 7–8 weeks before the scheduled cystoplasty, patients underwent cystoscopic evaluation, and a bladder biopsy sample (1–2 cm^2) was obtained from the dome through a small suprapubic incision. Urinalyses and cultures were obtained before biopsy. Bacterial cultures were done on the samples when they arrived at the processing facility. The last three patients had 3D CT imaging (CTI Single Slice Helical Quick; GE, Milwaukee, WI, USA) to assess the size, shape, and volume of the pelvic cavity and the state of the preoperative bladder.

Cells were cultured according to existing protocols.[20,36,37] Muscle fragments were plated on culture dishes (Becton Dickinson, Franklin Lakes, NJ, USA) with explant techniques, and cells were expanded in Dulbeccos Modified Eagles Medium (Sigma, St Louis, MO, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA). Urothelial cultures were expanded with keratinocyte growth medium (Gibco). The cells were maintained in a humidified 5% CO_2 incubator at 37°C until seeding. Small vials of early passage cells were stored in a liquid nitrogen tank as backups. The cells and the media constituents were examined regularly for infectious agents until implantation. The cells used for this study required no more than five passages each, with about three or four doublings per subculture stage.

The initial size of the scaffolds used to design the patient's bladder mould ranged from 70 cm^2 to 150 cm^2. Two different types of matrices were created. A scaffold made of homologous decellularised bladder submucosa, which was processed using previously described techniques,[2] was used for the first four patients. A biodegradable composite scaffold made of collagen and PGA (a material used as a mesh for hernia repair; Sherwood Medical, St Louis, MO, USA) was shaped like a bladder with polyglycolic sutures, using a template created specifically for each of the last three patients (figure 1). Using morphometric analyses (Vitrea-2, Vital Images, Plymouth, MN, USA), the volume of the composite bladder construct was calculated by estimating the average expected bladder size according to the patient's age and the dimensions of the pelvic cavity obtained from the CT scan. The total thickness of the scaffolds was about 2·0 mm. All scaffolds were sterilised with ultraviolet light followed by ethylene oxide.

About 70 plates (150 mm diameter) of each cell type, containing about $10{\times}10^6$ cells per plate, were processed to constitute one tissue-engineered bladder. The cells were collected in a 50-mL tube and suspended in a pre-calculated volume of medium to avoid spillage. The exterior surface of the scaffold was seeded with the smooth muscle cells at

a concentration of 50×10^6 per cm^3 with a sterile pipette. The cells seeded on the bladder scaffold were allowed to settle and aggregate in the incubator for 1 h, followed by addition of medium to cover the entire scaffold. Dulbeccos Modified Eagles Medium (Sigma) was changed every 12 h. After 48 h, the urothelial cells were seeded by coating the inside of the scaffold at a concentration of 50×10^6 per cm^3 with the same techniques as for the muscle cells. The scaffold seeded with cells was placed in a sealed container filled with Dulbeccos Modified Eagles medium and keratinocyte growth media mixed at a 1:1 ratio. The cell-seeded scaffold was maintained in a 37°C CO_2 incubator until surgical implantation (3 or 4 days). A small tissue sample from the edge of the scaffold was obtained and examined with phase contrast microscopy and trypan blue analyses before surgical intervention; this assessment confirmed the attachment of the cells in all samples.

The time elapsed between the bladder biopsy and the engineered bladder implantation was about 7–8 weeks. The patients were on a 7-day oral antibiotic course preoperatively. Each patient was anesthetised, and a vertical midline incision was made from one to two finger-breadths below the xiphoid process to the pubis. The bladder was opened with a cruciate incision. Two catheters were placed, one suprapubically that exited through the native bladder tissue and one transurethrally. The cell-seeded scaffold was anastomosed to the native bladder with running 4–0 polyglycolic sutures (figure 1), and fibrin glue prepared from the institutional blood bank was applied to the exterior surface of the scaffold with a dual syringe. A silicone drain was placed in the retroperitoneal cavity. Three patients who had a collagen scaffold implanted did not have an omental wrap surrounding the construct. One patient with the collagen scaffold and the three with the composite scaffolds had their implants fully covered with omentum (figure 1).

