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Authors
Piechota, Hans J
Gleason, Curtis A
Dahms, Stefan E
et al.

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ORIGINAL PAPER

Hans J. Piechota · Curtis A. Gleason
Stefan E. Dahms · Rajvir Dahiya · Lora S. Nunes
Tom F. Lue · Emil A. Tanagho

Bladder acellular matrix graft: in vivo functional properties of the regenerated rat bladder

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Abstract The purpose of this study was to determine whether the rat urinary bladder augmented by an acellular matrix graft can restore the bladder's low-pressure reservoir function and preserve normal micturition. After partial cystectomy (> 50%) and grafting with the bladder acellular matrix graft (BAMG), storage and voiding functions were monitored in 20 rats by means of a specially designed "micturition cage," leak-point cystography, and cystometry. After 4 months, sections ($n = 6$) were examined histologically to evaluate regeneration of bladder wall components within the BAMG. Bladder capacity and compliance increased progressively and were significantly higher in the grafted animals than in controls (partial cystectomy only), and volumes per void were significantly higher than in either control or normal animals. At 4 months, the regenerated urothelium, smooth muscle, blood vessels and nerves within the BAMG were qualitatively identical to normal bladder wall. Augmentation cystoplasty with the homologous BAMG leads to morphologic *and* functional rat bladder regeneration, thus enhancing low-pressure reservoir function and preserving normal micturition.

Key words Bladder · Transplantation · Homologous · Graft function · In vivo

Introduction

Bladder augmentation has been established as a successful surgical option in the treatment of voiding disorders. To restore or improve the low-pressure reservoir function of the urinary bladder, a great many synthetic and naturally derived biomaterials have been tried over the past century. Synthetic materials have been limited by foreign body-type reactions [5]; naturally derived materials have often retracted with time [4, 16]; and the alloplastic prosthetic bladder is still at an investigational stage in animals [11]. In contrast, autoaugmentation cystoplasty with gastrointestinal segments is now widespread because of its urodynamic benefits, availability and feasibility. The use of gastric, ileal and colonic segments, however, still bears well-known complications such as metabolic abnormalities, rupture, increased mucous production, infection, urolithiasis, osteoporosis, and malignancy [17, 23]. Most of these complications can be attributed to the presence of intestinal mucosa with its original secretory and absorptive function.

Detubularized gastrointestinal segments may enhance bladder capacity, but they cannot participate in the normal voiding process because of their different muscular organization and innervation. They may therefore occasionally simply replace low-capacity, high-pressure voiding disorders with others based on incomplete bladder evacuation [6, 12]. For this reason, investigators continue to seek another material or technique for *functional* bladder augmentation that will provide low-pressure enlargement without compromising voiding.

Previous research has demonstrated that collagen-based or, preferably, biodegradable materials have the best potential for regenerative and functional capacities [1, 2, 19, 32, 33]. Belonging to this group of biomaterials, the homologous bladder acellular matrix graft (BAMG) has recently been shown to promote complete regeneration of all bladder wall components in the rat [27, 28, 29]. The BAMG-augmented bladders showed complete luminal epithelialization, rapid angiogenesis, perfect spatial reconstruction of detrusor smooth muscle as well as muscularis mucosae, and regeneration of nerve fibers with no signs of scar formation at the site of anastomosis. These results were confirmed by Sutherland et al. [31] with a similarly prepared homologous bladder and

H. J. Piechota · C. A. Gleason · S. E. Dahms · R. Dahiya
L. S. Nunes · T. F. Lue · E. A. Tanagho
Department of Urology,
University of California School of Medicine,
San Francisco, CA 94143-0738, USA

H. J. Piechota (✉)
Klinik und Poliklinik für Urologie,
Westfälische Wilhelms-Universität Münster,
Albert-Schweitzer-Strasse 33, D-48129 Münster, Germany

gastric acellular tissue matrix in rats. It therefore appears that the BAMG could be a viable alternative to the use of bowel in urinary reconstruction. We designed the present study to investigate further the in vivo storage and voiding functions of the BAMG-regenerated rat bladder.

