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FREE URETERAL REPLACEMENT IN RATS: REGENERATION OF URETERAL WALL COMPONENTS IN THE ACELLULAR MATRIX GRAFT

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ABSTRACT

Objectives. To evaluate ureteral replacement by a free homologous graft of acellular matrix in a rat model.
Methods. In 30 male Sprague-Dawley rats, a 0.3 to 0.8-cm midsegment of the left ureter was resected and replaced with an acellular matrix graft of equal length placed on a polyethylene stent. The animals were killed at varying intervals, and the grafted specimens were prepared for light and electron microscopy.
Results. In all animals, the acellular matrix graft remained in its original position without evidence of incrustation or infection, and histologic examination showed complete epithelialization and progressive infiltration by vessels. At 10 weeks, smooth muscle fibers were observed; at 12 weeks, nerve fibers were first detected; at 4 months, smooth muscle cells had assumed regular configuration.
Conclusions. The ureteral acellular matrix graft appears to promote the regeneration of all ureteral wall components. UROLOGY **50:** 818–825, 1997.

Ureteral replacement has long been a subject of interest to researchers, and many surgical procedures and materials have been tried.[1–4] However, an entirely satisfactory method has yet to be found. Previous research has demonstrated that collagen-based materials, such as porcine small intestine submucosa (SIS), have the best potential regenerative capability.[5] In this group of biomaterials, a new acellular matrix has recently been shown, in the bladder of the rat model, to serve as a scaffold consisting of collagen and elastin fibers for the ingrowth of all bladder wall components.[6,7] In addition, the contractility of graft-regenerated bladders has been observed in vivo (preliminary results). We designed the present study to determine whether this acellular matrix could be used as a free ureteral graft in a rat model.

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MATERIAL AND METHODS

Animals and Surgical Technique

Male Sprague-Dawley rats (n = 30), weighing 260 to 360 g, were housed three to a cage at constant temperature and humidity with a 12-hour light and dark cycle. Rats were given free access to standard labaratory chow and tap water. The technique for preparation of the matrix graft, described previously for the bladder,[7] was modified as follows: the ureter from a Sprague-Dawley rat was excised and placed on a polyethelene stent (inner diameter [ID] 0.28 mm; outer diameter [OD] 0.61 mm) for further processing. Because the tiny rat ureter is not suitable for inversion, for the ureteral matrix the mucosa was not scraped off as it was in the preparation of the bladder matrix graft. Partial cell lysis (in 10 mM PBS), complete cell lysis (in 1 M sodium chloride containing 2000 Kunitz units DNase), and solubilizing of the lipid membranes in 50 mL of 4% sodium desoxycholate containing 0.1% sodium azide (repeated once) were performed. The resulting ureteral acellular matrix graft was stored in 10% neomycin sulfate at 4°C until grafted (Fig. 1).

At the time of surgery, animals were anesthetized with sodium pentobarbital (6 mg/100 g body weight intraperitoneally) and, through a midline lower abdominal incision, the left ureter was exposed. A 0.3 to 0.8-cm midsegment of the ureter was resected and replaced with an equal graft segment placed on a polyethylene stent through the open ends of the host ureter (Fig. 2). The stent was used to prevent obstruction at the level of the graft. The graft segment was trimmed to conform to the remaining host ureter and sutured in place with monofilament 10.0 nonabsorbable Dermalon sutures to identify the matrix borders. To promote growth, the acellular matrix graft was surrounded with retroperitoneal fat. When satisfactory closure was achieved, the abdominal wall

