Tissue reactions of the rabbit urinary bladder to cadaveric human fascia lata and polypropylene surgical mesh

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Objective: *To evaluate and compare histological tissue reactions of the urinary bladder to human cadaveric fascia lata (CFX) slings and synthetic mesh.*

Methods: *Thirty rabbits were randomized to three groups: group A (multifilament - Surgipro Mesh®) 12 animals, group B (CFX) 12 animals, and group C (surgical controls) 6 animals. A piece of Mesh or CFX was fixed in direct contact with the anterior bladder neck wall. The control group simply underwent sham bladder manipulation. The animals were sacrificed at 6- and 12-week intervals, and their bladders were collected for histological analysis.*

Results: *Group A showed fibrosis within the detrusor of the bladder wall in half of specimens, including marked trans-mural inflammation in one case. The graft was incorporated within a plate of fibrous tissue with a foreign body-type granulomatous reaction to the synthetic material. In group B, no fibrosis or inflammation was seen within the bladder wall. The graft showed marked inflammation (mixed cell infiltrate) in all specimens, whereas foci of devitalized collagen with associated foreign body-type granulomatous changes and dystrophic calcification were present in more than half of specimens. No histopathological alteration was found in the control group.*

Conclusion: *Significant histopathological changes within the bladder wall occur in response to synthetic mesh as compared to CFX over two tested time intervals. We believe these differences are related to the lack of reported adverse outcome with the use of cadaveric fascia lata slings in regard to graft erosion or infection when compared to complications occurring with the use of artificial materials.*

Key Words: urinary bladder, incontinence, slings, allograft, rabbit

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Introduction

Sling procedures for the treatment of urinary incontinence require different materials of varying lengths and attachments to support the bladder neck and urethra. The constant factor, however, is close contact between the materials used and the bladder neck and proximal urethra. Reports on these

procedures reveal a similar long-term continence rate (73%-86%) whatever material is used, but a higher complication rate with synthetic materials compared to homologous fascia lata.[1]

The commercial availability of cadaveric fascia lata allografts has considerably simplified the procedure and shortened hospital stay, sparing patients time and possible complications of harvesting, which has been associated with a local complication rate of up to 40%.[2] To our knowledge, no histopathological study regarding bladder wall tissue response to this type of graft is available.

The objective of the present work was to assess the histological appearance of the female rabbit urinary bladder in response to cadaveric fascia lata xenografts (CFX) and polypropylene surgical mesh in comparison to surgical controls at two time intervals.

Materials and methods

Thirty female New Zealand rabbits (2 kg-2.5 kg) were randomized to three groups: group A (polypropylene multifilament - Surgipro Mesh® - Auto Suture) 12 animals, group B (Tutoplast® processed CFX - Mentor) 12 animals, and group C (surgical controls) 6 animals.

The rabbits were anesthetized with a mixture of intramuscular Ketamine 50 mg/kg, Xylazine 5 mg/kg and Acepromazine 1 mg/kg. After shaving and prepping the abdomen, a laparotomy was performed under aseptic conditions. The bladder was exposed, avoiding traumatic manipulation. A 0.5 cm x 1 cm piece of CFX or polypropylene mesh was fixed in direct contact with the anterior bladder neck wall using 6.0 Vicryl sutures at the four corners of the graft. In the control group, the bladder was simply manipulated with a smooth end forceps for 5 minutes, and no material was applied. The abdominal wall was closed with 3.0 Vicryl, and the skin was sealed with a buried continuous 4.0 cat gut suture. The animals underwent standard postoperative recovery in our animal care facility.

At 6 weeks, half the number of animals in each group was sacrificed, and their urinary bladders collected for pathological assessment. The other half was sacrificed at 12 weeks from the time of surgery. These time intervals were chosen because adverse tissue reactions to sling materials usually occur in the first 3 months among patients undergoing sling surgery.[3]