Materials and methods

Preparation of the bladder acellular matrix graft (BAMG)

Bladders from Sprague-Dawley rats were freshly obtained from our institution's tissue-sharing program. The homologous full-bladder-size BAMGs were prepared as described previously [29] by a method adapted from Meezan et al. [24]. In brief, bladders were placed in a 35-mm Petri dish containing 50 ml of 10 mM phosphate-buffered saline (PBS, pH 7.0) and 0.1% sodium azide. The mucosa was scraped off with a pair of glass slides. The remaining lamina propria and detrusor muscle were treated with 50 ml of 10 mM PBS-0.1% sodium azide and stirred for 5–6 hours for partial cell lysis. Bladders were then washed with 40 ml of PBS before treatment with 50 ml of 1 M sodium chloride containing 2000 Kunitz units DNase (Sigma, St Louis, Mo.) and stirred for 6–8 hours. With this, cell lysis was complete and all the intracellular components were released. The samples were then treated with 50 ml 4% sodium desoxycholate containing 0.1% sodium azide and stirred for 5–6 hours to solubilize the lipid bilayer cell membranes and intracellular membrane lipids; this was repeated once. The resultant BAMG was washed three times with 50 ml PBS and stored in 10% neomycin sulfate at 4°C until grafted.

Trichrome, hematoxylin and eosin (H & E), alpha-actin and protein gene product 9.5 (PGP 9.5) staining was used in nine BAMGs to confirm the grafts' acellularity and thus the effectiveness of the matrix preparation process. Scanning and transmission electron microscopy were performed in five BAMGs to study the texture and ultrastructure of the graft material and to demonstrate again the absence of any cellular components (Fig. 1).

Surgical technique

Male ($n = 16$) and female ($n = 15$) Sprague-Dawley rats (age 2.9 ± 0.19 months; weight 284.5 ± 14.7 gm) were anesthetized with pentobarbital, 40 mg/kg intraperitoneally. Through a midline lower abdominal incision, the bladder was exposed and partial cystectomy (> 50%) was performed. The full-bladder-size BAMG (9 × 6 mm undistended) was grafted to the remaining host bladder with a continuous polyfilament-coated 8-0 absorbable suture for both the anterior and posterior wall (Fig. 2). The orientation of the graft's inner luminal surface and outer serosal surface was maintained. To identify the matrix borders, four nonabsorbable monofilament 7-0 button sutures were placed, one each at the anterior, posterior, left and right sides, 3-4 mm apart. The control animals ($n = 13$ [6 male/7 female]; age 2.6 ± 0.17 months; weight 266.0 ± 15.7 gm) underwent partial cystectomy to allow differentiation between the natural course of bladder autoregeneration/dilation and the effect of BAMG augmentation cystoplasty.

Suture integrity was tested by instillation of saline through a urethral tube in the female rats and by puncturing the posterior host bladder wall with a 27-gauge hypodermic needle in the male. When satisfactory closure was obtained, the abdominal wall and the skin were closed in two layers. No drainage was used and no drugs were administered. For the duration of the study, standard laboratory conditions were observed and the principles of laboratory animal care [NIH publication No. 86–23 (1984)] were followed.

Two grafted rats were killed at 2.5 months and two more at 8.5 months [urethane anesthesia (1.1 g/kg intraperitoneally) followed by bilateral thoracotomy]. All remaining grafted animals ($n = 16$ survivors) were killed 4 months postoperatively. At the date of killing, cystometric measurements and cystograms were performed as described below. Some of the grafted bladders ($n = 6$) were then saved for histologic evaluation only. They were gently filled with 10% buffered formalin after ligation of both ureters and the urethra, thus allowing fixation in a distended state. The specimens were excised en bloc, i.e., with the prostate and seminal vesicles in males and the ventral part of the uterus and vagina in females. The remaining bladders were subjected to immediate tissue bath studies [27].

Evaluation of the micturition pattern

Voiding frequency and volumes per void (= functional bladder capacity) were evaluated noninvasively in all animals preoperatively and at 3 and 7 days and 1, 2, 3 and 4 months after surgery. Because rats are nocturnal and about two-thirds of all voids occur at night [9], micturition patterns were studied for 12 hours overnight. The "micturition cage," custom-made for this purpose, was designed as follows: The housing cage, with a metal grid bottom, was placed on a rack over a siliconized cloth (to prevent stool particles from falling into the collecting tray while not hindering free passage of urine). The collecting tray, which covered the entire area under the cage, was suspended by four nylon strings to isometric force displacement transducers (range of detection 0.1 to 65 g of urine). A signal processor supplied the excitation voltage to the transducers and fed the analog force data to an analog-to-digital converter, connected to a personal computer. All animals