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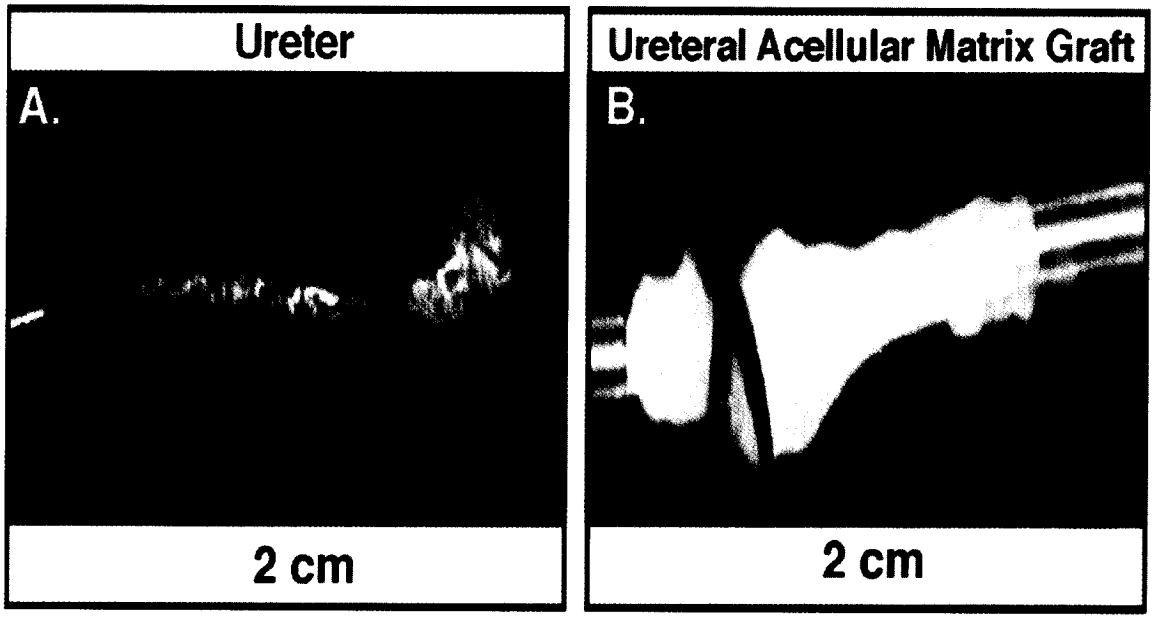


FIGURE 1. *Photographic documentation of a rat ureter placed on a polyethylene stent before (A) and after (B) processing into a ureteral acellular matrix graft.*