Immediately after sacrifice in a carbon dioxide chamber, the urinary bladder was filled with 10% buffered formaldehyde injected by syringe through the urethra. The urethral orifice was closed with a silk suture and the specimen was then promptly immersed in 10% buffered formaldehyde for 24 hours. The urinary bladder was step-sectioned in continuity at 2- to 3-mm intervals, including the associated graft. Four micron-thick paraffin embedded sections, including the graft in continuity with the underlying bladder wall, were stained with hematoxylin-eosin and Masson's trichrome. Specimens were histologically assessed by an uropathologist (LRB) in a single blind fashion without information the grafting status. Care was taken to avoid including the sutured corners in the histological analysis in order to prevent contamination of our results by possible tissue reaction to the Vicryl sutures. Specimens were specifically assessed for the following: injury and/or repair changes within the bladder wall; status of the graft per se, including its integrity (i.e., recognizable outline and shape, tinctorial quality of native collagen for the cadaveric fascia, and superimposed inflammation and/or fibrosis) and nature of contiguous tissue; and the interface between the graft and bladder wall. Fibrosis within the bladder wall was semiquantified as follows: grade 1 if less than 50% penetration of the muscularis propria and less than 20% (surface area) involvement of the bladder wall in contact with the graft; grade 2 if more than 50% penetration (yet without full penetration) of the muscularis propria and/or more than 20% (surface area) involvement of the bladder wall in contact with the graft; grade 3 if penetration beyond the muscularis propria (i.e., involving lamina propria).

Results

Two animals from group B were lost during the experiment. One died during the induction of anesthesia. The other one had to be sacrificed 4 hours post-operatively because of early abdominal wound dehiscence. We attributed this complication to initially using non-buried sutures, which the animal managed to undo.

Group A (polypropylene mesh) at 6 and 12 weeks

The graft: In all specimens, the synthetic graft was incorporated within a moderately cellular fibrous tissue with preservation of the graft shape/outline Figure 1a. The outer aspect was covered by a serosa which was continuous with the serosa at the bladder edge, including varying degrees of subserosal fibrosis at the edge. Foreign body-type multinucleated giant cells (histiocytes) were found in apposition with the synthetic material.

The bladder wall and interface: In 4 of 6 specimens at 6 weeks, the interface was characterized by a thin layer of loose fibrovascular tissue making the graft

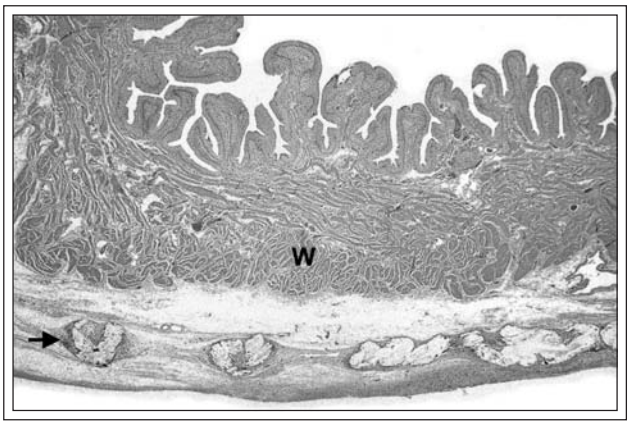

Figure 1a. The polypropylene surgical mesh graft (arrowhead) at 6 weeks is sharply demarcated and loosely adherent to the bladder wall (W) without any fibroinflammatory alteration of the latter.

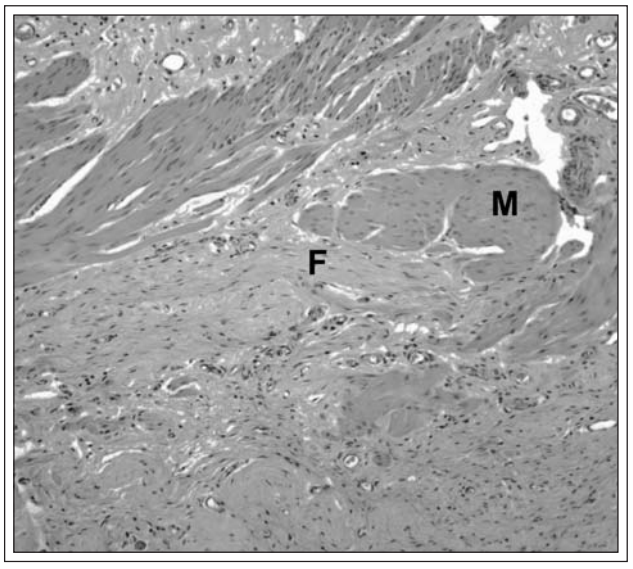

Figure 2a. Interface of polypropylene surgical mesh graft and bladder wall at 12 weeks showing fibrosis (F) invading the outer aspect of muscularis propria (M).