Unlike routine enterocystoplasty, patients did not need prolonged post-operative nasogastric drainage and most started an oral diet within the first 24 h. Both urinary catheters were open to drainage. The drain was removed 3–5 days after surgery. Three weeks after surgery, a cystogram was done, one of the urinary catheters was removed, and bladder cycling was initiated. Bladder cycling was done by clamping the urinary catheter intermittently throughout the day and opening it for drainage for 1 h in between each scheduled clamping. The catheter was clamped every other hour on day 1, for 2 h on day 2, for 3 h on day 3, and for 4 h at a time on day 4. On day 4, and for 14 days thereafter, the catheter was open to drainage for 1 h out of every 5 h. The catheter was removed and clean intermittent catheterisation was restarted.

Serial serum analyses (creatinine, electrolytes, arterial gases), renal ultrasounds, voiding cystograms, and urodynamic testing were done post-operatively. Acidosis was defined as arterial bicarbonate less than 22 mmol/L or arterial pH less than 7·35, or both. For each urodynamic study, a 7 Fr triple lumen catheter (Cook Urological; Spencer, IA, USA) was inserted into the bladder, which was filled (10–15 mL/min) with 0·9% saline at 37°C until capacity was reached (when abdominal fullness was noted or urethral leakage occurred). Pressures were monitored continuously on a Dantec Urodyn 5000 (Dantec [now Medtronic], Minneapolis, MA, USA). We recorded abdominal leak point pressures (the intravesical pressure at which the patient leaked urine from the urethra). Studies were done regularly up to 5 years postoperatively. Patients underwent six to eight serial cystoscopic evaluations with cold-cup biopsies of the native and engineered bladder segments. Engineered bladder tissue samples were obtained from the dome, the posterior and anterior lateral walls, and the trigone near the bladder neck region.

Histological staining was done with hematoxylin and eosin. Anti-α smooth muscle actin (1:100 dilution, Novocastra, Newcastle, UK), which labels smooth muscle actin, and anti-pancytokeratins AE1/AE3 (1:100 dilution, Dako, Carpinteria, CA, USA), which react against intermediate filaments in epithelia, were used for immunohistological analyses. Immunolabelling was done with the avidin-biotin detection system and stained by DAB kit (Vector Laboratories, Burlingame, CA, USA). Sections were counterstained with Harris's haematoxylin.

Role of the funding source

There was no external funding source. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Primary cultures of urothelial and smooth muscle cells were routinely expanded and passaged. There was no incidence of cessation of growth or abnormal changes in morphology. The cells were in culture for about 6 weeks for all patients.

All patients tolerated the augmentation cystoplasty procedure. The duration of hospital stay ranged from 23 to 34 days with a mean of 28·5 days (SD 3·8). Postoperatively, one patient had a urinary yeast infection, which was managed appropriately. No other postoperative surgical complications were observed. The patient follow-up ranged from 22 to 61 months with a mean of 46 months (SD 15). Radiographic cystography 3 weeks postoperatively did not show any evidence of urinary leakage. Preoperatively, the voiding cystograms showed reflux in two patients, both of whom had grade 2/5 reflux bilaterally, and neither one underwent ureteral reimplantation at the time of augmentation. Postoperatively, the reflux persisted, but did not progress.

About 3 weeks postoperatively, the patients underwent engineered bladder cycling for 3 weeks followed by catheter removal and the initiation of intermittent catheterisation. All patients were restarted on anticholinergic medication postoperatively, in accord with the routine postcystoplasty protocol. None of the ultrasounds showed any abnormalities

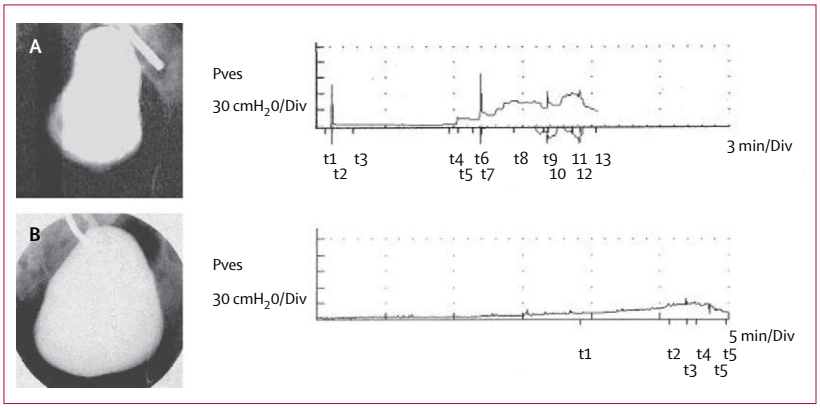

***Figure 2*: Preoperative (A) and 10-month postoperative (B) cystograms and urodynamic findings in patient with a collagen-PGA scaffold engineered bladder**