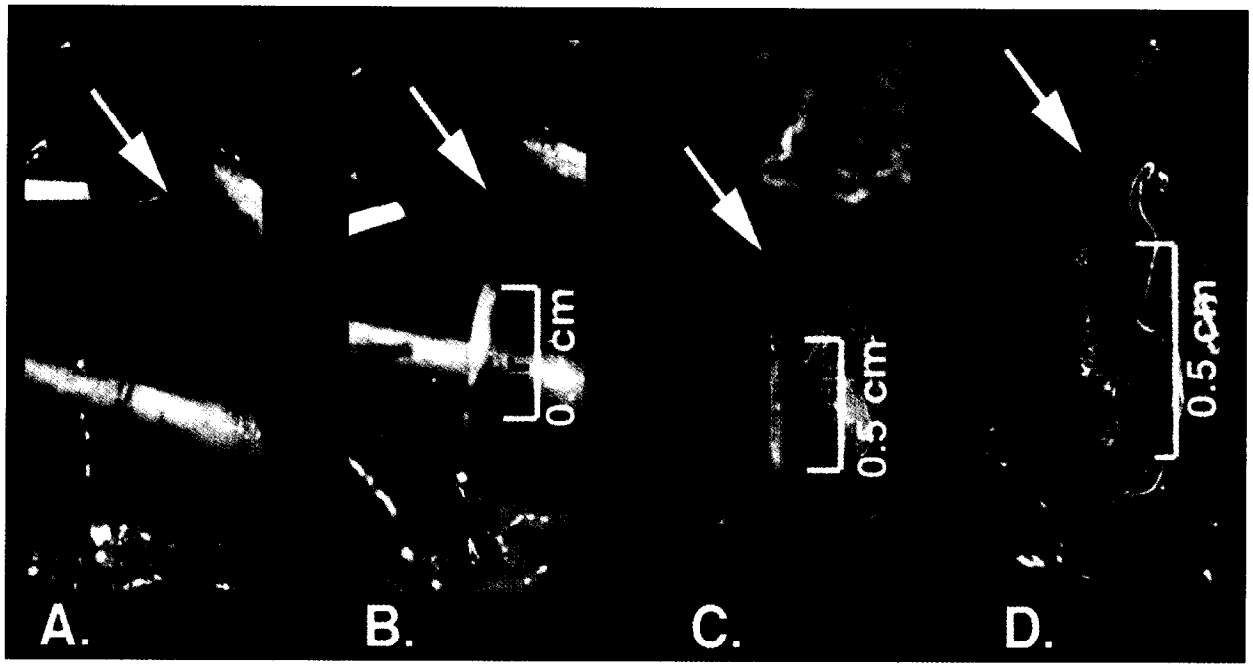


FIGURE 2. *Surgical procedure of partial ureteral replacement by the ureteral acellular matrix graft: (A) exposure and temporary clamping of the left ureter; (B) ureterotomy and intubation by the stented graft in the direction of the urinary bladder (arrows); (C) distal anastomosis with interrupted sutures; (D) intraoperative view after implantation.*

and the skin were closed. For microsurgery, an Olympus binocular operating microscope (10× to 40×) was used. No drugs were administered postoperatively.

Light Microscopic and Ultrastructural Evaluation

The animals were killed at the following times: 4 days (n = 3), 3 weeks (n = 5), 6 weeks (n = 3), 10 weeks (n = 7), 3 months (n = 5), and 4 months (n = 5). Host ureter and graft were identified, freed from the surrounding tissue under a dissecting microscope, and collected. All specimens were rinsed with saline solution to remove excess intraluminal urine. Tissues were fixed at the time of accession and processed for light and transmission electron microscopy.

Light Microscopy. Specimens were fixed in 10% buffered formalin for at least 24 hours. After dehydration in graded ethanol solutions, the specimens were embedded in paraffin, sectioned (5 μm), and stained with trichrome for collagen and smooth muscle, hematoxylin and eosin (H & E) for nuclei, alpha-actin for smooth muscle, and protein gene prod-

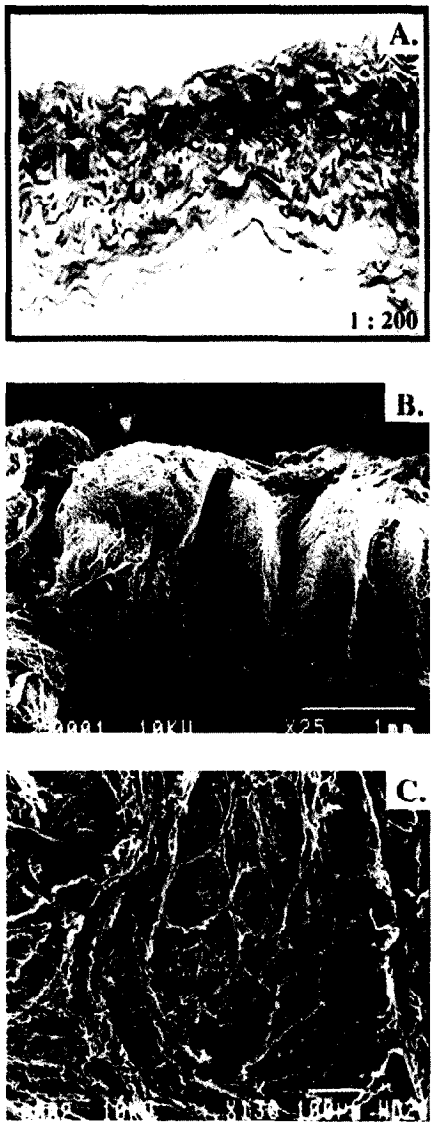


FIGURE 3. *Confirmation of the acellularity of the matrix graft and its structure as a framework of elastin and collagen fibers: (A) H & E staining at an original magnification of ×200; (B) scanning electron microscopy at low magnification (original magnification ×25) shows the intact surface of the ureteral acellular matrix; (C) at higher magnification (original magnification ×130), the mesh-like structure of fibers (which may serve as a scaffold), without evidence of cellular elements, is demonstrated.*

