RESEARCH ARTICLE

Long-term durability, tissue regeneration and neo-organ growth during skeletal maturation with a neo-bladder augmentation construct

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Aims: To comparatively evaluate bladder regeneration following 80% cystectomy and augmentation using a synthetic biopolymer with autologous urothelial and smooth muscle cells (autologous neo-bladder augmentation construct [construct]) or autotransplantation of native bladder (reimplanted native urinary bladder [reimplant]) in canines. **Materials & methods:** Voiding function, urodynamic assessment and neo-organ capacity-to-body-weight ratio (C:BW) were assessed longitudinally for a total of 24 months following trigone-sparing augmentation cystoplasty in juvenile canines. **Results:** Within 30 days postimplantation, hematology and urinalysis returned to baseline. Constructs and reimplants yielded neo-organs with statistically equivalent urodynamics and histology. Linear regression analysis of C:BW showed that constructs regained baseline slope and continued to adapt with animal growth. **Conclusions:** Constructs and reimplants regained and maintained native bladder histology by 3 months, capacity at 3–6 months and compliance by 12–24 months. Furthermore, construct C:BW demonstrated the ability of regenerated bladder to respond to growth regulation.

Keywords: enterocystoplasty, neo-bladder, organ regeneration, organ repair, regulative development, tissue healing

The process of regeneration is associated with maintenance or restoration of the original structure and function of a tissue or organ [1]. However, an injury that exceeds the regenerative capacity of a tissue triggers another mechanism, healing by repair, which covers a wound with a scar of fibrous tissue and structural elements that are different from the original [1]. The body's response to injury is the sum of several factors including intention, immunological competence, age, tissue/organ and the urgency to restore homeostasis [1,2].

Regenerative medicine's goal is to develop products that consistently and effectively restore function and structure to damaged tissue and whole organs without the side effects associated with transplantation or the scar associated with healing by repair. Regenerative medical products must also demonstrate durability and adaptability, particularly for pediatric applications, where outcomes are measured in years and are subjected to growth-related changes [3]. Regenerative medicine seeks outcomes superior to those achieved currently with transplantation or simple tissue engineering. Regenerative medicine approaches have been applied clinically to neurogenic bladder disease that is refractory to medical treatment [4]. Neurogenic bladder disease affects the urinary bladder wall and causes bladder noncompliance and elevated intravesical pressure. When anticholingeric medications and clean intermittent catheterization are ineffective, patients are at risk for hydronephrosis and/or end-stage renal disease. Augmentation enterocystoplasty uses a segment of autologous bowel for bladder reconstruction and capacity increase, but can be associated with significant morbidity and metabolic complications [5].

Tissue-engineering approaches to repair large bladder defects began in the early 1900s. Avoiding or overcoming dominant signals that lead to repair by fibrosis has been a challenge [6–8]. A regeneration milestone was achieved for the urinary bladder in canines [9] and humans [4] by using a synthetic biocompatible scaffold material seeded with autologous urothelial cells (UCs) and smooth muscle cells (SMCs). However, characterizations of the structural and functional outcomes in animal models have not exceeded 1 year [9,10]. Our purpose was to evaluate long-term safety, durability and the bladder capacity-to-body-weight (C:BW) ratios of neo-bladders over a 24-month period of growth from juvenile to adult in an established canine model of augmentation cystoplasty [9]. A poly-DL-lactide-co-glycolide (PLGA)-based biodegradable mesh scaffold with autologous UCs and SMCs (autologous neo-bladder augmentation construct [construct]) from normal bladder was the manufactured tissue substitution test implant. Reimplantation of cystectomized bladder (reimplanted native urinary bladder [reimplant]) was chosen as the comparative control implant

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because enterocystoplasty in canines with normal bladders would have unnecessarily introduced morbidity and metabolic consequences that would have compromised longevity and animal growth. Scaffold alone was not evaluated as a control implant in this study because a PLGA-based biodegradable mesh scaffold without cells induced a fibrotic healing response in previous shorter term studies [10]. Omentum provided a source for vasculature remodeling and tissue oxygenation for constructs and reimplants.

We hypotheszied that constructs would elicit equivalent or superior bladder healing and regeneration, durability and growth in the recipient animal compared with reimplants based upon previously published findings involving shorter study durations and fewer animals [9,10].

Experimental protocol

Animals, study design & surgery

Animal procedures were performed in accordance to Institutional, State and Federal regulations [11]. Purpose-bred, standard laboratory male and female mongrel canines (32 animals each gender) were randomly assigned to construct and reimplant groups (Table 1). Animals were anesthetized with isoflurane inhalant anesthetic during surgery. Cystectomy removed approximately 80% of native bladder tissue and spared only the trigone. Constructs were produced from scaffolds of approximately 70 ml volume (see Construct preparation below); reimplant volume was not measured. Implants were attached with resorbable sutures and wrapped with omentum. Prior to closure, implants were tested for leaks using a Foley catheter. Omentum was secured with suture or surgical adhesive (fibrin based) at the discretion of the surgeon to achieve a leak-free implant and omental approximation to the construct. Post-surgery, animals received analgesic therapy (buprenorphine, 0.01 mg/kg, subcutaneous) for up to 3 days, voided spontaneously and regained continence within 1 week of Foley/suprapubic catheter removal. At 1, 3, 6, 9, 12, 18 and 24 months postimplantation, animals were clinically evaluated and euthanized for histological evaluations.

Construct preparation

3D bladder-shaped scaffolds of 67 ml were formed from nonwoven PGA felts having bulk density values ranging from 70 to 100 mg/cc and thickness values ranging from 2.5 to 3.5 mm (Biomedical Structures, Warwick, RI, USA) and PLGA 50:50 (Sigma-Adrich, St Louis, MO, USA), packaged, sterilized with ethylene oxide and stored in a desiccator until seeding. Bladder tissue (1–4 cm^2) was harvested from the 32 animals in the construct group by transmural bladder biopsy. SMCs and UCs were isolated,

Table 1. Study design and postsurgical course.

