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BLADDER AUGMENTATION IN RABBITS WITH ANIONIC COLLAGEN MEMBRANE, WITH OR WITHOUT UROTELIAL PRESERVATION. CISTOMETRIC AND HYSTOLOGIC EVALUATION

FÁBIO G. O. LEPPER, TAMARA M. RAMOS, JOSÉ C. S. TRINDADE FILHO, FABIANA R. VALE, CARLOS R. PADOVANI, GILBERTO GOISSIS

Department of Urology, Botucatu School of Medicine, State University of São Paulo (UNESP), Botucatu, São Paulo, Brazil

ABSTRACT

Introduction: The use of bowel segments to perform bladder augmentation is associated with several metabolic and surgical complications. A great variety of synthetic materials, biodegradable or not, have been tested. Collagen-based biomaterials have shown effectiveness for the regeneration and obtainment of a functional bladder.

Objective: Assess the functional and histological response of the rabbit bladder to anionic collagen membrane (ACM), either when it is anastomosed to the bladder or it is placed onto bladder after vesicomyectomy.

Materials and Methods: In 15 male rabbit a partial cystectomy was performed. After 4 weeks they were divided in 3 groups. Group 1 (G1) – bladder augmentation with ACM. Group 2 (G2) ACM is placed onto bladder after vesicomyectomy. Group 3 (G3) control group. Maximal bladder capacity (MBC) and weight were assessed with 4 (M1), 8 (M2) and 12 (M3) weeks after partial cystectomy. In M3 was performed the sacrifice and extraction of the bladder and kidneys for anatomopathologic study.

Results: There were neither bladder stones, nor implant extrusion in M3. There was a significant increase in MBC in G1 and G2 ($p<0.05$), but no statistical differences in G3 ($p=0.35$). There is no significant difference comparing G1 and G2. In M3, both groups have shown a bigger MBC than G3 ($p<0.05$). The microscopic assessment showed an inflammatory reaction in the bladder augmented, with urothelium preserved.

Conclusions: The ACM was effective for the increase of MBC. The bladders with preservation of the urothelium have shown an extensive inflammatory process.

Key words: bladder; transplant; collagen; rabbit model; urodynamics
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INTRODUCTION

Several congenital or acquired diseases promote anatomic or functional alterations, turning difficult or precluding the reservoir function of the bladder (1). When conservative therapies are not effective, bladder augmentation or urinary diversion are recommended as alternative therapeutics (2). Today, enterocystoplasty is the method most utilized to bladder augmentation using ileum, colon or stomach segments (3). In spite of the success obtained by these techniques, aggression to gastrointestinal tract may lead to nutritional and electrolyte alterations, peritoneal adherences, abscesses, enteric fistulae, excessive mucus production, bacterial colonization and cancer (4,5).

Figure 1 - *Anionic collagen membrane graft ready for implantation.*

In order to minimize these complications, a variety of biologic or synthetic materials have been used in experimental assays, as alternative methods for bladder augmentation (6). Most of them presented complications, as lithiasis formation, infections, graft rejection or extrusion, and no adequate bladder reconstruction (7). Collagen-based biodegradable materials showed effectiveness to regenerate and normalize bladder's functional capacity (8,9).

With this aim, we have investigated anionic collagen membrane to bladder augmentation, evaluating histologic and urodynamic aspects. We have also tried to evaluate if maintaining the urothelium intact would help on bladder regeneration.

MATERIALS AND METHODS

Acellular anionic collagen membrane (Figure-1) was obtained through processing bovine serosa, by a technique previously described, and maintaining it in sterile recipients, from 4 to 10°C (10). For the performance of surgical procedures, animals were anesthetized with 3,5% pentobarbital sodium, 1 mL/kg by parenteral route.

Fifteen male rabbits were utilized, whose weight varied from 3.1 to 4.2 (mean 3.6±0.3kg), initially submitted to subtotal cystectomy (incision 1.0 cm above both ureteral meatus), with bladder dome excision. Bladder edges and the abdominal wall were sutured with running 4-0 chromic suture in separated layers. After 04 weeks, at moment 0 (M0), the animals were distributed in three experimental groups with five rabbits each. In Group 1 (G1) we performed detrusorectomy according to the technique described (11) and, over the mucosa, we have sutured the collagen membrane, with approximately 4.0x4.0 cm. In Group 2 (G2), we performed a partial cystectomy and bladder augmentation with anionic collagen membrane, with the same dimension (Figure-2). In Group 3 (G3) we have performed only cystometric evaluation.

