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The constitution of functional model rabbit bladder with acellular matrix

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Abstract

Aim: In this study, for the useable of bladder acelluar matrix in urinary tissue engineering, we compared the urodynamic and histopathological results between normal, partial cystectomy and bladder acellular matrix allograft (BAMA) augmented cystoplasty groups in rabbit model.

Material and methods: There were five groups each consisting of three rabbits. Urodynamic investigation was applied to all animals at the beginning of the study. Then, in the first control group, the rabbits were sacrified and the bladders were removed for histopathological examination. In the second control group the same procedures were realized at the end of 24th week. In the third group, partial cystectomy was performed to the animals and followed for 24 weeks. Partial cystectomy and BAMA augmented cystoplasty were performed to the animals in the 4th and 5th groups. The animals were sacrified at the end of 12th and 24th weeks, respectively. In the 3th, 4th and 5th groups urodynamic investigations were performed before sacrification. Then bladders underwent histopathological examination.

Results: There was no significant difference in term of bladder capacity between both control and BAMA augmented groups. Detrusor presures were no statistical different in all groups. However bladder capacity has significantly reduced in partial cystectomy group. In histopathological evaluations there were no differences between control and partial cystectomy groups. In BAMA augmented groups urothelium and vascularization were nearly the same as control groups however muscle layer was less organized and was thinner than control groups. Collagen depositon and innervation in BAMA augmented bladder were more prominent than that of the control group.

Conclusion: In our animal study, although the detrusor muscle had less organized with more collagen deposition, the BAMA augmented bladders had near normal capacity and compliance with normal detrusor presures.

Key words: bladder acellular matrix, augmented bladder, urodynamic result, histopathologic result

Introduction

The detrusor muscle consisting of smooth muscle fibers is responsible for the functions of the bladder. The main functions of the bladder are urine storage and voiding [1]. Pathological changes in the detrusor muscle result in bladder's contraction disorder. Neurological diseases, trauma, pelvic organ cancers, pelvic radical surgery, pelvic radiotherapy, infravesical obstructions and inflammatory disease can be the ethilogic factors [2].

The loss of detrusor contraction with low compliance and impaired muscle coordination affect the functions of bladder's urine storage and periodic urine voiding. This condition also affects the lower and upper urinary system, resulting in recurrent urinary tract infections, incontinence, urolithiasis, damage to the renal parenchyma and leads to renal failure [3]. If there is no benefit from conservative treatment in bladder pathologies, there are three types of surgical treatment alternatives; urinary diversion (ileal

loop), augmentation cystoplasty and replacement cystoplasty using intestinal segment [4].

Gastrointestinal segment has been used for urinary tract reconstruction in various benign and malignant urological diseases. But no ideal segment has been found that will allow low-pressure urine storage and protect the upper urinary system from side effects [4]. So urinary tissue engineering has got importance in reconstructive urologic operations. Many sentetic materials, natural tissues and autologus/ alternative cell types are used in experimental and clinical practice for the reconstruction of urogenital organs (bladder, üreter, ürethra, penis). Bladder, small intestine, skin, pericardium and plesanta are used for acellular matrix in natural tissues. Silicon, polyvinyl spanc, teflon, polylactic-glycolic acid and polylacticacid-caprolactone are used for sentetic materials [5].

Even though, the beginning of the experimental use of a bladder graft goes to 1961, in recent years many researchers dealing with bladder graft have done different types of study. Autologous graft, acellular matrix allograft, acellular available xenografts, molded collagen and alternative cell sources (bone marrow stem cells, urine-derived stem cells and cells from buccal mucosa) are used for the bladder tissüe engineering [6]. These natural or sentetic grafts develop a functional structure by creating the scaffold together with the bladder wall components. The scaffold can be also seeded by autologus urothelial cell or alternative cells. These cells have been grown in vivo or in vitro cultures [7]. Some studies recomended the pre-seeding of scafolds with cell can mitigate graft shrinkage [8,9].