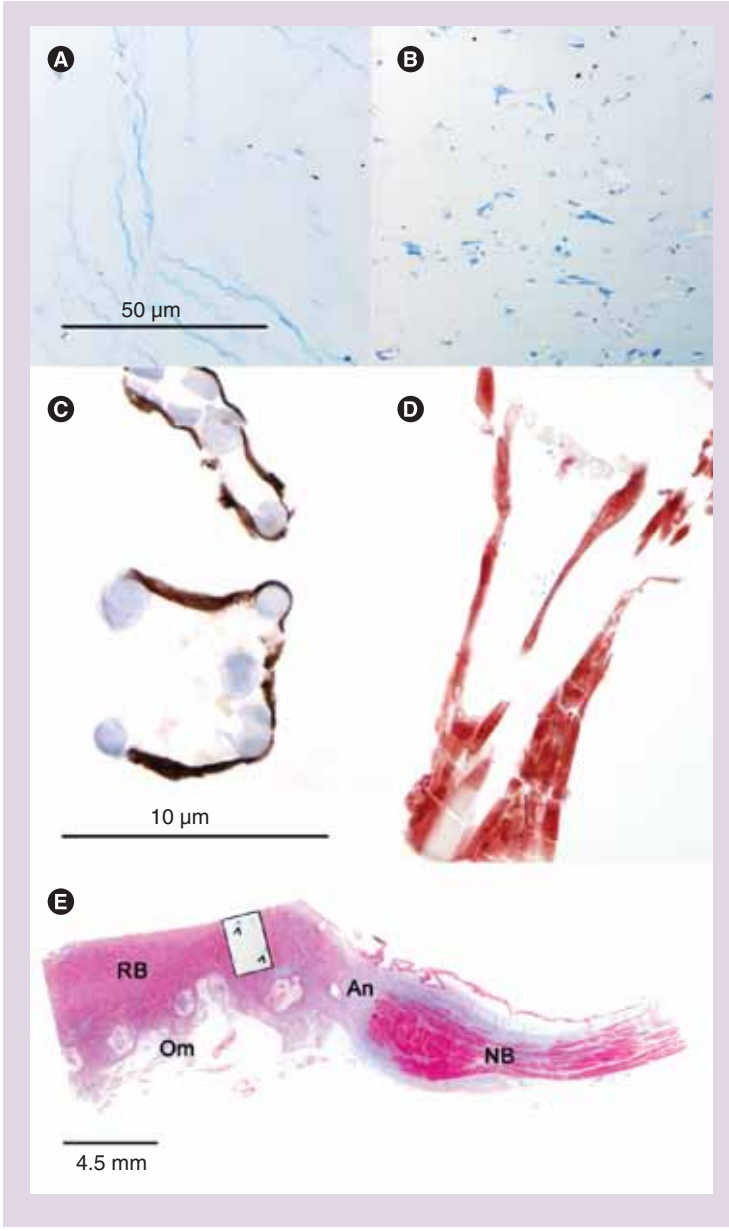
		Construct	Reimplant (control)
Total n		32 (16M/16F)	32 (16M/16F)
Age		Less than 1 year at implantation	
n at necropsy; planned (actual)	1 mo	4 (4)	4 (4)
	3 mo	4 (4)	4 (4)
	6 mo	4 (4)	4 (3*)
	9 mo‡	8 (8)	8 (8)
	12 mo	4 (4)	4 (4)
	18 mo	4 (4)	4 (4)
	24 mo	4 (4)	4 (4)
Urethral Foley catheter		Removed within 7 days p.i.	
Suprapubic indwelling catheter		Removed within 21 days p.i.	
Post-surgical observations of decreased appetite and lethargy		Resolved within 14 days p.i.	
Clinical pathology: urinalysis and hematology		Hematuria and inflammatory response consistent with surgery and acute-phase response to implant – normalized by 30 days p.i.	

**One reimplant group animal was euthanized at 4 days p.i. due to bladder wall perforation/rupture of undetermined cause.*

‡The cohort of 64 animals was studied in two groups. 9 months was the final necropsy of the first group and the first necropsy of the second group.

p.i.: Postimplantation.

Figure 1. Construct preparation and early regeneration.



(A & B) Histological sections of scaffold stained with Toluidine blue. Unseeded scaffold **(A)** and construct composed of scaffold and microcolonies of urothelial cells and smooth muscle cells at seeding **(B)**. **(C & D)** Histological sections of autologous cell-seeded scaffolds. Immunohistochemical with pancytokeratin AE1/AE3 stains urothelial cells brown **(C)**. Smooth muscle cells are stained red by Masson's Trichrome **(D)**. **(E)** Histological section of RB at 1 month postimplantation. An, NB and Om are labeled. Inset represents the actual size of a construct at the time of implantation.
Arrowheads (^) indicate colonies of urothelial and smooth muscle cells at time of implantation.
An: Anastomotic junction; NB: Native bladder wall; Om: Omentum; RB: Regenerating bladder wall.

characterized, expanded separately and seeded onto sterile scaffolds according to previously published protocols [9,12,13]. SMCs were isolated by dissecting the smooth muscle layer of biopsy into fragments for explant culture and UCs were disassociated manually from the mucosa into culture medium. SMCs and UCs were cultured in Dulbecco's modified eagle's medium with fetal calf serum and serum-free keratinocyte medium with EGF and bovine pituitary extract (BPE; Invitrogen, Carlsbad, CA, USA) for 5–7 weeks. Bovine-derived medium components are sourced from bovine spongiform encephalopathy-free or low-risk countries and assured through vendor qualification and verification of certificates of analysis, and residual levels are reduced to 1:1,000,000 concentration of input with EGF-free, BPE-free and fetal bovine serum-free medium prior to implantation.