loosely adherent to the bladder wall Figure 1a. In 2 of 6 specimens at 6 weeks and in all specimens at 12 weeks, the interface was characterized by a layer of dense fibrous tissue abutting on the muscularis propria and making the graft tightly adherent to the bladder wall. No inflammation within the bladder wall was observed in 11 of the 12 specimens. In 1 specimen at 6 weeks, there was a severe cystitis characterized by numerous polymorphonuclear eosinophils, transmural florid granulomatous inflammation and fibrosis with entrapped synthetic material, and perivesical abscesses. Varying degrees of fibrosis within the bladder wall (contiguous to the graft) Figure 2a were apparent, as summarized in Table 1. In all specimens, the urothelium was unaltered.

Group B (CFX) at 6 and 12 weeks

The graft: In 2 animals sacrificed at 12 weeks, no graft was found attached to the bladder wall, whereas focal subserosal fibrosis was seen and considered to be indicative of spontaneous detachment during this longer time interval. Otherwise, in all specimens, the graft showed moderate to severe inflammation, including polymorphonuclear eosinophils and lymphocytes with formation of lymphoid follicles (and a superimposed plasma cell constituent in 1 case). In 5 of 8 specimens, there were geographical foci of devitalized (necrotic) collagen with an associated foreign body-type granulomatous reaction and dystrophic calcification Figures 1b and 2b. The outer aspect was covered by a serosa, which was continuous with the serosa at the bladder edge, including varying degrees of subserosal fibrosis.

The bladder wall and interface: In 2 of 8 specimens (one at 6 weeks and one at 12 weeks), the graft was tightly adherent to the bladder wall all along the plane of apposition, including a thin layer of dense fibrous tissue at the interface. In 6 specimens, the graft was incompletely adherent to the bladder wall. In some of these, the graft had a pedunculated configuration as it was loosely attached to the edge with a thin layer of fibrovascular tissue at the interface. No inflammation or fibrosis was present within the bladder wall of any specimen. The urothelium was unaltered.

TABLE 1. **Results for the polypropylene mesh group at 6 and 12 weeks**

	Inflammation	**No inflammation**	**Fibrosis grade**			
			0	**I**	**II**	**III**
6 weeks	1/6	5/6	4	0	0	2
12 weeks	0/6	6/6	2	1	3	0
Total	1/12	11/12	6	1	3	2

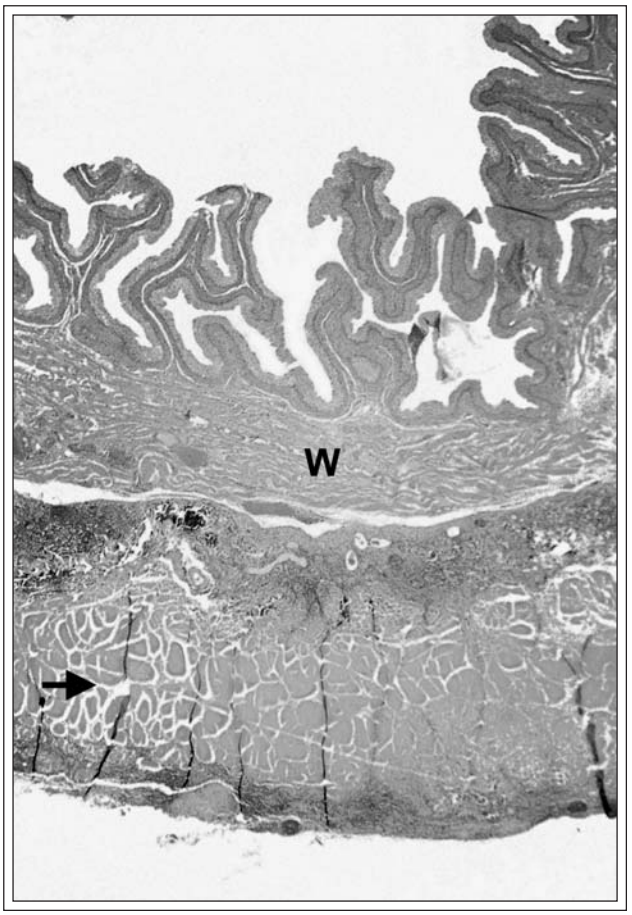

Figure 1b. The cadaveric human fascia lata graft (arrowhead) at 6 weeks is sharply demarcated yet tightly adherent to the bladder wall (W) without fibroinflammatory changes in the latter. (Both hematoxylin and eosin staining, 20x original magnification).