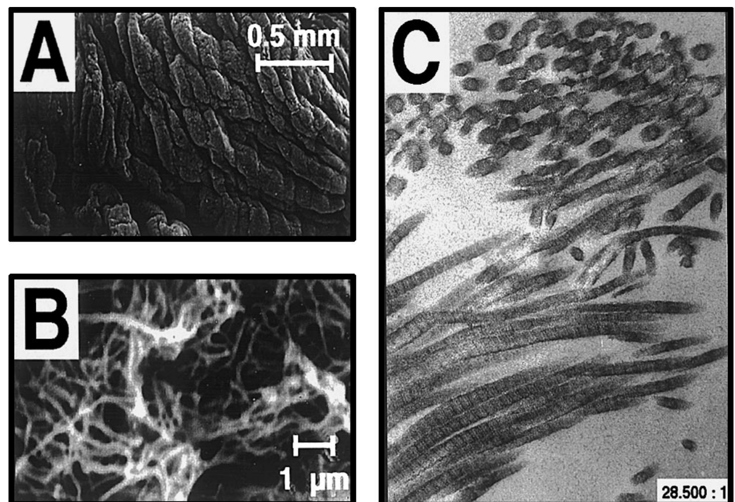

Fig. 1 Scanning electron microscopy at 20 × (**A**) and 5000 × (**B**) magnification and transmission electron microscopy at 28 500 × (**C**) magnification reveal the complete acellularity of the BAMG. It presents as a spongy framework of predominantly collagen fibers that, on longitudinal cuts, show their characteristic cross-striated pattern

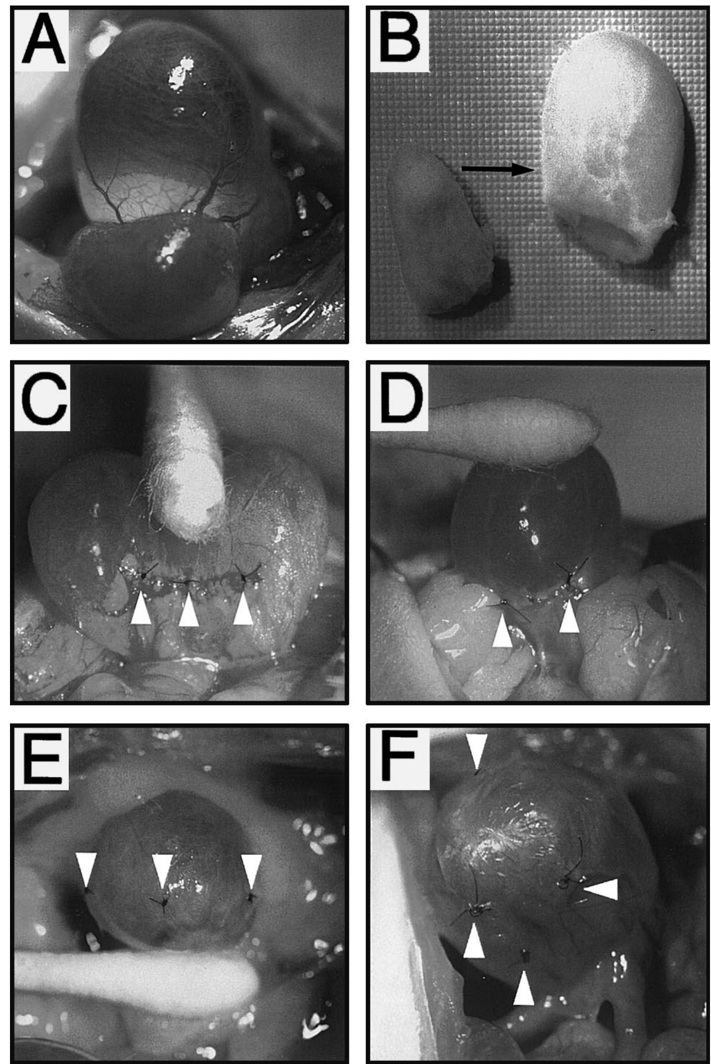

Fig. 2 (**A**) Intraoperative view of a native male rat bladder and part of the prostate. (**B**) Bladder body freshly resected (*left*) and after processing into the BAMG (*right*). (**C**) Male rat bladder immediately after partial (> 50%) cystectomy: note the huge prostate and the small bladder remnant with the resection line marked by three identification sutures (*white arrowheads*). (**D**) Male rat bladder immediately after grafting, showing the translucent, watertight BAMG and two of the marking sutures (*white arrowheads*) at the posterior bladder wall. (**E**) Female rat bladder showing increase in size and volume 4 months after partial cystectomy. (**F**) BAMG-regenerated female rat bladder 4 months after grafting. The graft has been smoothly integrated into the bladder body, and small blood vessels are sprouting on its surface. The graft surface looks smaller than at surgery, suggesting dilation/autoregeneration of the host bladder

were allowed to equilibrate in the housing cages for 8 to 10 hours with food and water given ad libitum. To enhance diuresis during the overnight study, no food but a sweetened orange-flavored instant drink (Tang, Kraft General Foods, White Plains, N.Y.) was offered ad libitum [28].