Sterile scaffolds were hydrated in culture medium, seeded with approximately 1.5×10^8 of each UCs and SMCs a few days before implantation, and seeded cell viability was confirmed by measuring metabolite consumption.

Urodynamics

Bladder capacity and intravesical pressure was measured on dual-lumen, catheterized animals following removal of residual urine. One line was connected to a pressure-monitoring device and sterile saline (~37°C) was infused at 20 ml/min until fluid leakage was observed around the cathether (leak point). Volume (ml) of instilled saline (capacity) and intravesical pressure (cm H_2O) was recorded at leak point. Compliance values were calculated by dividing the change in bladder capacity by the change in bladder pressure from baseline to leak point.

Histological evaluation

At necropsy, neo-bladders were distended to the same volume and pressure measured by prenecropsy urodynamics and fixed in 10% buffered formalin (Sigma-Adrich). Sections from bladder walls were stained with Masson's Trichrome to visualize stromal and muscle components.

Statistical analyses

Means, medians, standard deviations, frequency distributions, regression analyses, p- and f-values, and 95% confidence intervals (CI) were calculated with Excel (Microsoft) and JMP™ (SAS Institute, Cary, NC, USA).

Results

The purpose of this study was to longitudinally evaluate the postsurgical course, durability and the ability of neo-bladder capacity to adapt to

Table 2. Hematology and serum chemistry: construct group.

			Construct group				
			Baseline	***0–3 months***	***3–6 months***	***6–12 months***	***12–24 months***
Hematology							
	Number of measurements		66	274	167	204	157
	WBC	Mean ± SD	9.96 ± 2.71	13.21 ± 3.96	10.53 ± 1.78	9.47 ± 1.85	8.38 ± 1.68
		95% CI	9.30–10.61	12.74–13.68	10.26–10.80	9.21–9.72	8.11–8.64
	HCT	Mean ± SD	43.07 ± 9.81	44.43 ± 5.26	48.02 ± 3.87	49.30 ± 4.25	52.25 ± 4.33
		95% CI	40.82–45. 32	43.81–45.05	47.43–48.60	48.72–49.89	51.58–52.93
Serum chemistry							
	Number of measurements		70	280	167	205	157
	ALP	Mean ± SD	81.16 ± 31.94	70.73 ± 29.42	46.51 ± 16.43	42.36 ± 15.17	32.76 ± 12.11
		95% CI	73.68–88.64	67.29–74.18	44.02–49.00	40.28–44.43	30.86–34.65
	CREAT	Mean ± SD	0.813 ± 0.166	0.856 ± 0.153	0.956 ± 0.182	0.924 ± 0.136	0.996 ± 0.143
		95% CI	0.774–0.852	0.838–0.874	0.928–0.983	0.906–0.943	0.973–1.018

ALP: Alkaline phosphatase; CI: Confidence interval; CREAT: Creatinine; HCT: Hematocrit; SD: Standard deviation; WBC: White blood cell.

animal growth over 24 months following augmentation cystoplasty with a combination of PLGA-based biodegradable mesh scaffold and autologous UCs and SMCs (construct) or a reimplanted autologous native bladder (reimplant).

Construct production

Biopsies yielded up to 6.5×10^6 UCs and over 3×10^6 SMCs for primary culture and expanded to an average of 1.5×10^8 cells in serial passages. Figure 1 shows representative stages of construct production and regenerated tissue at 1 month postimplantation.

Implantation of constructs (test group) & reimplants (control group)

