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EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Collagen-1 Membrane for Replacing the Bladder Wall

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We studied the possibility of using membrane fabricated from type 1 collagen isolated from cattle tissues (group 1) or porcine tissues (group 2) for replacement of the resected bladder wall defect in rabbits in order to retain functional volume of the organ. Satisfactory take of both types of collagen membranes with formation of competent anastomosis was observed. Histological studies revealed inflammatory process in the bladder wall at the site of contact with the implanted membrane (more pronounced in case of membranes from cattle tissues) that decreased by day 21 of the experiment. Bladder tissue ingrowth into the implant from was observed starting from day 14. The bladder capacity decreased in 7 days after surgery in both groups, presumably because of increasing tone of the organ wall resulting from surgical trauma and inflammation. In group 2, the bladder volume increased by day 14 after surgery and returned to normal by day 21, whereas in group 1 it remained below the control despite a trend to increase. These findings confirm good prospects of using collagen-1 membranes for plastic repair of the urinary bladder, the membranes from porcine collagen being more preferable.

Key Words: *augmentation cystoplasty; collagen membranes; histological changes; bladder volume*

It is sometimes essential in urological practice to restore the functional volume of the urinary bladder after its severe contraction (e.g. in interstitial cystitis) [4,6]. Different vascularized segments of the gastrointestinal tract (small or large intestine, greater curvature of the stomach) are commonly used for augmentation cystoplasty. However, operations of this kind are highly traumatic and fraught with a high risk of surgical complications; one more important problem is the development of metabolic disorders associated with different functions of the mucosa of the transplanted segment of the intestine or stomach [8,14].

The search for alternative variants of augmentation cystoplasty, *e.g.* with the use of synthetic materials, is still in progress. Collagen from animal tissues is promising material in this respect, because it exhibits no toxicity and carcinogenic activity and is characterized by low antigenic activity, high mechanical strength, and enzyme resistance; the rate of collagen lysis can be regulated *in vivo*, it forms complexes with bioactive substances, and stimulates regeneration of own tissues [5,11]. An important characteristic of collagens is their phylogenetic relatedness in various animal species and humans allowing the use of xenogenic collagen for designing biomatrixes [1]. Collagen is a promising material for creation of guiding substrate for regeneration at the expense of gradual replacement with the adjacent tissues, which makes it preferable in comparison with synthetic polymers used

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in reconstructive surgery [2]. Collagen-based preparations have been used for plastic repair of the urinary bladder with promising results [3,12,15]. However, these studies are solitary and performed on different collagen preparations; hence, by the present time accumulation of the relevant data is essential.

We studied the possibility of using membranes from type 1 collagen isolated from cattle and porcine tissues as the graft for cystoplasty preserving bladder volume and preventing leakage; we also compared the reaction of bladder wall to implanted collagens.

MATERIALS AND METHODS

The study was carried out on 20 adult male Chinchilla rabbits (3.5-4.0 kg). The animals were narcotized with intravenous thiopental (40-50 mg/kg) after premedication (2 ml ketamine, 2 ml droperidol, and 2 ml relanium subcutaneously 20 min before the operation) and resection of the bladder wall (2×2 cm) was carried out with replacement of the defect with a collagen membrane (Fig. 1). Membranes from collagen isolated from cattle tissues were implanted to group 1 animals (n=10) and membranes from porcine tissues to group 2 animals (n=10). The membranes were sutured to the wall of the resected bladder with Prolene 5.0 by single continuous blanket suture using an atraumatic needle. The bladder was drained with a catheter over 3-4 days.

Collagen membranes were prepared as follows. Sterile transparent neutral solution of collagen isolated from animal tissues (VISKOLL; Imtek; 1 ml) in a sterile culture dish (2 cm^2) was incubated in a CO_2 incubator at 37°C until the formation of a hydrogel. Then, the hydrogel was dried under aseptic conditions at 20-25°C for 7 days until the formation of rigid glass-like material. The resultant membranes were packed in individual sterile packages with appropriate label.

The status of the bladder was evaluated in 7, 14, and 21 days after surgery. The abdominal cavity was opened under general anesthesia, the bladder was mobilized, its appearance and intensity of adhesions were evaluated, and the maximum volume was measured by filling it through a catheter. The membrane with the adjacent tissues was resected for histological studies, the specimens were routinely processed and stained with hematoxylin and eosin. The animals were sacrificed by sodium thiopental overdose.

The data were statistically processed by the Mann–Whitney U test.