Note irregular bladder on cystogram, abnormal bladder pressures on urodynamic study preoperatively, and improved findings postoperatively.

except for the presence of a bilateral and a unilateral hydronephrosis in the two patients with pre-existing reflux.

Preoperatively, all patients had a poorly compliant bladder urodynamically. The data were analyzed according to the matrix and procedure used. The postoperative urodynamic studies on patients implanted with the engineered bladders, up to a 61-month follow-up, showed varying degrees of contractility, capacity, and compliance (figure 2, table).

	Patient (implant type)						
	1 (C)	2 (C)	3 (C)	4 (C+O)	5 (PC+O)	6 (PC+O)	7 (PC+O)
Preoperative							
Capacity (mL)	187	335	50	438	201	139	100
Leak point pressure (cm H_2O)	55	45	38	42	51	67	82
Compliance (cm H_2O/mL)	3·4	7·4	1·3	10·4	3·9	2·1	1·2
3–12 months postoperative							
Capacity (mL)	128	..	90	700	245	190	150
Leak point pressure (cm H_2O)	45	..	35	30	30	45	40
Compliance (cm H_2O/mL)	2·8	..	2·6	23·3	8·2	4·2	3·8
13–24 months postoperative							
Capacity (mL)	128	366	60	..	..	180	130
Leak point pressure (cm H_2O)	48	18	54	..	..	44	40
Compliance (cm H_2O/mL)	2·7	20·3	1·1	..	..	4·1	3·3
25–36 months postoperative							
Capacity (mL)	162	..	..	456	250	..	150
Leak point pressure (cm H_2O)	45	..	..	42	25	..	43
Compliance (cm H_2O/mL)	3·6	..	..	10·9	10	..	3·5
37–48 months postoperative							
Capacity (mL)	..	..	63	..	..	..	..
Leak point pressure (cm H_2O)	..	..	36	..	..	..	..
Compliance (cm H_2O/mL)	..	..	1·8	..	..	..	..
49–61 months postoperative							
Capacity (mL)	..	..	78	450	309	480	..
Leak point pressure (cm H_2O)	..	..	45	18	24	47	..
Compliance (cm H_2O/mL)	..	..	1·7	18	13	10·2	..

C=collagen. C+O=collagen plus omental wrap. PC+O=composite collagen-PGA plus omental wrap.

***Table*: Preoperative and postoperative urodynamic findings**

The mean pre-operative leak point pressure for all patients ranged from 38 to 82 cm H_2O (mean 54 cm H_2O, SD 15·5). The mean leak point pressure at capacity (46 cm H_2O, 8·5) decreased postoperatively by 12% to 40·8 cm H_2O (SD 11) in the collagen engineered bladders without an omental wrap. The leak point pressure of the collagen engineered bladder with an omental wrap at capacity decreased from 42 cm H_2O by 29% to a mean of 30 cm H_2O (12). The mean leak point pressure at capacity (67 cm H_2O, SD 15·5) decreased post-operatively by 56% to 37·5 cm H_2O (8·8) in the composite engineered bladders with an omental wrap (table).

Preoperatively, the mean maximum bladder capacity for all patients was 207 mL (SD 136). Postoperatively, the mean maximum bladder capacity in the collagen engineered bladders without an omental wrap (191 mL, 142·5) showed a 30% decrease in volume to a mean volume of 134 mL (100·2). The collagen engineered bladder with an omental wrap showed a 1·22-fold increase in volume from 438 mL to a mean of 535 mL (142·6) postoperatively. The mean maximum bladder capacity in the composite engineered bladders wrapped with omentum showed a 1·58-fold increase in volume from 147 mL (51) to 232 mL (109·7; table).