All rabbits were submitted to cystometric study in the moment of bladder augmentation, and every four weeks until sacrifice (M3). M0: bladder augmentation; M1: 4 weeks; M2: 8 weeks; and M3: 12 weeks. Cystometric study was performed with awaken animals, which were submitted to urethral dual-lumen (8F) and retal catheterism (8F). After the drainage of vesical content, saline solution was infused at room temperature, with continuous fill rate of 2mL/min by infusion-pump through the proximal lumen of the urethral catheter. Vesical, detrusor, and abdominal pressure curves were continuously registered during procedure, by a pressure transducer connected to distal lumen of the urethral catheter. Maximum bladder capacity (BC) was determined in the moment of urine leakage around the catheter, and registered in an urodynamics device - Dantec™ (Duet).

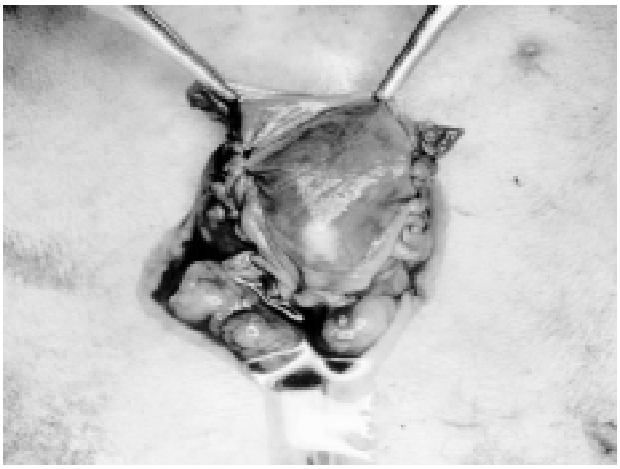

Figure 2 - *Bladder augmentation with anionic collagen membrane graft (Group 2).*

Table - *Bladder capacity in different moments and groups*

		Bladder Capacity (mL) Moments		
		M1	**M2**	**M3**
Groups	G1	24.0 ± 7.8	32.8 ± 3.6	35.5 ± 5.1*
	G2	19.0 ± 12.2	25.2 ± 9.9	31.2 ± 6.5*
	G3	16.2 ± 5.2	20.0 ± 8.5	18.8 ± 8.2

**p<0.05*

At M3, after cystometric study, anesthesia was performed and we proceeded to explore the abdomen. Subsequently, we proceeded to intravesical instillation of 10% formaldehyde and, afterwards, to total cystectomy and bilateral nephrouretectomy. The animals were sacrificed with 3,5% pentobarbital sodium 3mL/kg by parenteral route. After 15 days, we proceeded to bladder section, obtaining serial longitudinal sections, spaced in about 0,5cm. At the histopathological study using hematoxylin and eosin (HE), at least 8 segments per bladder were evaluated. The tissue segments were evaluated for presence or absence of implant, fibrosis, inflammatory process, foreign body reaction, calcification, and other alterations.

For descriptive and statistical analysis of variables, we used mean, standard deviation, and analysis of variance (ANOVA) with 2 factors for repeated measures; Friedman and Newman-Kuels tests. For all analysis performed the results were considered significant when $p<0.05$ (12).

RESULTS

At macroscopic evaluation (inspection) we observed, at M0, the presence of calcium concretions adhered to chromic catgut suture, within the bladder, in most of the rabbits. At the moment of sacrifice

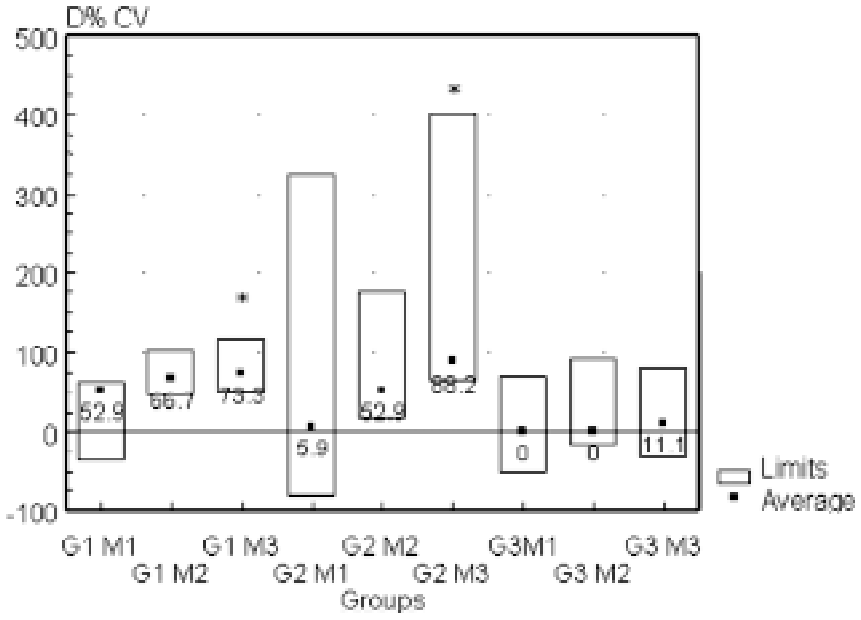

Figure 3 *- Percent variation of bladder capacity (BC), in different groups and moments, with average and superior and inferior limits.*

(M3) neither urinary lithiasis, nor membrane extrusion was observed.