Until today, besides small and large animal studies for urinary tissue engineering studies, many studies have been done on human urinary system disease. The results of these studies differ in general. So the studies of urinary tissue engineering that have been done so far are not clinically sufficient [10]. However, urinary tissue engineering will continue to be a source of hope to improve the quality of life for patients with urinary tissue insufficiency and in need of urinary reconstruction.

In this animal study, for the useable of bladder acelluar matrix (BAM) in urinary tissue engineering, we compared the bladder's urodynamic and histopathological results between the control, partial cystectomy and bladder acellular matrix allograft (BAMA) augmented cystoplasty groups in rabbit model.

Material and methods

One year old 15 adult female New Zhaland rabbits, obtained from Erciyes University Experimental Research and Application Center (DEKAM), were randomly separated into five groups, each containing 3 rabbits. All animals were housed in a temperature and light controlled room with ad libitum access to water and rabbit chow, except from 8 hours prior to 24 hours after the operation during the whole study.

All surgical procedures were performed under xylasine (2 mg/kg) and ketamine (4 mg/kg) anaesthesia. Each animal was positioned supine and f ixed, and then sterilely prepared and draped. Skin and other layers of the lower abdomen were passed with a midline vertical incision. The fascia was opened and the muscles were slipped off with blunt dissection. The bladder was exposed after opening the peritoneum. After filling the bladders with steril saline, the bladder fundus was excised comprising 40 - 50 % of total bladder volüme. Preprepared acellular matrix were sutured with two layer by using 5/0 vicryl sutures. Four nylon corner sutures were inserted at every 90 degrees. Then, the abdomen was closed separately through peritoneal and fascia sutures with 3/0 chromized catgut and the skin was closed with 3/0 silk suture. 5 mg/kg IM ciprof loxacin was applied as surgical prophylaxis preoperatively. For the first group (first control group), after completion of urodynamic examination under anaesthesia, one-year old three animals were sacrificed and the bladders were extracted for histological examination. For the second group (second control group), after completion of urodynamic examination under anaesthesia, one and a half-year old three animals were sacrificed and the bladders were extracted for histological examination.

For the third group (partial cystectomy only group), after completion of urodynamic examination under anaesthesia, almost half of the bladders were resected from the fundus. The remnant bladders were closed. 6 months later urodynamic examinations under anaesthesia were repeated and the animals were sacrificed and the bladders were extracted for histological examination.

For the fourth group (early BAMA augmented cystoplasty group), after completion of urodynamic examination under anaesthesia, almost half of the bladders were resected from the fundus. BAMA was augmented to the remnant bladders. 3 months later urodynamic examinations under anaesthesia were repeated and the animals were sacrificed and the bladders were extracted for histological examination.

For the fifth group (late BAMA augmented cystoplasty group), after completion of urodynamic examination under anaesthesia, almost half of the bladders were resected from the fundus. BAMA was augmented to the remnant bladders. 6 months later urodynamic examinations under anaesthesia were repeated and the animals were sacrificed and the bladders were extracted for histological examination.

Urodynamic examinations were performed under anaesthesia by insertion of two 186 intracath catheters from the bladder fundus. While filling the bladders with sterile saline with a velocity of 2 ml/min from one of intracath catheters, intravesical pressures were measured from the other ones. Bladder volumes were calculated. The voiding phases in urodynamic investigations were not recorded becuase of the anaesthesia.

The extracted bladders were filled with formol solution and rested for one night. After dehidratation with ethanol, the specimens were embedded in paraffin. And then 5pm thick sections were obtained. Samples from original bladder, peripheral and central matrix tissue, anastomosis line were preferred. Anti-smooth muscle a actin staining was used for examination of muscle layer. S 100 staining was used for the detection of neural regeneration. The matrixes were stained and examined for the confirmation of acellularity.