RESULTS

All animals well tolerated the surgery; no appreciable complications were recorded. Collagen membrane anastomoses with the bladder wall were hermetic in all cases. Three rabbits developed suppuration of the skin suture without negative consequences.

In all group 1 animals, the bladder tightly adhered to the anterior abdominal wall and adjacent intestinal loops, which impeded its mobilization in some cases. The bladder was diffusely hyperemic and looked hypertensive (Fig. 2, *a*). The adhesion process was significantly less intensive in group 2 animals. The bladder was easily separated from the adjacent adhesions and its wall was relaxed. Slight hyperemia was observed only close to the sutured in collagen membrane (Fig. 2, *b*).

Dissection of the urinary bladder and examination of its cavity showed tight union between the sutured in membrane and the bladder wall in both groups. The mucosa in the zone of contact with the membrane was edematous, with formation of bullous protrusions early after the operation. The edema was the maximum on day 14 and reduced by day 21 of observation. Edema was more severe in group 1 animals during all periods

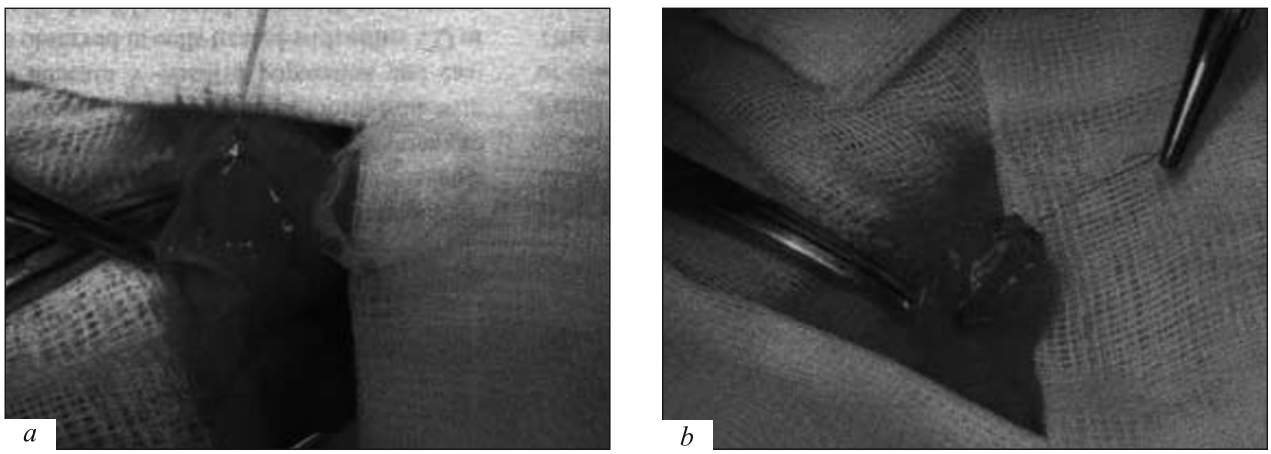

Fig. 1. Resected bladder before repair of the defect with collagen membrane (*a*) and after its suturing (*b*).