BC at M0 was 17.5±6.1mL, with no significant difference among the groups (p=0.62). We have observed a significant elevation only in moment 3 (M3) for Groups G1 and G2, when we analyzed absolute values of bladder capacity ($p<0.05$) (Table). Evaluating percent variations of BC related to M0, we have observed within the groups, significant elevations in G1 and G2 at M3 ($p<0.05$). When we compared the groups, we observed that between G1 and G2 there was no significant difference at various moments. Both G1 and G2 presented significant difference when compared to G3 at M3 ($p<0.01$) (Figure-3).

When we evaluate microscopic findings in bladder, at G1 we observed the presence of a chronic inflammatory reaction, like a foreign body reaction: a lymphoplasmocyte infiltrate in bladder submucosa, muscular wall hypertrophy in 60% of the cases, and presence of scarring tissue (Figure-4). At G2, we have observed absence of inflammatory process, apparently normal muscular wall, and some regions of scarring at the wall (Figure-5). In rabbits at G3, we observed absence of inflammation and scarring tissue. In one rabbit we have seen glandular cystitis, and in another one an atrophic urothelium. At observation the kidneys presented few significant alterations: two cases of mild chronic pyelonephritis, one at G1 and the other at G2, and one case of focal acute tubular necrosis (G3).

DISCUSSION

Acellular matrices are prepared from bowel, stomach or bladder, through mechanical or chemical manipulation, in order to extract cells from the tissue, maintaining the membrane of extracellular homogeneous matrix. Studies with these materials implanted in bladder have shown excellent results, with total epithelization in 4 days, and evidence of muscular and vascular regeneration in two weeks (13). However, some complications can also be observed, as lithiasis formation in up to 63% of the cases (14).

In our experiment, we have done bladder augmentation with ACM with and without preserved urothelium. We used the group with preserved urothelium to evaluate possible differences in calcification of the material, or bladder calculi formation, for it is well know that this animal's urine has alkaline pH, which favors salt precipitation in the presence of foreign bodies, with greater risk of developing bladder calculi or graft calcification (15). We did not observe such complication, even when ACM has remained in direct contact with urine.

We have to perform some specific studies to evaluate whether the material presents or not less lithogenic potential. We know that the main difference that distinguishes it from other collagen derivatives is that this anionic membrane presents negatively charged molecules in neutral pH, due to selective hydrolysis of the carboxyamide groups of asparagine

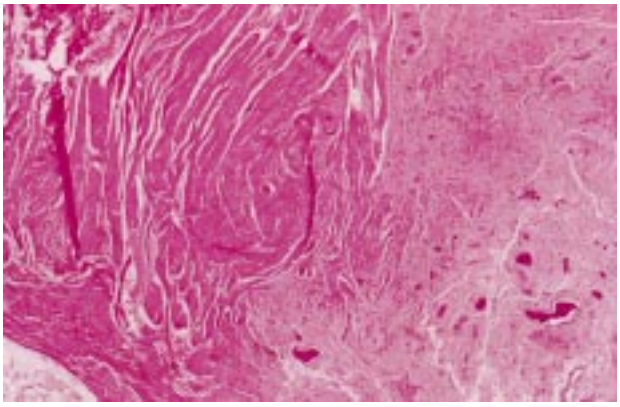

Figure 4 - *Bladder section from group 1 where we may observe detrusor hypertrophy. (HE, X60.)*

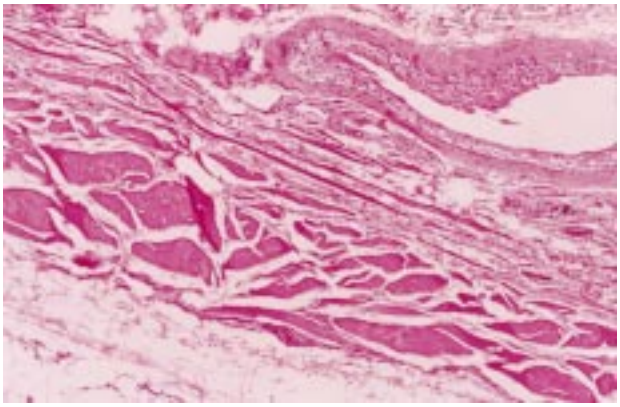

Figure 5 - *Bladder section from Group 2 with normal aspect. (HE, X100.)*

and glutamine, which supplies improvements in dielectric properties, compared to native collagen (10).