Cystometric measurements

Cystometric evaluation was performed just before death according to a method modified from Malmgren et al. [22] and Dörr [8].

All rats were anesthetized with urethane as described and placed supine on a warmed operating table. In female rats, transurethral cystometry was performed by means of a 24-gauge angiocatheter connected by polyurethane tubing (PE-90) to a pressure transducer (Baxter Uniflow, Baxter Healthcare, Irvine, Calif.). A signal processor supplied the excitation voltage to the transducer and fed the analog pressure data to an analog-to-digital converter. A personal computer and the LabView software program (National Instruments, Austin, Tex.) were used for data acquisition and saving in spreadsheet format. In male rats open cystometry was performed. The tubing (with a small cuff at its tip) was inserted through a small incision in the lower ventral host bladder wall, where it was fixed with a purse-string suture. Before the experiments got underway, the pressure transducers with tubing and angiocatheter attached were zeroed to the atmosphere during infusion. Because aspiration of residual urine was hampered by urinary calculi in most of the animals, the bladders were gently squeezed manually before each measurement. After an equilibration period of 15 minutes, each rat underwent at least five consecutive cystometric measurements during infusion of warm saline solution (37°C) at 0.2 ml per minute with a Harvard Apparatus 22 pump (Harvard Apparatus, Millis, Mass.).

Upon infusion, capacity was determined as the volume at which any kind of leakage occurred. Baseline (intravesical pressure of emptied bladder), bladder-opening (pressure at first leakage), and peak pressures (maximum pressure at micturition) were measured. Compliance (C) was calculated according to the formula:

$$C = \frac{P_2 - P_1}{V_2 - V_1}\ (\mathrm{cmH_2O/ml})$$

with P_2 representing the bladder-opening pressure and V_2 the infused volume at that time and P_1 representing the baseline pressure and V_1 the infused volume at that time. Hence, high values reflected poor compliance and low values good bladder compliance.

Cystographic evaluation

Changes in bladder morphology and capacity were assessed by leak-point cystograms, which were performed in female rats at the end of surgery and at the date of killing after cystometric evaluation.

A General Electric mobile X-ray unit, Kodak Lanex regular X-ray film, and undiluted Conray-43 contrast medium (Mallinckrodt, St Louis Mo.) were used. X-ray parameters were set at 50 kV, 1.5 MAS, and 25-inch film-focus distance. After a plain image was obtained and the bladder emptied, warmed contrast medium was slowly administered through the 24-gauge urethral angiocatheter. The cystographic image was obtained exactly when urethral leakage around the angiocatheter occurred (leak-point cystogram). Capacity was determined as the volume at which leakage occurred.

Staining

The specimens for light microscopic examination were fixed in a distended state as described (see above) for at least 24 hours. They were then bisected along a diagonal between two of the four identification sutures and immersed overnight in 10% buffered formalin. After dehydration in graded ethanol solutions, clearing in histoclear, and embedding in paraffin, the specimens were oriented for precise sectioning with the two marking sutures at both ends of the grafted area. Sections (5 μm) were cut, air-dried onto precoated slides, and stained with trichrome for collagen and smooth muscle, H & E for nuclei, alpha-actin for smooth muscle, and PGP 9.5 for nerves. (The monoclonal antibody, anti-alpha-smooth muscle actin, recognizes alpha-smooth muscle cells exclusively [30]; PGP 9.5 represents a major protein component of the neural cytoplasm and therefore labels more nerve fibers than do other general nerve markers [7, 14].) Specimens were also prepared for scanning and transmission electron microscopic evaluation. Bladder calculi were analyzed elsewhere for their chemical composition.

Statistics

An unpaired *t*-test was used to compare volume per void and cystometric values in the control and grafted animals; P values <0.05 were considered statistically significant. All data are presented as mean ± SEM.

Results

Mortality

Of the 31 rats, 11 died postoperatively; 8 of these (4 male/4 female) died within 48 hours from uremia consequent to urinary leakage into the abdominal cavity. The 4 male rats were found to have complete obstruction of the bladder neck and proximal urethra by a staghorn stone-like plug formed of coagula and defurfurated fibers of the BAMG. Leakage occurred at the site of anastomosis only; a rupture of the graft itself was never seen. Two of the 11 (1 male/1 female) died after 6 and 10 days of infection (abscess, sepsis); 1 male rat died after 2 months from a Corona virus infection. Twenty surviving rats (10 male/10 female) were available for evaluation.