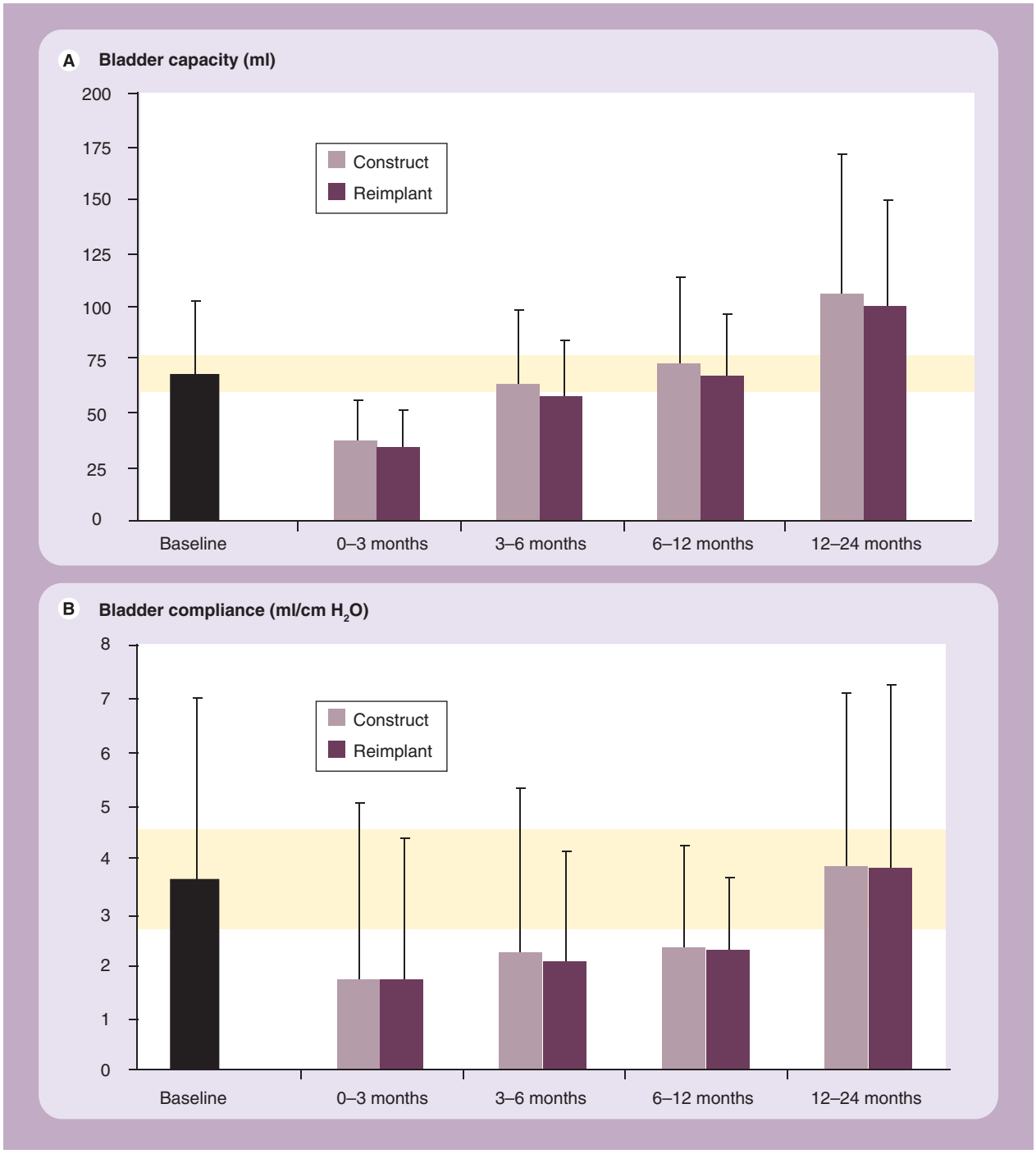
Study design, group characteristics, necropsy schedule and postsurgical course are summarized in Table 1. Prior to surgery, baseline urodynamics were obtained from 54 out of 64 animals. In all canines, the trigone was spared, leaving an intact sphincter for continence. Bladders were transected less than 1 cm above the trigone. In reimplant animals, the transected tissue was immediately reattached after resection. In construct animals, the scaffold with cells was anastomosed. Omentum was used to approximate a vascular source and form a leak-free barrier for

Table 3. Hematology and serum chemistry: reimplant group.

			Reimplant group				
			Baseline	***0–3 months***	***3–6 months***	***6–12 months***	***12–24 months***
Hematology							
	Number of measurements		66	260	161	207	156
	WBC	Mean ± SD	9.96 ± 2.71	10.83 ± 2.56	10.89 ± 2.12	9.59 ± 2.13	8.71 ± 1.82
		95% CI	9.30–10.61	10.52–11.14	10.56–11.22	9.30–9.88	8.42–8.99
	HCT	Mean ± SD	43.07 ± 9.81	44.72 ± 3.40	47.24 ± 3.74	47.39 ± 3.88	49.70 ± 4.31
		95% CI	40.82–45.32	44.30–45. 13	46.66–47. 82	46.86–47.92	49.02–50.38
Serum chemistry							
	Number of measurements		70	266	162	209	156
	ALP	Mean ± SD	81.16 ± 31.94	82.55 ± 31.27	52.30 ± 20.55	43.95 ± 15.17	36.28 ± 11.96
		95% CI	73.68–88.64	78.79–86.31	49.13–55.46	41.89–46.00	34.41–38.16
	CREAT	Mean ± SD	0.813 ± 0.166	0.936 ± .708	0.944 ± 0.127	0.909 ± 0.211	0.926 ± 0.127
		95% CI	0.774–0.852	0.851–1.021	0.924–0.963	0.880–0.938	0.906–0.946

ALP: Alkaline phosphatase; CI: Confidence interval; CREAT: Creatinine; HCT: Hematocrit; SD: Standard deviation; WBC: White blood cell.

Figure 2. Bladder capacity and compliance.



Mean values for bladder capacity **(A)** and compliance **(B)** assessments at baseline (presurgery, all animals), 0–3 months, 3–6 months, 6–12 months and 12–24 months postimplantation are presented. Error bars = standard deviation. The 95% confidence interval for baseline measurements is represented by the yellow-shaded area.

both constructs and reimplants. Absence of leakage was confirmed in all animals prior to closing the abdominal incision and skin.

Postsurgical course was similar between groups. The most frequent observations were a temporary lack of appetite and minimal lethargy during the first 2 weeks following postimplantation. In most cases, and regardless of group, animals spontaneously urinated and were continent within 1 week after suprapubic catheter removal (Table 1).

Table 4. Bladder capacity and compliance: statistical summary.

		Baseline	0–3 months		3–6 months		6–12 months		12–24 months	
			Construct	*Reimplant*	*Construct*	*Reimplant*	*Construct*	*Reimplant*	*Construct*	*Reimplant*
	Measurements*	57	79	84	71	67	100	99	74	75
	Animals‡	64	28–32	27–31	24–28	23–27	12–24	11–23	4–12	3–11
	Mean ± SD	67.5 ± 35.5	36.3 ± 20.9	33.9 ± 18.2	63.3 ± 36.3	57.5 ± 27.2	73.6 ± 41.1	67.9 ± 29.6	106.1 ± 66.6	100.2 ± 51.0
Capacity (ml)	Median	66	33	29.5	54	52	66	66.0	79.5	94
	95% CI	58.1–76.9	31.6–41.0	30.0–37.9	54.7–71.9	50.9–64.2	65.4–81.7	62.0–73.8	90.7–121.5	88.5–112.0
	Mean ± SD	3.6 ± 3.4	1.7 ± 3.3	1.7 ± 2.6	2.2 ± 3.1	2.1 ± 2.1	2.3 ± 1.9	2.3 ± 1.4	3.9 ± 3.3	3.8 ± 3.4
Compliance (ml/cm H_2O)	Median	2.65	1.103	1.146	1.257	1.579	1.793	1.978	2.540	3.035
	95% CI	2.7–4.5	1.0–2.5	1.1–2.3	1.5–3.0	1.6–2.6	1.9–2.7	2.0–2.5	3.1–4.6	3.0–4.6

**Measurements were taken presurgery (baseline), monthly and just prior to necropsy. The number of measurements within each time interval is not an exact multiple of viable animals because occasionally a measurement could not be obtained owing to technical difficulties.*

*‡Interim sacrifices at 1, 3, 6, 9, 12, 18 and 24 months reduced the number of animals remaining during each interval (see **Table 1**).*

CI: Confidence interval; SD: Standard deviation.