Postoperative mean compliance increased from 4·0 mL per cm H_2O (3·1) to 4·6 mL per cm H_2O (11; 15%) in the collagen engineered bladders without an omental wrap; from 10·4 mL per cm H_2O to 17·4 mL per cm H_2O (6·4; 67%) in the collagen engineered bladder with an omental wrap; and by 2·79-fold from 2·4 mL per cm H_2O (1·4) to 6·7 mL per cm H_2O (3·7) in the composite engineered bladders with an omental wrap (table).

The intravesical pressures generated by bladder filling remained below 40 cm H_2O postoperatively in all patients, except the first, who had progressively increasing intravesical pressures over time, and required a reoperative cytoplasty 4 years later, which was uneventful. This patient had a 30% smaller collagen matrix reservoir created than the other individuals, and no omental wrap was used.

Preoperatively, the patients leaked urine frequently despite a strict regimen of clean intermittent catheterisation. Postoperatively, the maximum average daytime dry intervals ranged from 1·5 h to 3·5 h for the patients implanted with the collagen engineered bladders with no omental wrap, from 2·5 h to 4·0 h for those implanted with the collagen engineered bladder with an omental wrap, and from 3·0 to 7·0 h for those implanted with the composite engineered bladders with an omental wrap.

Renal function remained stable. Serum creatinine was normal preoperatively (mean 52·8 mmol/L, SD 9·68) and post-operatively (61·6 mmol/L, 14·96). No metabolic abnormalities were noted. All patients had normal serum sodium, potassium, chloride, phosphorus, and arterial blood gases preoperatively and postoperatively. They did not have any evidence of renal or bladder calculi throughout

the study. The mucus production usually evident with the use of gastrointestinal segments for cystoplasty was absent, as expected.

Postoperatively, the margin between the composite matrix-based engineered segments and the native bladders were grossly indistinguishable during cystoscopic evaluation. All the full-thickness biopsies showed a tri-layered structure, consisting of a urothelial cell-lined lumen surrounded by submucosa and muscle, with all the expected components of bladder tissue present. Immunohistochemical analysis confirmed the presence of phenotypically normal smooth muscle and urothelia (figure 3).

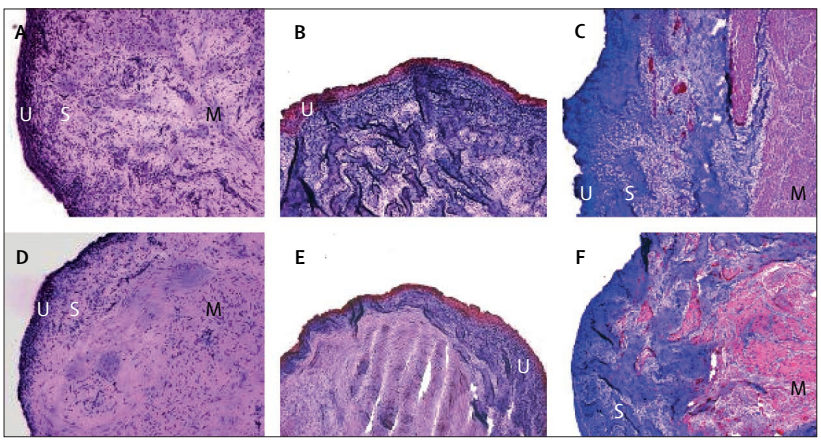

Figure 3: **Morphological analysis of implanted engineered bladders**
(A), (B), (C): cystoscopic biopsies of implanted engineered bladders 31 months after augmentation shows extent of regeneration. Engineered bladder tissue showed tri-layered structure, consisting of lumen lined with urothelial cells (U) surrounded by submucosa (S) and muscle (M). Haemotoxylin and eosin (A), immunocytochemical analysis with anti-pancytokeratin AE1/AE3 antibodies (B), and anti-α smooth muscle actin antibodies (C) showed presence phenotypically normal urothelium and smooth muscle. (D), (E), (F): native bladder tissue. Original magnification: ×100.

Discussion

We engineered human bladder tissues for patients with end-stage bladder disease by isolating autologous bladder urothelial and muscle cells, expanding the cells in vitro, and attaching them to biodegradable three-dimensional matrices.