None of the 13 control rats died of causes related to the surgery. However, three died within 2 hours postoperatively of respiratory failure while suffering from Corona virus infection. Ten surviving rats (5 male/5 female) were available for evaluation.

Bladder stone formation

Within 4 months, bladder stones occurred in 3 of 10 control rats (33%) and in 16 of 20 grafted rats (80%). Although the incidence of stone formation was significantly higher in the BAMG group, the numbers of stones in the individual stone formers did not differ significantly between the grafted (range 1 to 7, mean 1.6 ± 0.45) and control (3 to 9 stones, mean 1.7 ± 0.98) animals. The composition (struvite [60%–100%], apatite [40%–100%], Newberyite [20%–100%] and brushite [10%–100%]) and size of the calculi did not differ between groups.

Micturition pattern

As rat bladder capacity and volumes per void change with age and body weight, normative volume per void values were established by studying the micturition pattern of 156 untreated male and female Sprague-Dawley rats at age 2 to 10 months. The results obtained from the treated animals were then compared with these.

Three days after grafting, volumes per void decreased by half in both treated and control groups; they were gradually restored within two months in all animals and exceeded preoperative values thereafter (Table 1). At the end of the 4-month observation period, volumes per void in the grafted animals (1.04 ± 0.06 ml) were significantly higher than in both the control rats after partial cystectomy (0.89 ± 0.04 ml; $P = 0.04$) and

Table 1 Volumes per void after partial cystectomy and in BAMG-regenerated rat bladders within 4 months of surgery

Time of measurement	Rat BAMG ($n = 18$)		Partial cystectomy ($n = 10$)	
Preoperative	0.64 ± 0.05		0.68 ± 0.07	
Postoperative				
3 Days	0.31 ± 0.02	(−48%)[a]	0.34 ± 0.04	(−50%)[a]
1 Week	0.42 ± 0.04		0.48 ± 0.02	
1 Month	0.6 ± 0.04		0.56 ± 0.07	
2 Months	0.76 ± 0.08		0.81 ± 0.05	
3 Months	0.98 ± 0.12		0.88 ± 0.08	
4 Months	1.04 ± 0.06*	(+63%)[a]	0.89 ± 0.04	(+31%)[a]

All data are expressed as mean ± SEM (in milliliters)
* Significant difference ($P < 0.05$) when compared with the partial cystectomy group at the same time
[a] Percentages of preoperative values

in the age-, weight- and sex-matched untreated rats (0.81 ± 0.03 ml; $P = 0.01$). Volume per void values did not differ significantly between the control and untreated rats ($P = 0.21$).

Cystometric measurements

Baseline and bladder opening pressures were practically identical in all animals at any time after surgery (Table 2). In contrast, the peak pressure at micturition was more than 50% higher in the controls after 4 months (45.4 ± 1.2 vs. 27.4 ± 1.3 cmH_2O in the grafted; $P = 0.008$). In the grafted animals, the peak pressure exceeded bladder-opening pressure by 5.0 ± 0.9 cmH_2O at 2.5 months, by 7.2 ± 1.2 cmH_2O at 4 months, and by 8.7 ± 0.7 cmH_2O at 8.5 months after surgery. However, this progressive rise in peak pressure was not statistically significant. Bladder capacity was significantly smaller in the control animals (1.52 ± 0.07 vs. 2.45 ± 0.07 ml in the grafted; $P = 2.53 \times 10^{-8}$). Within the grafted group, bladder capacity almost doubled from 2.5 to 8.5 months after surgery. The BAMG-regenerated bladders were approximately three times more compliant than the control bladders (8.4 ± 0.4 vs. 24.8 ± 0.9 cmH_2O/ml; $P = 3.18 \times 10^{-4}$). Furthermore, compliance of the grafted bladders improved from 10.5 ± 0.5 cmH_2O/ml at 2.5 months to 8.4 ± 0.4 cmH_2O/ml at 4 months and 5.6 ± 0.5 cmH_2O/ml at 8.5 months after surgery. The changes in both bladder capacity and compliance within the grafted group were statistically significant (Table 2).