Time course of bladder healing

Urinalysis

Proteinuria and hematuria were evident in both groups at 2 weeks postimplantation. White blood cell (WBC) counts in urine were higher in construct animals at 2 weeks postimplantation. By 8 weeks, all profiles returned to baseline.

Hematology & serum chemistry

Except for a slightly more elevated WBC count at 0–3 months in construct animals (Table 2), hematology and serum chemistries were similar between construct and reimplant (Table 3) animals. Hematocrit, alkaline phosphatase (ALP) and creatinine levels stayed within normal ranges. ALP levels decreased over time in both groups, as expected for a 24-month study of animals implanted at less than 1 year of age experiencing skeletal maturation and growth plate fusion associated with regulative development of the skeletal system [14].

Urodynamic assessment of construct & reimplant neo-organs

Postimplantation capacity and compliance profiles for constructs and reimplants were equivalent (Figure 2; Table 4). Capacity and compliance were decreased in the initial 3 months postimplantation, but steadily increased over time. Neobladder capacities achieved levels within the 95% CI of baseline at 3–6 months postimplantation and exceeded baseline at 12–24 months postimplantation. Baseline compliance was regained at 12–24 months for both groups (Figure 2) and remained constant to study completion.

Neo-bladder wall histology

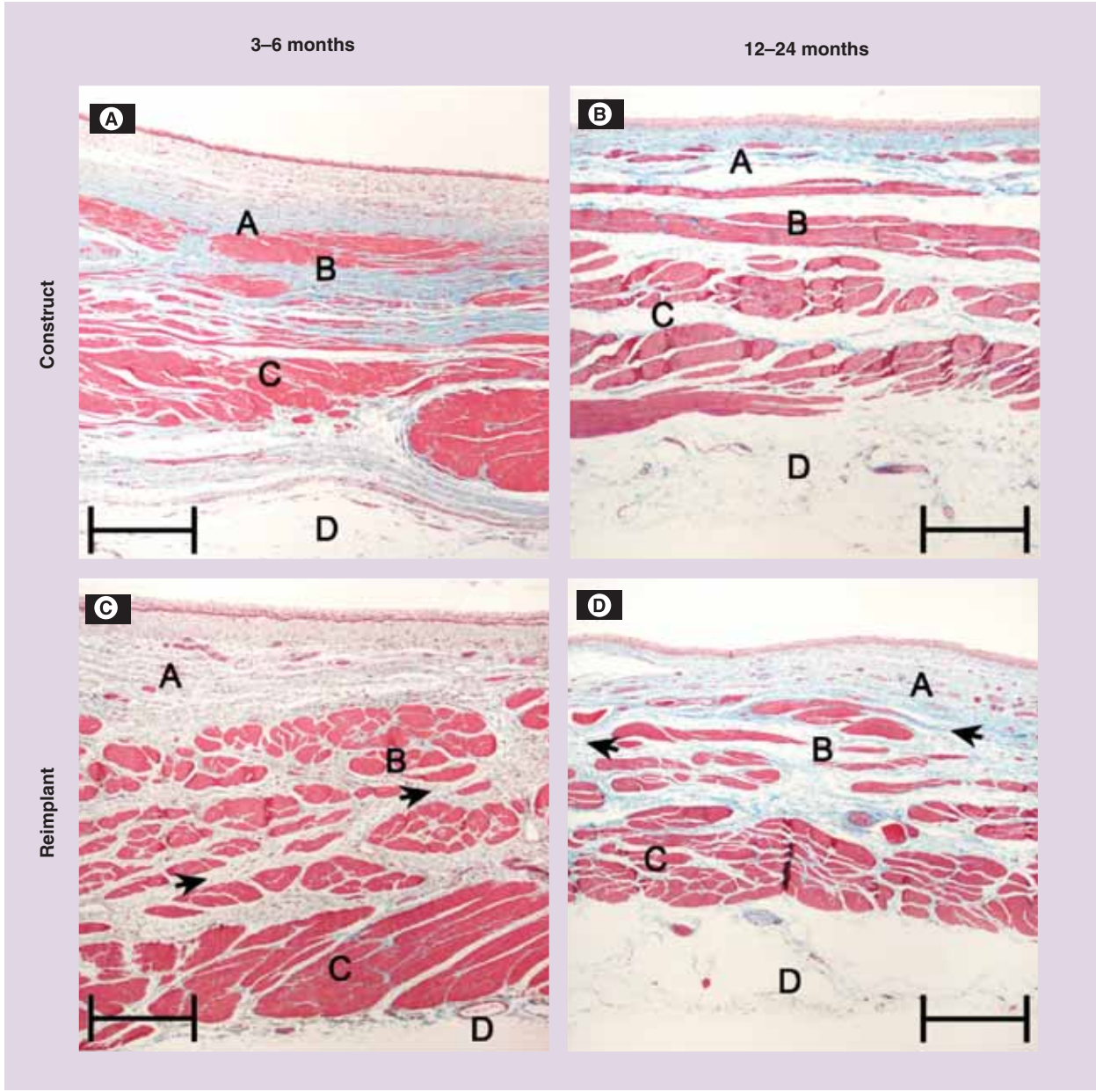
All three muscle layers: an inner and outer longitudinal and a middle circular were present in the bladder wall of both groups, including a characteristic histological pattern of interweaving muscle layers [15] by 6 months. Construct and reimplant neobladders during the 12–24-month period exhibited a normal tissue structure. Detrusor muscle tissue of the construct's middle circular layer (Figure 3A & B, label B) appears linear and continuous. By contrast, detrusor muscle bundles in reimplant middle circular muscle layer (Figure 3C & D, label B) appears divided by cicatrization (blue-staining tissue found between the red-stained muscle in Trichrome; indicated by arrowheads in Figure 3C & D).