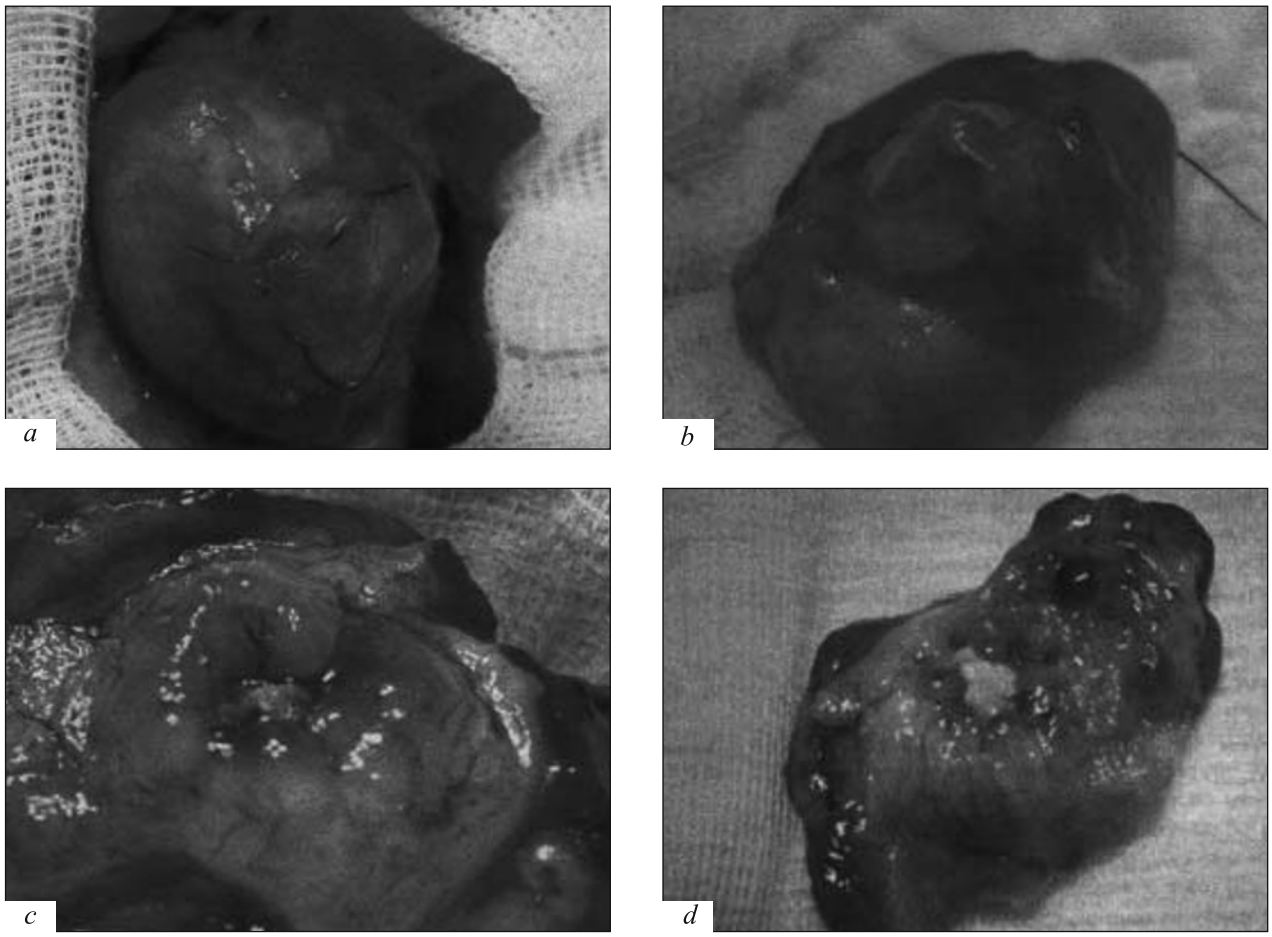

Fig. 2. General view of the bladder and aspect from the mucosa in 14 days after implantation of the membrane from cattle (*a*, *c*) and porcine (*b*, *d*) collagen.

of observation (Fig. 2, *c*, *d*). No mucosal hyperemia was observed in both groups. In contrast to the external surface of implanted membranes, which was completely covered by adventitial tissue, the inner surfaces were not completely epithelialized even on day 21. The inner surface of the sutured in membrane without epithelium was covered with a light-yellow deposition. However, visually the area of this site shrank with prolongation of observation.

Histological studies on day 7 showed signs of marked inflammation in the anastomosis zone in both groups: massive leukocytic infiltration and dilatation of vascular lumen in the submucous zone. In group 2, the signs of inflammation regressed with prolongation of observation, while in group 1 the intensity of inflammatory reaction remained sharply pronounced by day 14; by day 21, though reduced, it remained more intense than in group 2 animals (Fig. 3, *a*, *b*). Tissue structures growth into the collagen membrane matrix was observed from day 14; the process was more intense in group 2.

Bladder capacity (volume) after replacement of the resected site with a collagen membrane showed phasic changes. The bladder volume after resection without membrane repair decreased from 46-50 to 29-35 ml (by 30-37%) in 3 animals. Immediately after repair with collagen membranes, the bladder volume was 43±3 ml in group 1 and 46±3 ml in group 2, *i.e.* close to normal values. On day 7, a decrease in bladder volume was observed in both groups (more pronounced in group 1), followed by a trend to recovery

TABLE 1. Dynamics of Bladder Volume (ml) after Bladder Wall Plasty with Collagen Membrane

Group	Before resection	Day after resection		
		7	14	21
1	48±2 (100%)	34±3 (71%)	36±2 (75%)	41±2 (86%)
2	48±2 (101%)	37±3 (77%)	43±2 (90%)	49±2* (100%)

Note. *$p<0.05$ in comparison with group 1.