Bladder acellular matrix allograft production method

Freshly prepared almost half of the bladder tissue from the fundus region of the 6 partially cystectomized control rabbits were placed in a Petri dish containing 150 mL of 10 mmol/L PBS (pH 7.0) and 0.1% sodium azide, and stirred for 24 h to produce partial celilysis. The bladder segments were washed with 150 mL of PBS for five times and treated with 150 mL of 1 mol/L sodium chloride containing 2500 Kunitz units of DNase (Sigma Chemical Co., St. Louis, MO) and stirred for 24 h. Lysis was then complete and all the intracellular components were released. The samples were treated with 150 mL of 4% desoxycholate containing 0.1% sodium azide, and stirred for 24 h to solubilize the lipid celi membrane and intracellular membrane lipids. This treatment was repeated twice. The resultant graft was washed five times with 150 mL PBS and stored in 10% gentamycin sulphate at + 4 °C until transplantation of graft.

Statistical analyses

The tests were conducted by using the SPSS 10.0 software program. The data were presented as mean (range). Mann Witney U test was used for the comparisons of volume and pressure means among the both of the Controls and among the both of the augmentation groups. Kruskal Wallis variance analysis was used for the comparisons of pressure and volume means among the control, partial cystectomy and augmentation groups. Mann Witney U test was also used for the twos comparisons betvveen Controls, partial cystectomy groups and augmentation groups. Kruskal Wallis test was used for the analyses of vveight differences among the control group II, partial cystectomy only group (Group III) and augmentation group (Group V) during the 24-week period. P<0.05 considered as indicating statistical signif icance.

Results

One animal of the second control group (Group II) had died at 4 weeks follow up. Another animal of the early matrix augmented group (Group IV) had died at early postoperative period. No another complication was observed in all the animals. All other animals recovered well from the procedures.

Comparison of weight differences of the animals during the experimental period of 24 weeks were showed in Table 1. Weights of the animals were measured at the beginning and completion of the study. Weight differences of the animals of the group II, III, and V during the 24 weeks period were compared by using Kruskal Wallis test, and no differences were observed in Table 1.

Comparison of pressure and volume measurements between the control groups (group I and II), and BAMA augmented cystoplasty groups (group IV and V) were showed in Table 2. There was no statistically significant difference between the pressure and volume measurements of the both of the control groups (Group I and II). Table 2 also confirms no difference between pressure and volume values of BAMA augmented cystoplasty early (Group IV) and BAMA augmented cystoplasty late (Group V) groups.

Comparison of pressure and volume measurements before and after partial cystectomy in Group III and before and after BAMA augmented cystoplasty in Group IV and Group V were showed in Table 3. While, there was no difference in the values of pressure, the change in the volume of the bladders was significantly differed before and after partial cystectomy (p=0.04). Contrastingly, there was no significant difference in the mean values of pressure and volume before and after BAMA augmented cystoplasty early and late groups (Group IV and V).

Comparison of pressure and volume measurements between the control groups (Group I + Group II), partial cystectomy only group (Group III) and the BAMA augmented cystoplasty groups (Group IV + Group V) were showed in Table 4. After confirmation of no statistical difference of pressure and volume measurements within the control groups and the BAMA augmented cystoplasty groups, comparisons of pressure and volume values between the control groups, the partial cytectomized group and the BAMA augmented cystoplasty groups were performed. While, there were no significant difference for pressure measurements, but volume measurements differed significantly (p=0.04).

Table 1 Comparison of weight differences of the animals during the experimental period of 24 weeks.

	Group II (n:2) mean (range)	Group III (n:3) mean (range)	Group V(n:3) mean (range)	P (KruskalVVallis test)
VVeight difference	650(600-700)	500(450-700)	500(500-700)	0.18

Group II: second control group, Group III: partial cystectomy only group, Group V: BAMA augmented late group

Table 2 Comparison of pressure and volume measurements between the control groups (group I and II) and the BAMA augmented cystoplasty groups (group IV and V).

	Group I (n:3) mean (range)	Group II (n:2) mean (range)	P*	Group IV(n:2) mean (range)	Group V (n:3) mean (range)	P*
Pressure**	7(5-16)	10.5 (6-15)	0.27	7.5 (5-10)	6(3-10)	0.39
Volüme***	24 (22-37)	22.5 (20-25)	0.17	26.5 (20-33)	26 (24-31)	0.27

Group I: first control group, Group II: second control group, Group IV: BAMA augmented early group Group V: BAMA augmented late group, *: Mann VVhitney U test, **: cmH2O, ***: ml

Table 3 Comparison of pressure and volume measurements before and after partial cystectomy in Group III and before and after BAMA augmented cystoplasty in GroupIV and GroupV.