Growth of neo-organs during skeletal maturation

Regulative development of postimplant neobladders was investigated by regression analysis,

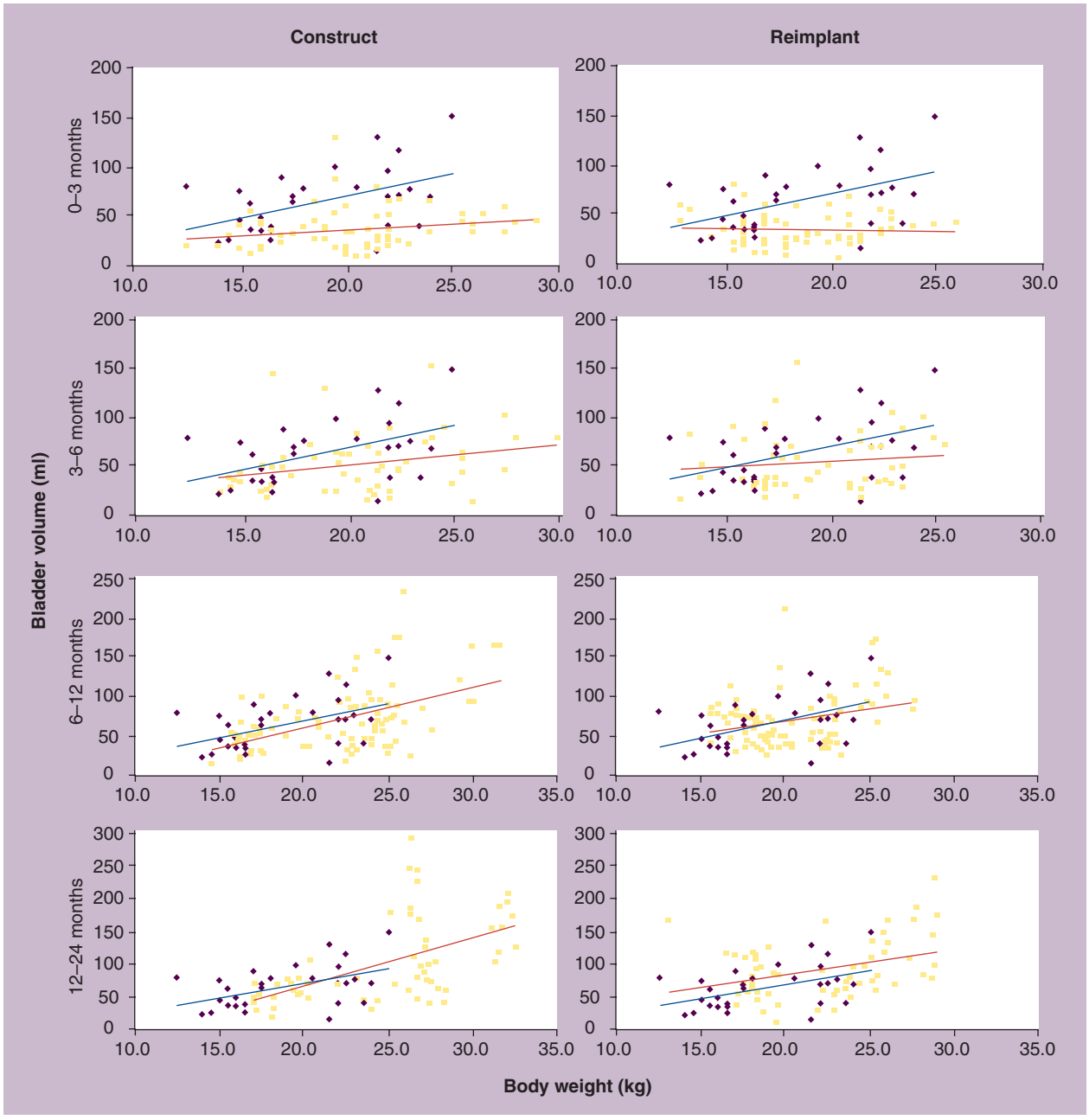
measuring bladder capacity and body weight at baseline, 0–3 months, 3–6 months, 6–12 months and 12–24 months postimplantation (Figure 4). At baseline, native bladder capacity and body weight showed linearity (Table 5), with significant correlation ($p < 0.01$). At 3–6 months, the linearity between construct neo-bladder capacity and body weight again reached significance ($p < 0.05$) and construct and reimplant animals reached and maintained significance at 6–12 months to 12–24 months ($p < 0.0001$ and $p < 0.005$, respectively).

Figure 3. Bladder wall histology.



Bladder-wall samples fixed at leak point were obtained from necropsied bladders and stained with Masson's Trichrome as described in the experimental protocol. Representative bladder-wall histology from construct **(A & B)** and reimplant **(C & D)** neo-organs at 3–6 months **(A & C)** and 12–24 months **(B & D)** postimplantation. The three muscle layers of canine bladders: inner longitudinal **(A)**, middle circular **(B)** and outer longitudinal **(C)** are indicated, as is the underlying serosa **(D)**. Luminal surface of bladder wall is at the top of each panel.
Bar = 0.5 mm

Figure 4. Regression analysis of bladder capacity and body weight.