Cystographic evaluation

Leak-point cystograms proved helpful to illustrate macromorphologic changes in bladder configuration over time. Bladder capacity thus determined closely corresponded to the bladder capacity values obtained from the above cystometric measurements. Compared with the oval configuration of the normal rat bladder, both control and grafted bladders were more round 4 months after surgery. Stone formation occasionally led to a distension of the bladder outlet region. Dissection of the grafted animals revealed that the site of anastomosis of the BAMG had shifted from the bladder base towards the bladder dome region. Although the site of anastomosis could easily be identified on cystography immediately after grafting, it was no longer distinguishable at 4 months, indicating smooth integration of the BAMG into the whole bladder body without diverticular or scarred stricture formation (Fig. 3).

Histologic examination

At bladder harvest, no macroscopic signs of hydronephrosis or other upper tract deterioration were noted in any of the animals. Only mild to moderate adhesions to the surrounding tissue were found in the grafted as well as the control animals. The adhesions were located next to the identification sutures rather than within the matrix area itself.

Histologic staining of BAMG-regenerated bladders (see Fig. 4) showed a bladder wall structure that was qualitatively identical to the host, in conformity with our previous histologic findings [27, 29]. All three layers of normal rat bladder were present: the inner surface of the grafts was covered by a uniform urothelial lining with a differentiated muscularis mucosae. Most animals had hyperplastic urothelium in the native bladder and BAMG regenerate, resulting from stone formation. Distinct bundles of well developed, spatially oriented, detrusor smooth muscle were evident throughout the grafted area. The thickness and number of the muscle bundles appeared to decrease towards the central area of the BAMG, however. The number of well-formed small- and large-diameter blood vessels was greater in the BAMG regenerates than in the host bladder. The opposite was true for PGP-positive nerve fibers. These findings were consistent with our previous quantitative histologic studies [29]. Unlike smooth muscle, the number of neural elements appeared to increase further between 4 and 8.5 months. There were no mononuclear inflammatory cell infiltrates and other histologic signs of infection or rejection in any of the BAMG-regenerated bladders.

Table 2 Postoperative cystometric findings after partial cystectomy only and in BAMG-regenerated rat bladders

	Baseline pressure (cmH_2O)	Bladder opening pressure (cmH_2O)	Peak pressure (cmH_2O)	Capacity (ml)	Compliance (cmH_2O/ml)
Rat BAMG-regenerated Bladder					
2.5 Months ($n = 2$)	3.5 ± 1.2	22.5 ± 0.5	27.5 ± 0.5	1.93 ± 0.18	10.5 ± 0.5
4 Months ($n = 16$)	3.1 ± 0.5	19.9 ± 1.5	27.4 ± 1.3*	2.45 ± 0.07*	8.4 ± 0.4*
8.5 Months ($n = 2$)	3.4 ± 0.9	17.0 ± 0.3	25.4 ± 0.5	3.7 ± 0.17**	5.6 ± 0.5**
Partial Cystectomy Only					
4 Months ($n = 10$)	3.2 ± 1.4	20.7 ± 1.3	45.4 ± 1.2	1.52 ± 0.07	24.8 ± 0.9

All data are expressed as mean ± SEM
* Significant difference ($P < 0.05$) when compared with the partial cystectomy group at the same time
** Significant difference ($P < 0.05$) when compared with the other BAMG-regenerated bladders harvested earlier