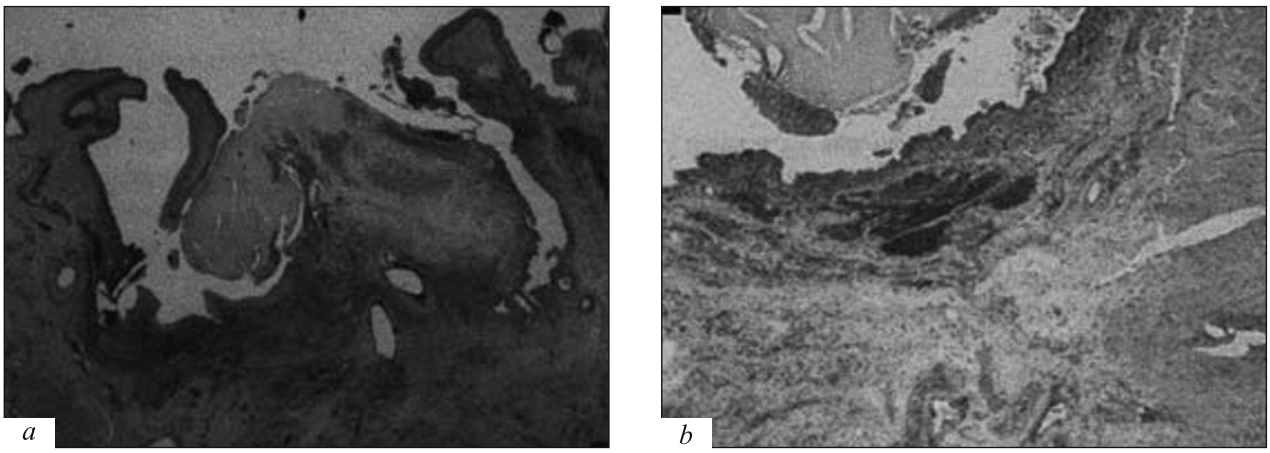

Fig. 3. Histological picture of the implantation site on day 14 after implantation of the membrane from cattle (*a*) and porcine (*b*) collagen.

of the organ volume (Table 1). In group 1, the bladder volume increased only by day 21 but still remained below the normal. In group 2, the bladder volume increased in 14 days and returned to normal in 21 days after surgery.

The study showed good prospects of cystoplasty with the use of collagen 1 membranes. The membranes exhibited good integration with the bladder tissues and satisfactory biological inertness; porcine collagen membranes showed better characteristics. Implantation of these membranes led to recovery of the bladder volume after artificial constriction. Transitory decrease in bladder volume at the early terms after surgery seemed to be caused by surgical trauma and inflammatory process (the latter was more intense after application of cattle collagen membranes) impeding normalization of the organ volume.

The use of membranes of both types was associated with bladder tissue growth into the collagen implant indicating its good integration into the adjacent tissues. The collagen matrix guides the regeneration process and is gradually replaced by host tissues [2] presumably due to stimulation of cell adhesion to the collagen surface demonstrated *in vitro* [13].

According to some data, immunogenic activity of collagen 1 preparations is low [1,11]. However, our experiments showed that implantation of collagen membrane induced reactive inflammation in the adjacent tissues, though not so intensive as to cause complications; this inflammation ceases by day 21 after implantation. No suture incompetence or bladder leakage was recorded. Membranes from porcine collagen exhibited better biocompatibility and integration in the adjacent tissues.

Incomplete epithelialization of the inner surface of implanted membranes can be explained by a short period of observation in our study. It was previously reported that cystoplasty with Collost, a collagen 1 preparation, was followed by complete epithelialization of the inner membrane surface in 3-6 months after its implantation [3].

Our results confirm good prospects of collagen membranes for anatomic reconstruction of the bladder, which is in line with the results of other authors, demonstrating migration of cells and blood vessels into the collagen implant [12,15]. However, other authors (including some pilot clinical studies) reported no appreciable improvement in the function of the reconstructed bladder after cystoplasty with collagen membranes despite its anatomic restoration [7,10,11]. This problem prompts further research in this direction, *e.g.* the use of collagen preparations with incorporated bioactive substances stimulating cell regeneration and angiogenesis (FGF, VEGF, products of cultured stem cell secretion, *etc.*) [9,11]. This possibility will be explored in our further studies.

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