The goal of the study was to improve the functionality of diseased bladders by decreasing the intravesical pressures, and improving bladder compliance and continence. Patients with a poorly compliant bladder may incur renal damage over time; thus, avoiding any progressive renal disease was also a goal of the study. Additionally, augmentation is usually done with gastrointestinal segments, which can lead to metabolic abnormalities such as acidosis or alkalosis, depending on the segment used, an increased rate of calculi formation, increased mucus production, and enhanced risk of malignant disease.[4,5,38,39] Therefore, we also aimed to avoid any metabolic abnormalities, reduce the incidence of urinary stone formation, and avoid the production of enteric mucous. The engineered bladders were able to be implanted within 7–8 weeks after a bladder biopsy to harvest cells. All patients who received the omental-wrapped collagen-PGA matrix bladders were able to benefit, as defined by the goals we have stated.

The bladders reconstructed with the engineered tissues made with composite-scaffolds were able to show improved function. Follow-up urodynamic studies showed that bladder compliance was improved and capacity was increased. The urodynamic results were similar to those that would be expected with the use of gastrointestinal tissue. However, the patients did not experience any of the ill effects associated with gastrointestinal tissues. Renal function was preserved for the duration of the study. Urinary continence improved markedly in all patients. Histologically and immunohistochemically, tissue biopsies of the engineered bladder segments showed an adequate structural architecture and phenotype.

A major challenge in the engineering of tissues or organs for clinical use has been to develop biodegradable three-dimensional constructs that can accommodate adequate amounts of cells for functional tissue formation. The scaffolds should have the appropriate biomechanical and structural properties needed for the preservation of tissue integrity long-term.[26,29–33,35,40] The use of a composite scaffold, composed of PGA, which supports structural integrity, and collagen, which supports cell growth and survival, proved to be optimal for the engineering of bladder tissue. Another challenge for the engineering of tissues and organs involves the need for the constructs to be sufficiently vascularised to support and maintain the transplanted cells.[41–43] The ideal construct should allow for tissue survival through the delivery of sufficient nutrients and oxygen for enhanced neovascularization. Omentum, commonly used in reconstructive surgery to enhance vascularization of flaps and grafts due to its rich blood supply, was eventually used as a wrap over the bladders at the time of implantation, and this proved to be important for the ultimate success of the engineered bladders.

All patients were on intermittent catheterisation preoperatively, and had abnormal innervation because of their myelodysplasia. When patients with this diagnosis and history undergo cystoplasty, intermittent catheterisation needs to be continued for adequate bladder drainage. Therefore, as in patients undergoing cystoplasty with bowel segments, our patients needed intermittent catheterisation. Although studies in dogs showed that the engineered bladders were reinnervated, leading to normal emptying, the animals had started out with normally innervated bladders that had normal voiding contractions. On the basis of these results, it would be expected that if a patient had a normally innervated bladder to begin with that could empty spontaneously preoperatively, the same would be true postoperatively, although this supposition remains to be tested clinically.

We have shown that tissue engineering techniques can be used to generate bladders that can be implanted in patients requiring cystoplasty. The implanted composite engineered bladders showed improved functional

parameters that were durable over a period of years. Although follow-up of longer than 5 years is reported, additional studies will be needed before this procedure can be used widely.

Contributors

A Atala contributed to design of clinical study, characterisation and analyses of cells, and preparation of bladder scaffolds. S B Bauer and A B Retik contributed to recruitment of patients, preoperative and postoperative assessments, surgery, and data analyses. S Soker was involved with characterisation and analyses of cells. J J Yoo was involved with preparation of bladder scaffolds, cell seeding, and data analyses.

Conflict of interest statement

None of the authors had any conflict of interest at the time of recruitment and selection of patients (1998–2001). Several patents relating to the technology described in this article were assigned to Children's Hospital and were licensed to Tengion in 2003. A Atala serves as a member of the board of directors and A B Retik, S Soker, and J J Yoo serve as consultants to Tengion.

Acknowledgments

We thank Jonathan Roth and Tae Gyun Kwon for their assistance.

References

1 Glassberg KI. Current issues regarding posterior urethral valves. *Urol Clin North Am* 1985; **12:** 175–85.

2 Kiddoo DA, Carr MC, Dulczak S, Canning DA. Initial management of complex urological disorders: bladder exstrophy. *Urol Clin North Am* 2004; **31:** 417–26.