Group C (control) at 6 and 12 weeks

No histopathological alteration was observed in specimens of the control group.

Discussion

Sling procedures are indicated for a number of incontinence conditions. They have become widespread in the treatment of women with stress urinary incontinence (SUI) due to intrinsic sphincter deficiency with or without bladder neck mobility.[4] They are also frequently used to address female neurogenic incontinence. The success rates of allograft and autograft pubovaginal slings are equally high, and no complications relating to the cadaveric origin of allograft fascia lata are documented in the literature.[5]

Synthetic grafts used for this purpose have included Gore-Tex, Marlex, and polypropylene mesh. Polypropylene is now widely used in the most recent forms of artificial slings (Tension free vaginal tapes, Supra-pubic arc, various trade marks of trans-obturator tapes etc.). They are attractive due to their unlimited availability.[6] The clinical experience correlates with a good cure rate in the range of 82% to 91%.[1,3,6,7] However, they are associated with increased infection and adverse localized tissue reactions. Bent et al[3] reported a 21% incidence of local tissue reactions in a review of 115 patients who received polytetrafluoroethylene (PTFE) slings: 23 slings

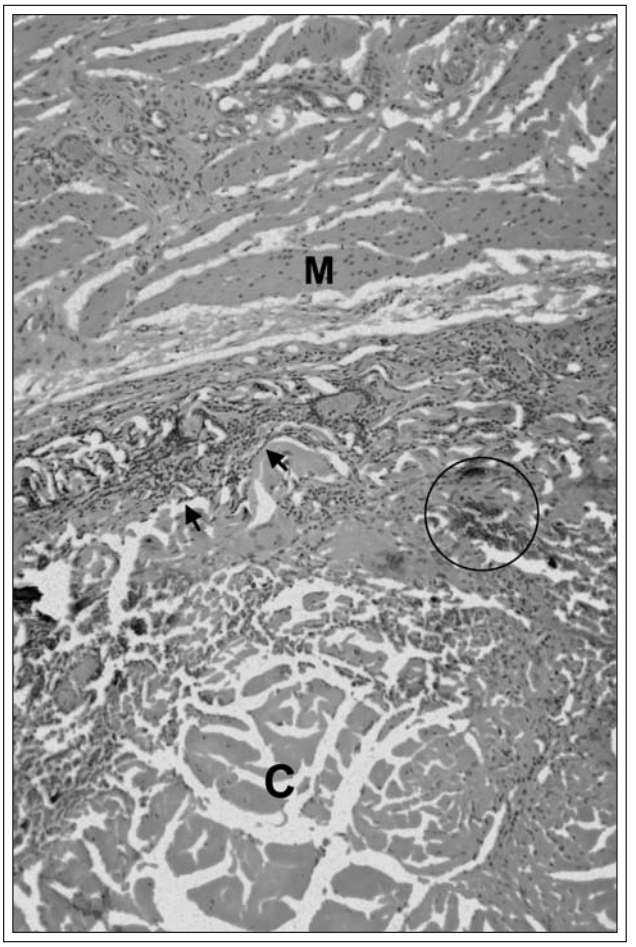

Figure 2b. Interface of cadaveric human fascia lata graft (lower two-third) and bladder wall at 6 weeks. The graft is characterized by mostly devitalized dense connective tissue (C) with associated inflammation (arrowheads) and dystrophic calcification (within circle). Note the absence of fibroinflammatory changes at the level of the muscularis propria (M). (Both hematoxylin and eosin staining, 100x original magnification).

required removal and all demonstrated gram-positive cocci. Histological assessment revealed a thin granular protein fibrin cell matrix, in association with neutrophils, granulocytes, erythrocytes, degenerated cells and pyknotic nuclei.[3] In another series, Choe and Staskin[1] demonstrated a low rejection rate of 5%, all of which required excision. They attributed this to using a smaller cross sectional area of the patch (1/6th that of the Bent sling).[1]