Another finding with ACM use was an increase in BC observed at the final moment (M3) in the groups augmented. Our results were equivalent to those obtained with other materials. A study performing only auto-augmentation in retracted bladders, with formalin, showed an increase in BC from 15 mL to 34 mL (16). Our animals presented mean increases from 17 to 35 mL, and this final result may be considered as the normal mean value of a rabbit's bladder. Another study evaluating BC of normal rabbits, with body weights similar to our rabbits, observed that these presented mean volumes between 33 and 37 mL (17).

In our experiment we have also tried to evaluate the presence of tissue regeneration. After 3 months we did not find traces of the primitive membrane, substituted by epithelium, and scarring tissue and normal muscle. What impressed us was that the group in which we have preserved bladder mucosa presented an intense inflammatory process in submucosa region. Other authors that augment bladder with bowel loops and preserve bladder mucosa did not report this alteration (18).

Atala et al. (19) used acelullar collagen membrane graft in bladder augmentation, and they observed at histological studies normal cell organization in augmented bladder, with epithelium, submucosa, and muscle neoformation. A relevant fact is the occurrence of greater bladder capacity in animals receiving graft with seeded cells. Other authors using bladder acellular collagen membrane have not only confirmed regeneration of bladder muscle, as also regeneration of innervation, as confirmed by electric stimulation as well as muscarinic receptors (20). In the literature, several authors consider that the state-of-the-art graft should be biodegradable, i.e., should be completely eliminated after acting as a backbone to bladder tissue regeneration. It should neither be rejected by the host, nor promote adherences or mutagenic alterations, and should be readily available at low cost. Anionic collagen membrane is biodegradable, and in our study it has turned possible bladder tissue regeneration. It is important to report that this experiment was performed in normal bladder, and further studies will be necessary to evaluate the responses in pathological bladders.

CONCLUSIONS

With our study we could to conclude that collagen anionic matrix showed effectiveness for bladder augmentation, demonstrating no significant difference in BC when bladder augmentation was performed with or without urothelium preservation. Bladders with urothelial preservation presented extensive inflammatory process, and we did not observe lithiasis formation with the use of the collagen anionic matrix.

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Correspondence address:
Dr. Fábio Gustavo Oliveira Lepper
Rua Coronel Manoel Luiz dos Santos, 465 / 24
Botucatu, SP, 18.603-310, Brazil
Fax: + 55 14 6822-0421
E-mail: jcarlos@fmb.unesp.br

EDITORIAL COMMENT

The drawbacks associated with current augmentation modalities have created an interest in developing better materials for bladder augmentation. Nowadays, investigators are attempting to engineer virtually every human tissue. The first stage of tissue engineering begins with the design and fabrication of a porous scaffold. This scaffold serves as a three-dimensional template for initial cell attachment and subsequent tissue formation. The authors have applied the principle of in vivo tissue-engineering strategies. They have used an anionic collagen membrane, which was elaborated from collagen gels prepared by the treatment of purified bovine serosa and tendon (reference 10 in the article).

Currently, numerous investigators have stated that better outcomes with the use of grafts for blad-

der augmentation can be obtained with the use of acellular matrices elaborated from small intestinal submucosa (SIS) or from bladder allograft (BAMA) (1). The assumption is that the appropriate extracellular environment provided by a scaffold, which contains almost all major components of the native extracellular matrix, is sufficient to effectively organize the regenerative capacity of the various components of the bladder wall. The efficacy of acellular matrix depends on its low antigenicity, its capacity for rapid vascularization, and its stability as a bladder template. These properties will be determined largely by the final composition of the acellular matrix. In this way, the authors' collagen membrane seems more appropriate to be used in other conditions than bladder augmentation.

Regardless of the strategy employed to develop a better bladder wall substitute, the incorporation of an acellular bladder augment involves 2 processes; one from the edge of the defect, and one from islands of cells in the midst of the defect. As the regeneration from the edges is more expressive than islands of cells, less encouraging outcomes at longer time are expected, if one uses a large patch (1). The authors have shown that the preservation of urothelium did not provide better results than the classical patch augmentation. However, the range of the bladder capacity was too wide in the group that collagen membrane was performed exclusively. Furthermore, the authors presented good short-term outcomes, but I am concerned about the efficacy of the anionic collagen membrane as a bladder substitute at long-term follow-up.

The challenge for more advanced scaffold systems is to arrange cells/tissue in an appropriate 3D configuration, and present molecular signals in an appropriate spatial and temporal fashion, so that the individual cells will grow and form the desired tissue structures – and will do so in a way that can be reproduced at low costs and on a large scale (2).

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Dr. E. Alexsandro da Silva
Urogenital Research Unit
State University of Rio de Janeiro
Rio de Janeiro, RJ, Brazil