3 Snodgrass WT, Adams R. Initial urologic management of myelomeningocele. *Urol Clin North Am* 2004; **31:** 427–34.

4 McDougal WS. Metabolic complications of urinary intestinal diversion. *J Urol* 1992; **147:** 1199–208.

5 Soergel TM, Cain MP, Misseri R, Gardner TA, Koch MO, Rink RC. Transitional cell carcinoma of the bladder following augmentation cystoplasty for the neuropathic bladder. *J Urol* 2004; **172:** 1649–51.

6 Neuhof H. Fascial transplantation into visceral defects: an experimental and clinical study. *Surg Gynecol Obst* 1917; **25:** 383.

7 Cheng E, Rento R, Grayhack JT, Oyasu R, McVary KT. Reversed seromuscular flaps in the urinary tract in dogs. *J Urol* 1994; **152:** 2252–57.

8 Gleeson MJ, Griffith DP. The use of alloplastic biomaterials in bladder substitution. *J Urol* 1992; **148:** 1377–82.

9 Kelami A, Ludtke-Handjery A, Korb G, Roll J, Schnell J, Danigel KH. Alloplastic replacement of the urinary bladder wall with lyophilized human dura. *Europ Surg Res* 1970; **2:** 195.

10 Probst M, Dahiya R, Carrier S, Tanagho EA. Reproduction of functional smooth muscle tissue and partial bladder replacement. *Br J Urol* 1997; **79:** 505–15.

11 Tsuji I, Ishida H, Fujieda J. Experimental cystoplasty using preserved bladder graft. *J Urol* 1961; **85:** 42–44

12 Vaught JD, Kropp BP, Sawyer BD, et al. Detrusor regeneration in the rat using porcine small intestinal submucosal grafts: functional innervation and receptor expression. *J Urol* 1996; **155:** 374–78.

13 Bona AV, De Gresti A. Partial substitution of urinary bladder with Teflon prothesis. *Minerva. Urol* 1966; **18:** 43.

14 Fujita K. The use of resin-sprayed thin paper for urinary bladder regeneration. *Invest Urol* 1978; **15:** 355.

15 Kudish HG. The use of polyvinyl sponge for experimental cystoplasty. *J Urol* 1957; **78:** 232.

16 Monsour MJ, Mohammed R, Gorham SD, French DA, Scott R. An assessment of a collagen/vicryl composite membrane to repair defects of the urinary bladder in rabbits. *Urol Res* 1987; **15:** 235–38.

17 Rohrmann D, Albrecht D, Hannappel J, Gerlach R, Schwarzkopp G, Lutzeyer W. Alloplastic replacement of the urinary bladder. *J Urol* 1996; **156:** 2094–97.

18 Tsuji I, Kuroda K, Fujieda J, Shiraishi Y, Kunishima K, Orikasa S. Clinical experience of bladder reconstruction using preserved bladder and gelatin sponge in the case of bladder cancer. *J Urol* 1967; **98:** 91.

19 Atala A. Tissue engineering in the genitourinary system. In: Atala A, Mooney D, eds. Tissue engineering. Boston: Birkhauser Press, 1997: 149.

20 Cilento BG, Freeman MR, Schneck FX, Retik AB, Atala A. Phenotypic and cytogenetic characterization of human bladder urothelia expanded in vitro. *J Urol* 1994; **152:** 665–70.

21 Lai JY, Yoon CY, Yoo JJ, Wulf T, Atala A. Phenotypic and functional characterization of in vivo tissue engineered smooth muscle from normal and pathological bladders. *J Urol* 2002; **168:** 1853–57.

22 Atala A, Vacanti JP, Peters CA, Mandell J, Retik AB, Freeman MR. Formation of urothelial structures in vivo from dissociated cells attached to biodegradable polymer scaffolds in vitro. *J Urol* 1992; **148:** 658–62.

23 Atala A, Freeman MR, Vacanti JP, Shepard J, Retik AB. Implantation in vivo and retrieval of artificial structures consisting of rabbit and human urothelium and human bladder muscle. *J Urol* 1993; **150:** 608–12.