	GroupIII'(n:3) mean(range)	GroupIII*(n:3) mean (range)	p*	Group IV+V' (n:6) mean (range)	Group IV+V* (n:5) mean (range)	p*
Pressure**	6(5-13)	3(2-6)	0.15	7(4-10)	6 (3-10)	0.16
Volüme***	23 (21-35)	15 (14-20)	0.04	25 (21-31)	26 (20-33)	0.24

Group III: partial cystectomy only group, Group IV: early BAMA augmented cystoplasty group Group V: late BAMA augmented cystoplasty group, *: Mann VVhitney U test, **: cmH20, ***: ml, ': before, *: after.

Table 4 Comparison of pressure and volume measurements between the control groups (Group I + Group II), partial cystectomy only group (Group III) and the BAMA augmented cystoplasty groups (Group IV +Group V).

	Group I, II (n:5) mean (range)	Group III (n:3) mean (range)	Group IV, V (n:5) mean (range)	P*
Pressure**	7(5-16)	3(2-6)	6(3-10)	0.14
Volüme***	24 (20-37)	15 (14-20)	26 (20-33)	0.04

Group I: first control group, Group II: second control group, Group III: partial cystectomy only group, Group IV: BAMA augmented early group Group V: BAMA augmented late group, *: Kruskal Wallis test, **: cmH20, ***: ml.

Table 5 Comparison of pressure and volume measurements of the BAMA augmented cystoplasty groups (Group IV + Group V) with the partial cystectomy only group (Group III) and control groups (Group I + Group II).

	Group I, II (n:5) mean(range)	Group IV, V (n:5) mean(range)	P*	Group III (n:3) mean(range)	Group IV, V (n:5) mean (range)	P*
Volüme***	24(20-37)	26(20-33)	0.59	15(14-20)	26(20-33)	0.04

Group I: first control group, Group II: second control group, Group III: partial cystectomy only group, Group IV: early BAMA augmented cystoplasty group, Group V: late BAMA augmented cystoplast group, *: Kruskal VVallis test, ***: ml.

Comparison of pressure and volume measurements of the BAMA augmented cystoplasty groups (Group IV + Group V) with the partial cystectomy only group (Group III) and control groups (Group I + Group II) were showed in Table 5. In order to determine values of which group contribute significantly, the comparisons of volume means between control groups and BAMA augmented cystoplasty groups were made, and there was no significant difference. Contrastingly, the difference of volume means value between partial cystectomy only group and BAMA augmented cystoplasty groups was significantly (p=0.04).

Histology

Macroscopic examinations of the partial cytectomized bladders revealed almost half of the total bladder volumes, disappearance of suture lines and no more contracture. Bladder wall thickness of the partial cystectomized animals was thinner than that of control animals. Moreover, microscopic appearances of urothelial mucosa, muscle organization and amount, vascularization and nerve structures were very similar to that of control animals.

The appearances of the early BAMA augmented cystoplasty group's bladders were macroscopically smaller than that of control groups's bladders, but bigger than that of partial cystectomized group's bladders. Suture lines and grafted areas cannot be identified macroscopically. Inserted marker nylon sutures were displaced to environmental tissues. Contractures on the fundus area were clearly obvious of the early BAMA augmented cystoplasty group's bladders. Microscopic examination revealed quite identical appearance of urothelial mucosa from both of the early BAMA augmented cystoplasty group's bladders and control group's bladders. Musculature was appeared as irregular sequences as individual thin bundles within broad fibrotic areas. Vasculature was more prominent at border lines. Neural proliferation was also more prominent than control group's bladders.