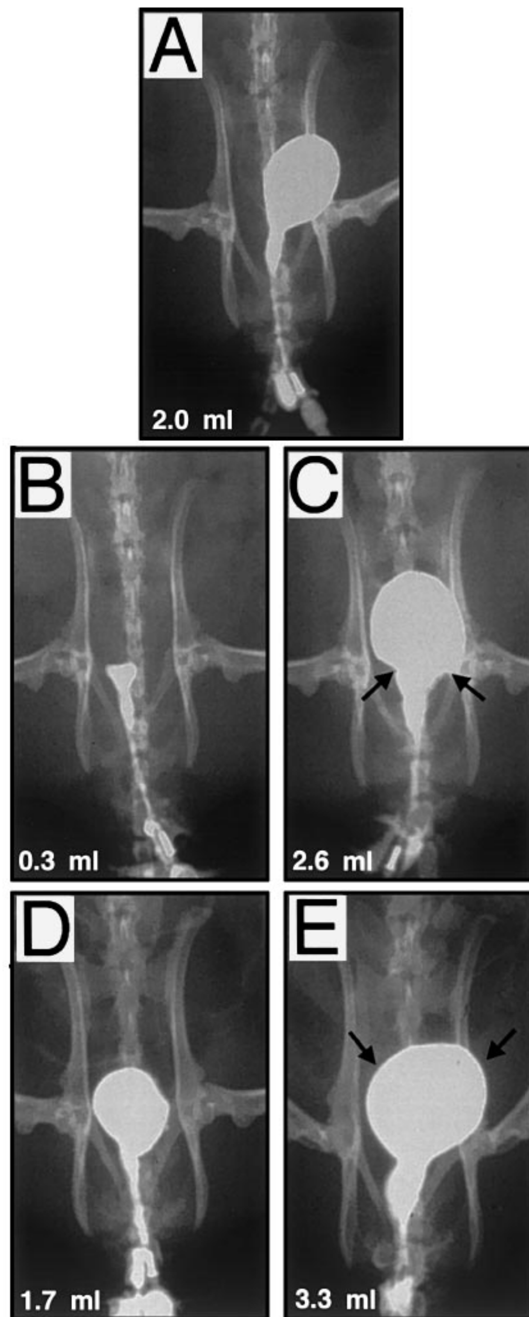

Fig. 3 Leak-point cystograms in female rat bladders preoperatively (**A**) and immediately after partial cystectomy (**B**) and BAMG augmentation cystoplasty (**C**). Within 4 months of surgery, capacity has increased in both the cystectomized (**D**) and the grafted (**E**) animals. The site of anastomosis (*black arrows*) in the grafted animals has shifted toward the bladder dome region where it can no longer be identified, thus demonstrating smooth integration of the BAMG into the bladder body

Discussion

Recently the homologous and heterologous BAMG has been demonstrated to serve as a scaffold for the ingrowth of all bladder wall components in the rat [27, 28, 29]. The present in vivo study was designed to evaluate whether BAMG augmentation cystoplasty assures true *functional* bladder regeneration by restoring low-pressure reservoir function and normal micturition in rats.

Among the 31 rats that underwent grafting with the homologous full-bladder-size BAMG, 8 of the 11 deaths occurred secondary to suture line leakage (in the male fostered by early bladder outlet plug formation). None of the animals died of causes related to immunogenic or clinical rejection of the graft. Cross-sections showed only a trivial number of leukocytes and lymphocytes, thus evidencing no histologic signs of rejection [20, 29].

The incidence of bladder stone formation was significant in both the grafted (80%) and control animals (33%), possibly favoring an underestimation of the total and functional bladder capacity values in both groups. The calculi were always free-floating and never adherent to or incorporated into the graft. Because struvite was the main component, an infectious cause for their formation is likely [25]. As a result of the scraping of the mucosa at the beginning of the matrix preparation process, some of the BAMG fibers could have served as a stone matrix when released during contact with urine. Furthermore, the rugged inner surface of the graft before it was covered by urothelium might have facilitated stone formation, even without urinary infection [31]. These mechanisms may have been relevant factors in the present study, especially since our full-bladder-size BAMGs had a much larger surface than the patches grafted in other comparable studies [18, 31, 32]. It has been shown, however, that any sort of bladder augmentation surgery in the rat predisposes it to stone formation [13, 18, 21, 31]. In contrast, in our ongoing preliminary studies in the dog, no free-floating calculi have been seen up to 7 months after BAMG homotransplantation (manuscript in preparation).

After partial cystectomy alone, autoregeneration and distension of the native rat bladder was evidenced by a progressive rise in total and functional capacity at decreasing voiding frequency over time. Although restoration of capacity after extensive bladder resection has been well documented in the literature [3, 26], there remains some controversy whether distension only or true bladder wall regeneration (or a combination) is responsible for this phenomenon [10, 15]. We have used a control group that underwent partial cystectomy only in order to differentiate the distension/autoregeneration of the native bladder from BAMG-induced rat bladder regeneration.

The choice of a suitable support structure for cell delivery and/or the ingrowth of bladder wall components is recognized as one of the key factors that determine regenerative capacities and graft function in augmentation cystoplasty. A variety of natural and artificial permanent support structures as well as degradable and nondegradable synthetic polymers have been utilized to fabricate such tissue engineering matrices [1, 2, 4, 5, 16, 19, 31, 32, 33]. A common finding with