24 Pariente JL, Kim BS, Atala A. In vitro biocompatibility assessment of naturally derived and synthetic biomaterials using normal human urothelial cells. *J Biomed Mater Res* 2001; **55:** 33–39.

25 Pariente JL, Kim BS, Atala A. In vitro biocompatibility evaluation of naturally derived and synthetic biomaterials using normal human bladder smooth muscle cells. *J Urol* 2002; **167:** 1867–71.

26 Nakanishi Y, Chen G, Komuro H, et al. Tissue-engineered urinary bladder wall using PLGA mesh-collagen hybrid scaffolds: a comparison study of collagen sponge and gel as a scaffold. *J Pediatr Surg* 2003; **38:** 1781–84.

27 Southgate J, Cross W, Eardley I, Thomas DF, Trejdosiewicz LK. Bladder reconstruction—from cells to materials. *Proc Inst Mech Eng [H]* 2003; **217:** 311–16.

28 Fraser M, Thomas DF, Pitt E, Harnden P, Trejdosiewicz LK, Southgate J. A surgical model of composite cystoplasty with cultured urothelial cells: a controlled study of gross outcome and urothelial phenotype. *BJU Int* 2004; **93:** 609–16.

29 Pattison MA, Wurster S, Webster TJ, Haberstroh KM. Three-dimensional, nano-structured PLGA scaffolds for bladder tissue replacement applications. *Biomaterials* 2005; **26:** 2491–500.

30 Schultheiss D, Gabouev AI, Cebotari S, et al. Biological vascularized matrix for bladder tissue engineering: matrix preparation, reseeding technique and short-term implantation in a porcine model. *J Urol* 2005; **173:** 276–80.

31 Danielsson C, Ruault S, Basset-Dardare A, Frey P. Modified collagen fleece, a scaffold for transplantation of human bladder smooth muscle cells. *Biomaterials* 2006; **27:** 1054–60.

32 Schoeller T, Lille S, Stenzl A, et al. Bladder reconstruction using a prevascularized capsular tissue seeded with urothelial cells. *J Urol* 2001; **165:** 980–85.

33 Kanematsu A, Yamamoto S, Noguchi T, Ozeki M, Tabata Y, Ogawa O. Bladder regeneration by bladder acellular matrix combined with sustained release of exogenous growth factor. *J Urol* 2003; **170:** 1633–38.

34 Nuininga JE, van Moerkerk H, Hanssen A, et al. A rabbit model to tissue engineer the bladder. *Biomaterials* 2004; **25:** 1657–61.

35 Sievert KD, Fandel T, Wefer J, et al. Collagen I:III ratio in canine heterologous bladder acellular matrix grafts. *World J Urol* 2006; **24:** 101–09.

36 Yoo JJ, Meng J, Oberpenning F, Atala A. Bladder augmentation using allogenic bladder submucosa seeded with cells. *Urology* 1998; **51:** 221–25.

37 Oberpenning F, Meng J, Yoo JJ, Atala A. De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. *Nat Biotechnol* 1999; **17:** 149–55.

38 Gilbert SM, Hensle TW. Metabolic consequences and long-term complications of enterocystoplasty in children: a review. *J Urol* 2005; **173:** 1080–86.

39 Mingin GC, Stock JA, Hanna MK. Gastrocystoplasty: long-term complications in 22 patients. *J Urol* 1999; **162:** 1122–25.

40 Brown AL, Brook-Allred TT, Waddell JE, et al. Bladder acellular matrix as a substrate for studying in vitro bladder smooth muscle-urothelial cell interactions. *Biomaterials* 2005; **26:** 529–43.

41 Cassell OC, Hofer SO, Morrison WA, Knight KR. Vascularisation of tissue-engineered grafts: the regulation of angiogenesis in reconstructive surgery and in disease states. *Br J Plast Surg* 2002; **55:** 603–10.

42 Nomi M, Atala A, Coppi PD, Soker S. Principals of neovascularization for tissue engineering. *Mol Aspects Med* 2002; **23:** 463–83.

43 De Coppi P, Delo D, Farrugia L, et al. Angiogenic gene-modified muscle cells for enhancement of tissue formation. *Tissue Eng* 2005; **11:** 1034–44.