The apperance of the late BAMA augmented cystoplasty group was similar to the appearance of the early BAMA augmented cystoplasty group. Suture lines and grafted areas cannot be identified macroscopically. Inserted marker nylon sutures were displaced to environmental tissues. Contractures on the fundus area were clearly obvious of the late BAMA augmented cystoplasty group's bladders. Microscopic examination revealed quite identical appearance of urothelial mucosa from both of the late BAMA augmented cystoplasty group's bladders and control group's bladders. Muscular bundles of the late BAMA augmented cystoplasty group's bladders were thicker, organized and orderly than that of the early BAMA augmented cystoplasty group's bladders. There were vascular proliferation and fibrosis at the serosa. Neural proliferation was also more prominent than control groups and had quite similar appearance to that of the early BAMA augmented cystoplasty group.

The bladder wall thickness of the early and late BAMA augmented cystoplasty groups were significantly thinner than the control groups and the partial cystectomy group. The histologic images were seen in figures (Figure 1-6).

Figure 1 - Normal bladder

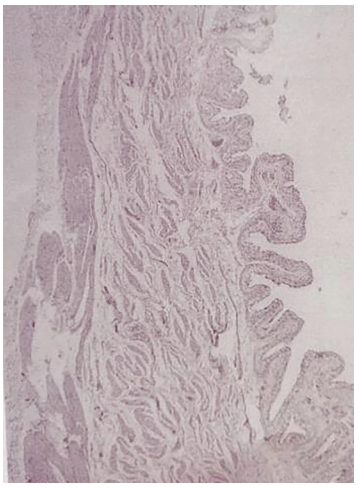

Figure 2 - Only parcial cystectomized bladder

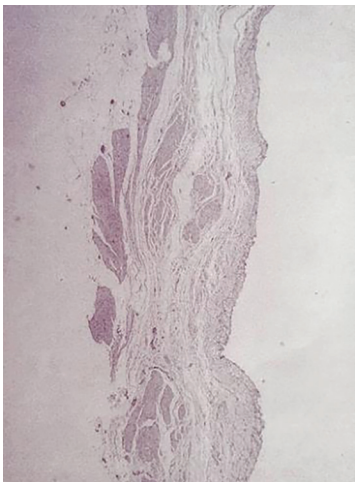

Figure 3 - early BAMA augmented bladder (12 week)

Figure 4 - early BAMA augmented bladder (12 week)

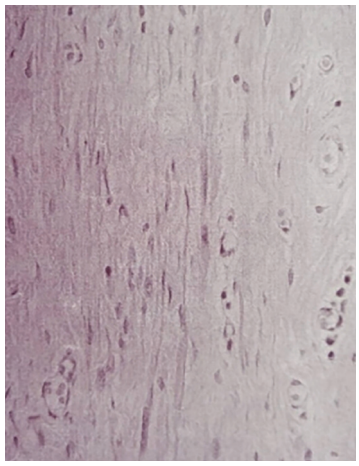

Figure 5 - late BAMA augmented bladder (24week)

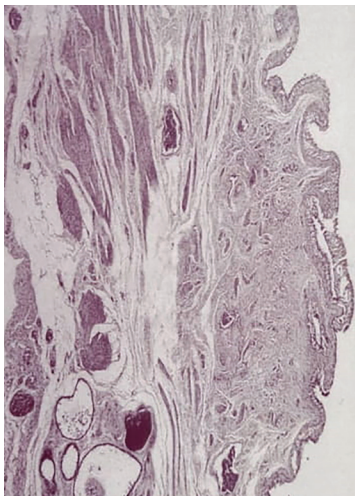

Figure 6 - late BAMA augmented bladder (24 week)

Discussion

Gastrointestinal segments are frequently used in orthotopic neobladder reconstruction and bladder augmentation. However, gastrointestinal segments have got the capability of absorption of diverse specific substances [11,12]. Contrastingly, neobladder tissues are composed of specialized cells for storage and disposal of the solutes. Because of those differences, in case of contact of those two structures, serious complications may arise. These complications may include infection, metabolic disorders, increased mucus production, stone formation, perforation and malignancy [13]. Due to the emergence of such problerns in the use of gastrointestinal segments, urinary tissue engineering has been performed to supply alternative materials and native tissues for the use of orthotopic neobladder reconstruction and bladder augmentation [5].