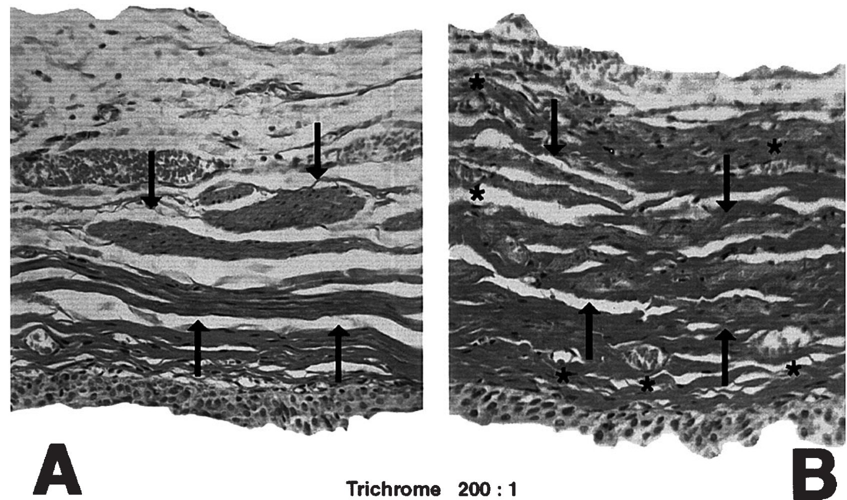

Fig. 4 Histologic appearance of the host bladder (**A**) and the BAMG-regenerated rat bladder (**B**). All three layers of normal bladder wall are present. There is still more collagen and less smooth muscle (*black arrows*) 4 months after grafting. Pronounced neovascularization (*stars*) can be found even in the center of the graft

these materials was an adequate histologic result, but with subsequent graft contraction and shrinkage [2].

In our study, the surface of the BAMG regenerates were not measured since they varied considerably with bladder filling and bladder stone volume. Although the native BAMG was full-bladder-sized, the area of the BAMG regenerate appeared smaller 4 months postoperatively suggesting significant autoregeneration and/or dilation of the host bladder and relative shrinkage of the BAMG. Nonetheless, BAMG-augmentation cystoplasty significantly enhanced total and functional bladder capacity and compliance in the rat model. The BAMG regenerates provided low-pressure enlargement of the bladder and supported normal micturition which besides the preservation of renal function are primary goals of augmentation cystoplasty in general. Hence relative graft shrinkage over time does not seem to compromise BAMG-induced rat bladder regeneration.

The grafted animals in this study showed regeneration of all bladder wall components with the BAMG serving as a scaffold. This resulted in true bladder augmentation as demonstrated by the rise in total bladder capacity, which was significantly higher than after partial cystectomy alone. Increasing volumes per void reflected the progressive rise in *functional* bladder capacity. Compliance, too, increased and was significantly higher in the BAMG-grafted animals, ensuring restoration of the low-pressure reservoir function of the bladder. Grafting did not affect baseline and bladder opening pressure, nor did it cause upper tract deterioration.

The aforesaid functional changes paralleled smooth muscle and nerve fiber development. As determined by our previous histologic studies focusing on the time course of BAMG-induced bladder wall regeneration [29], nerve regeneration is still ongoing while smooth muscle growth has already reached its maximum at about 3 months after surgery. However, it takes 1 month more until the difference in functional bladder capacity of the grafted bladders becomes significant in comparison with that of partial cystectomy alone. This seems to imply that the regenerated detrusor smooth muscle does not support micturition effectively until reconstitution of the appropriate nerve supply is ensured by the nerve fibers regenerating at a lower speed [27].

Previous in vitro studies following homologous BAMG augmentation cystoplasty in rats have shown that the BAMG regenerates exhibited contractile activity to electrical field stimulation and a qualitatively identical pattern of response to muscarinic, purinergic, alpha- and beta-adrenergic and nitric oxide drug administration. At 4 months after surgery, the absolute forces of contraction of the BAMG regenerates amounted from 60% to 80% of the host bladder. Histologic and immunohistochemical sections confirmed the presence of receptors for neurotransmitters which had been functionally demonstrated by the organ bath experiments [27].

Our in vivo and in vitro studies have thus shown that BAMG-augmentation cystoplasty can lead to morphologic and functional regeneration of the rat bladder, preserving its low-pressure reservoir function. Because BAMG-regenerated bladders exhibit functional innervation that is similar to normal, they can work in coordination with the host bladder components, thus generating adequate intravesical pressure to produce sustained voiding [27, 28].

We assume that the composition and texture of the graft material may be key factors in the extent, speed and quality of regeneration. We are performing ongoing functional and histologic studies with homologous (rat) and heterologous (hamster, rabbit, dog and pig) BAMG material for augmentation cystoplasty in rats [27, 28]. These suggest that bladder regeneration is facilitated by the closest possible structural match of the matrix graft and the host bladder wall interstitium. Although a complete understanding of the mechanisms involved in the normal development and regeneration of the urinary bladder remains to be elucidated, the findings of the present study – in which augmentation cystoplasty with the homologous BAMG effected functional bladder regeneration – suggest its important clinical potential in bladder reconstruction.

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