Each scatter diagram plots bladder capacity (y-axis) and body weight (x-axis) of construct (left panel: yellow) and reimplant (right panel: yellow) animals at 0–3 months, 3–6 months, 6–12 months and 12–24 months postimplantation. Baseline measurements (blue) are included in each panel for comparison. Equations of best fit, ANOVA test results and number of measurements plotted are summarized in **Table 4**.
ANOVA: Analysis of variance.

Discussion

The purpose of this study was to longitudinally evaluate postsurgical course, durability and regulative development following augmentation cystoplasty with a PLGA-based biodegradable mesh scaffold with autologous urothelial and smooth muscles cells (construct) as compared with reimplanted autologous native bladder (reimplant) in juvenile animals undergoing maturation over a 2-year period. Reimplanting autologous native bladder tissue corresponded to the best-case scenario of the transplantation paradigm for organ

Table 5. Linear regression analysis of bladder capacity and body weight.

	Baseline	0–3 months		3–6 months		6–12 months		12–24 months	
		Construct	*Reimplant*	*Construct*	*Reimplant*	*Construct*	*Reimplant*	*Construct*	*Reimplant*
Equation (y=)	4.44x - 18.8	1.26x + 10.4	-0.15x + 37.4	2.05x + 12.2	1.34x + 29.0	5.12x - 41.2	3.44x + 0.1	7.39x - 80.8	3.91x + 6.8
R^2	0.2275	0.0481	0.0007	0.0711	0.0208	0.2516	0.0974	0.3127	0.1310
F value	8.2471	3.3884	0.0436	4.1347	1.0606	29.915	9.2797	25.4778	9.0457
P value	0.0077	0.0701	0.8352	0.0469	0.3080	<0.0001	0.0031	<0.0001	0.0038
n (measurements)	30	69	62	56	52	92	88	58	62

healing. As expected, reimplanted bladders exhibited durable healing. In addition, construct neo-bladders also demonstrated durability. Therefore, the hypothesis that constructs would elicit equivalent or superior bladder healing and regeneration, durability and growth with the recipient animal compared with reimplants was supported.

Clinical pathology

Postsurgical urinalysis of red blood cells, WBCs and protein levels were consistent with the surgical procedure and returned to baseline within 8 weeks. Normal creatinine levels (Table 2) and lack of histological evidence of renal disease postsurgery (data not shown) support the conclusion that transient proteinuria was postrenal and related to the regenerative process occurring in the bladder wall, likely originating from newly developing blood vessels.

The pattern of hematocrit increase and ALP decrease over time is consistent with other long-term studies in canines of approximately the same age as this study [15]. ALP is a serum biomarker that shows a steady decrease during skeletal maturation in canines and reflects regulative development of the skeletal system in these animals [14]. ALP levels were similar and decreased by approximately 35 U/l over the study in construct and reimplant groups (Table 2), indicating that animals in both groups experienced equivalent skeletal growth and maturation.

WBC counts were elevated in the blood (Table 2) and urine in the first month postimplantation in both groups, but slightly more elevated in the construct group. PLGA material persists for up to 3 months postimplantation, and inflammatory cells are observed near PLGA material [10], suggesting that the acute-phase response was linked to the surgical procedure and the presence of PLGA material in the construct.

Continence & voiding

Implantation surgery in this study spared the trigone and left the sphincter intact; therefore, restoration of continence after suprapubic catheter removal was expected in both groups. Although the trigone-sparing transection during implantation defunctionalized the bladder, Machado demonstrated that defunctionalized bladders regained native tissue responses to electrical field stimulation and carbachol stimulation when re-anastomosed [16]. We conclude that similar mechanisms allowed the reimplant animals to spontaneously void by abdominal tone (valsalva). The observation that construct animals were also able to spontaneously void by valsalva is further support for the conclusion that the neo-bladder tissue healed by regeneration and not repair. Further studies are needed to elucidate the tissue structures responsible for construct animals' control mechanism for voiding.

Structure & function measurements

Urodynamic measurements assess the bladder wall's viscoelasticity, that is, its ability to structurally adjust and accommodate increasing volumes of urine. Baseline urodynamics in both construct and reimplant groups were restored by 6 months and maintained throughout the study's duration of 2 years. Histologically, the tissue structures of the organs in the construct and reimplant groups at 12–24 months postimplantation were indistinguishable from each other (Figure 3).

The observed favorable comparability of the construct neo-bladders with the reimplant bladders over the course of this study contrasts markedly with previous observations of cell-free PLGA-based biodegradable mesh scaffolds. Construct neo-bladders restore and maintain native urodynamics for 24 months. Diverging from this result, the tissue formed after implantation in a similarly cystectomized bladder of cell-free PLGA-based biodegradable mesh scaffold lacked extended durability [10]. These contrasting results demonstrate that construct cellular components are associated with regenerative healing. This study's functional and morphological findings support the conclusion that regenerative healing occurs following construct

implantation, which is distinct from the healing-by-repair process observed after implantation of cell-free scaffolds.

Neo-organ growth during skeletal maturation

Restoration of shape and size of organs after cell or tissue loss is a characteristic known as regulative development [2]. The determinants of vertebrate organ size are poorly understood, but recent studies have indicated that the progenitor cell pool (e.g., cell number, regulation of cell proliferation and apoptosis) is important for achieving the target size for a visceral organ [2]. During development and regeneration, the final shape and size of organs can be restored after cellular loss.