Kropp et al [14] prepared acellular matrix grafts using porcine small intestinal submucosa (SIS) and used for augmentation cystoplasty in dogs. While the mean bladder capacity was 51 ml preoperatively, the mean bladder capacity after surgery has been found to be 55 ml. Histological examination showed complete development of the urothelial layer and incomplete development of muscle layer, a few muscle bundles were observed among large pieces of connective tissue. In vitro contraction tests were also revealed that contractile responses of small intestine submucosa transplanted dog bladders were almost half of that of normal bladder tissues [14]. Although tissues resembling bladder tissue were obtained in small and large animal studies where partial bladder reconstruction using SIS was obtained, there was a significant difference between the augmented bladder tissues. It was concluded that this difference was related to the type of bladder injury [15,16]. In addition, SIS seeded with alternative cell or autologous cells had better urodynamic and histologic results [17,18].

Tanagho et al followed rats that were partially cystectomized and BAMA augmented for 20 weeks. While the urothelium was organized in the 1st week, the organization of the muscle layer was completed in the 20th week and nerve structures had increased continuously until the 20th week. Collagen and elastin was found to be increased in the BAMA augmented bladder tissue. Almost near normal bladder capacities had been reported in all BAMA augmented rats [19]. In an anohter similar study used porcine BAM for augmentation cystoplasty, macroscopic graft contracture was seen in the 22th week [20]. No tissue rejection potential and detection of almost normal bladder capacity and compliance levels were the positive aspects of the neobladders created by use of acellular matrix technique. Only the growth of the muscle layer was observed inadequeately [21]. Yoyo et al seeded BAM grafts with urothelial and smooth muscle cells for augmentation of the partial cystectomy in a canine model. Augmented bladder capacity increased %99; however, augmented bladder without unseeded matrices' capacity increased only 30% [22].

In an animal study, molded collagens seeded with urothelial and smooth muscle cells for augmentation of partial cystectomy in dogs had better urodynamic and histologic results than the other graft techniques [23]. In a similar study used porcine, molded collegens were used two layers and seeded with alternative cells. The urodynamic and histologic results were succesfull as a native bladder tissue [24]. Atala et al performed cystoplasty in seven patients with myelomeningocele causing neurogenic bladder. Urothelial and muscle cells were seeded in a scaffold. Scaffold was made of molded collagens. The results were unclear about augmented bladder capacity and compliance [25]. But the anohter urinary tissue engineering study did not support the use of biodegradable scaffold seeded with autologous urothelial and smooth muscle cells for augmented bladder tissue in augmentation cystoplasty [26].

In our study, macroscopic appearance and urodynamic capacity of partial cystectomized bladders were about up to half of normal control bladders. Capacity and compliance of the all BAMA augmented bladders were almost close to that of normal control bladders. But the macroscopic appearance was smaller

than that of normal bladder. By the histologic examination, bladder wall thickness was reduced in the all BAMA augmented bladders and only partial cystectomized bladders. Despite detection of normal development of the urothelial mucosa, blood vessels and nerve regeneration of all BAMA augmented bladders, the lack of amount of muscle structure and organization and excessive increase of collagen and fibrosis were observed in all BAMA augmented bladders. The amount of bladder's muscular structures and organization were more advanced in the late BAMA augmented bladder than that of the early BAMA augmented bladder. No tissue rejection complication was observed in any of BAMA augmented animals in our study.

Conclusion

In our animal study; although the detrusor muscle had less organized with more collagen deposition, the BAMA augmented bladders had near normal capacity and compliance with normal detrusor presures. A lot of treatment methods used in a variety of urologic diseases currently seems to be changed in the near future by production of urinary tissues with the use of tissue engineering techniques.

Ethics committee approval: Erciyes University Faculty of Medicine ethics committee gave approval (EU.192661/2007). One year old 15 adult female New Zhaland rabbits, obtained from Erciyes University Experimental Research and Application Center (DEKAM).

Authors' Contributions Erçin Altıok: wrote the article, data collections, involved in planning and performing the urodynamic test and operations. Engin Özbay: helped writing the manuscript. Oğuz Ekmekçioğlu: revised the manuscript.

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