Experience in the field of neurosurgery over a span of 20 years with allogenic and xenogenic fascia lata grafts in duraplasty has been reported to be remarkably good, with a success rate of about 97%.[8] Similar success rates have been reported with knee ligament surgeries.[9] No adverse outcomes with regard to erosion or graft infection were observed in two recent series using cadaveric fascia lata slings for the treatment of SUI.[2,5] An important concern about allograft fascia lata is the potential for disease transmission. The companies that make these products available commercially claim that the materials are inactive virally and immunologically. There has been only one reported case of a woman receiving a bone graft and contracting human immunodeficiency virus (HIV) from a seronegative donor since screening was started in 1985. The estimated risk of acquiring tissue from a properly screened donor infected with HIV is 1/1,667,600.[10] Nevertheless, it is recommended that surgeons obtain informed consent from their patients when using this material.[11]

Previous work by Pesch[12] has demonstrated that preserved collagen materials represent innert grafts in which cells have been destroyed. These cells act as "formers", stimulating the build-up of the body's own connective and support tissue. His work on the biotolerability of xenogenic fascia lata grafts and host tissue reactions of the abdominal wall in albino rats parallels our results. He also observed an initial infiltration of neutrophil granular leukocytes and macrophages; the graft became enclosed, sandwich-like, in resorbtive granulation tissue, and no vitalization of the graft occurred. He reported a foreign body-type granulomatous reaction only in the vicinity of the sutures. One-year follow-up of a similar group of animals, including those receiving multiple grafts, revealed similar reactions of abdominal wall tissue, but the grafts were enzymatically lysed and replaced by living connective tissue.[13] These grafts have also been studied in knee ligament surgery on experimental dogs. Histological tissue reactions again revealed tissue granulation and newly-formed connective tissue deposition. No rejection was found.[14]

Resistance to infection could differ in animals, depending on the type of graft used. Disa et al,[15] demonstrated significant differences in infection rates with fascia lata grafts in New Zealand rabbits, compared to synthetic PTFE for closure of abdominal wall defects. The rabbits were inoculated with 10^9 *S. aureus*. Seven of 12 animals in the PTFE group developed fatal necrotizing infections, as compared to only 2 of 12 animals with fascia lata grafts. Such differences in susceptibility to infection may generate varying local reactions at the site of graft implantation.

In our study, the polypropylene surgical mesh group showed significant encroachment of the muscularis propria (detrusor) by fibrosis at both time intervals, including marked fibrosis in 2 specimens at 6 weeks. In contrast, no fibrosis was observed in the CFX group. These changes parallel the previously-discussed clinical results where erosion and rejection were only reported with synthetic grafts in association with a significant inflammatory reaction.[5]

We acknowledge that suturing the graft to the bladder dome created a risk of contamination of our results because of the usual tissue reaction to Vicryl sutures. However, we took great care to make our histological sections in the middle of the graft, far from the sutured areas.

Although the present study is descriptive in nature and based on an animal model, it provides good insight into the type of tissue response that might possibly occur in the human urinary tract. Regarding the use of cadaveric fascia lata grafts, we acknowledge that our extrapolation is questionable since we used human allografts on an animal bladder. However, considering the fact that the local reaction was so mild, even with a possible cross species host versus tissue reaction, we assume that the reaction in humans would be even milder.

Finally, we also acknowledge that the difference between our model and a sling operation in clinical practice is that there is no tension in our implantation, and human slings are positioned around the urethra and not the anterior bladder neck. After having tried to realize a real sling on this animal model we decided to avoid contaminating our results with obligatory local reactions secondary to a quite extensive surgical dissection/manipulation. Furthermore, we think that the difference in anatomical structures between the bladder and the urethra should not make a difference for tissue reactions and that the lack of tension in our model is not an issue since the best sling outcomes are obtained with tension-free procedures.

Conclusion

Our study showed better tissue reactions of the bladder wall in the CFX group as compared to the surgical polypropylene group. These findings correlate well with the clinical data. CFX looks to be much better tolerated by urinary tract tissues than polypropylene surgical mesh. However, other issues regarding CFX, such as the significance of DNA fragments and CFX durability must be solved before their wide-spread use. Insofar as polypropylene surgical mesh is concerned, other new products such the mesh used for tension-free vaginal tape and the suprapubic arc (SPARC) should be studied in the same model, since their structures are different even if they are made with the same material. □

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