Although the internal regulation that maintains organ size is complex [2], a relationship between solid organ mass and body weight has been demonstrated by regression analyses in humans and animals [17,18]. In the current study, regression analysis of native bladder C:BW revealed a similar relationship and was used to investigate neo-bladder growth compensation in construct and reimplant animals.

Construct neo-bladders not only achieved a size consistent with the animals' body weights as early as 6 months, but bladder capacity continued to adjust to body weight at a rate consistent with that of native bladder (Figure 3). In earlier studies of shorter duration [9,10], cell-free scaffold implants healed with fibrosis and bladder capacities failed to return to baseline by 9 months. These findings suggest that the cellular composition of the construct was involved in eliciting a regenerative healing response that reconstituted native bladder-like size, structure and function. The reimplant group also regained linearity between bladder capacity and body weight, but was delayed slightly compared with the construct group (Figure 4; Table 5).

A complete analysis of regulative development would require a focused study with age-matched, untreated control animals; therefore, the bladder C:BW analysis conducted in this study has limited external validity. However, these results demonstrate that construct neo-bladders are able to grow with recipients during skeletal maturation from juvenile to adult in a manner consistent with a normal bladder-to-body-weight ratio.

Few studies have evaluated bladder capacity in postenterocystoplasty patients and the results are inconclusive. Greenwell *et al.* observed 'durable' capacity over time and postulated that augmentation cystoplasty may decrease bladder dysfunction by increasing the total capacity beyond that required between voidings [19]. McInerney *et al.* studied enterocystoplasty patients aged 15–50 years and found that although voided volume increased approximately 50%, residual urine volume more than doubled between 3 months and 2 years postaugmentation, relating inappropriate augmentation capacity with increasing age [20]. Therefore, the demonstration that construct neo-bladders grow with recipients during skeletal maturation has potential clinical relevance despite the external validity limitations.

Regeneration versus repair

Regeneration involves replacement and restoration of cellular components and fully developed tissue mass, in addition to organizational, architectural and functional characteristics for the particular organ. By contrast, repair involves incomplete tissue replacement frequently with collagen deposition and, in extreme cases, formation of scar tissue [21]. Evidence of repair is seen in the middle-circular layer of the reimplant bladder-wall tissue at 18 months postimplantation. Trichrome staining revealed that the middle-circular muscle (layers labeled B in Figure 3) disorganization is accompanied by higher levels of collagen (blue staining) in reimplant tissue (Figure 3D) compared with construct (Figure 3B). Although this structural difference between constructs and reimplants is not reflected in functional differences by urodynamic assessment (Figure 2; Table 4), it may account for the delayed adaptation of C:BW seen in reimplant tissues (Table 5). A potential mechanism for the slightly elevated reparative healing response in reimplant neo-organs is transient tissue ischemia occurring between the time of cystectomy (tissue resection and reattachment) and omental wrapping.

Conclusions

The inflammatory response to synthetic scaffold materials and their degradation products has been listed by some as a potential disadvantage and pathway to fibrosis [22]. However, in this and previous studies [9,10] PLGA-based synthetic scaffolds with autologous UCs and SMCs have elicited regenerative healing without fibrosis. The results of this study demonstrate that a construct consisting of a PLGA-based biodegradable mesh scaffold with autologous UCs

and SMCs is capable of regenerating urinary bladder structure and function as early as 6 months postimplantation. Furthermore, the neo-bladder elicited by construct implantation has long-term durability and adapts to the size of recipient animals during a period of skeletal maturation from juvenile to adult.

Financial & competing interests disclosure

MJ Jayo, D Jain, JW Ludlow, R Payne, BJ Wagner and TA Bertram are employees and stock option holders of Tengion, Inc. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Cell–scaffold combination technologies represent a foundation for regenerating complex organs such as the urinary bladder

- Recapitulating a complex internal organ with a cell–scaffold combination product occurs in ordered phases: scaffold degradation and microvascular formation, histogenesis with structural restoration and, ultimately, functional maturation.
- A regenerated organ will ideally adapt to growth and physiological changes in the recipient in a manner similar to a native organ.
- A cell–scaffold product is capable of regenerating a urinary bladder in growing animals.

Delivery of specialized cells in the spatial context of the scaffold results in regeneration of a new bladder that has native structure & functional characteristics.

- Urothelial cells and smooth muscle cells derived from a normal bladder are sufficient to regenerate bladder tissue when delivered to the body on a biodegradable scaffold.
- The cell–scaffold product composition allowed integrated growth of the detrusor muscle and connective tissue (stroma) so that the regenerated bladder developed with the proper viscoelastic structural and functional properties to store and release a normal amount of urine at low pressure (compliance).

Bladder regeneration follows a defined pathway of histogenesis & organogenesis that is closely regulated & maintained

- Restoration of the bladder wall's three histologically distinct layers (mucosa, connective tissue and muscle) occurred within 3 months of construct implantation, a functionally mature capacity was achieved by 6 months and normal compliance characteristics of a urinary bladder wall developed by 12 months.
- The cell–scaffold product regenerated bladders that were functionally and structurally stable up to 2 years postimplantation.
- The cell–scaffold product's volume at implantation was constant. Yet when it was placed into different-sized dogs, the regenerated bladder's capacity-to-body-weight ratio adapted to the recipient animal's size, demonstrating that the cell–scaffold product responded to homeostatic control of organ size.

Conclusions

- Regenerating native-like internal organs using cell–scaffold products has the potential to reduce morbidity and mortality associated with organ transplantation or reconstructive surgery.
- Rapid regeneration and long-term durability of regenerated organs holds the promise of replacing failed or lost tissues and organs.
- The body's ability to regulate development of a regenerated organ represents a threshold mechanism, suggesting that implantation of a cell–scaffold product made from normal bladder biopsies can safely endure and function in its recipient for years.

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