uct 9.5 (PGP 9.5) for nerves. The monoclonal antibody anti-alpha-smooth muscle actin recognizes alpha-smooth musle cells exclusively.[8] Protein gene product 9.5 represents a major protein component of the neural cytoplasm and therefore labels more nerve fibers than other general nerve markers.[9,10] Before implantation, pure graft specimens were also prepared for light and scanning electron microscopy to confirm the structure as an acellular scaffold consisting of collagen and elastin fibers and thus the effectiveness of the matrix preparation process.

Transmission Electron Microscopy. Specimens were immersed in a fixative (2.5% glutaraldehyde and 2.5% paraformaldehyde) in 0.15 M sodium cacodylate buffer. After the primary fixation, the samples were placed in a drop of fixative on dental wax and cut in approximately 3-mm segments. Specimens were postfixed with 2% osmium tetroxide, block stained in 2% uranyl acetate, and dehydrated in a graded series of ethanol and propylene oxide, after which they were embedded in resin. Thin sections (500 Å) were obtained, mounted on 200-mesh copper grids, stained in uranyl acetate and lead citrate, and examined in a Zeiss Model 10c electron microscope.

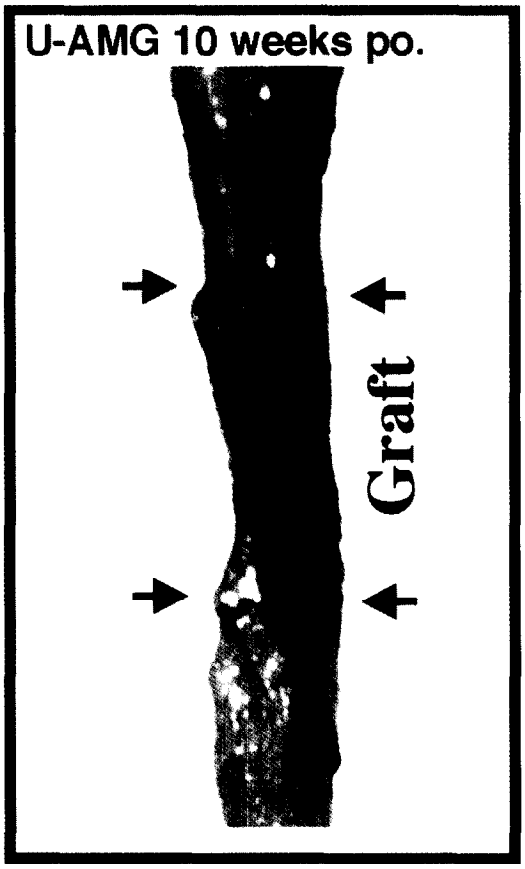


FIGURE 4. *Macroscopic view of the rat ureteral matrix graft 10 weeks after surgery. Arrows mark nonabsorbable sutures to identify the border between the matrix graft and host.*

RESULTS

MORTALITY

Of the 30 animals, 2 died 6 weeks after surgery from a coronavirus infection associated with severe respiratory tract obstruction and were not used for further microscopic evaluation. In contrast to our reported experience with the matrix grafted to the bladder,[7] stone formation was not observed in either the upper or lower urinary tract.

EVALUATION OF MATRIX TECHNIQUE

Light microscopy of the pure ureteral matrix demonstrated the effectiveness of the matrix preparation process. The acellularity of the graft as an intact framework consisting of elastin and collagen fibers was apparent (Fig. 3A). Scanning electron microscopy showed the intact nature of the ure-

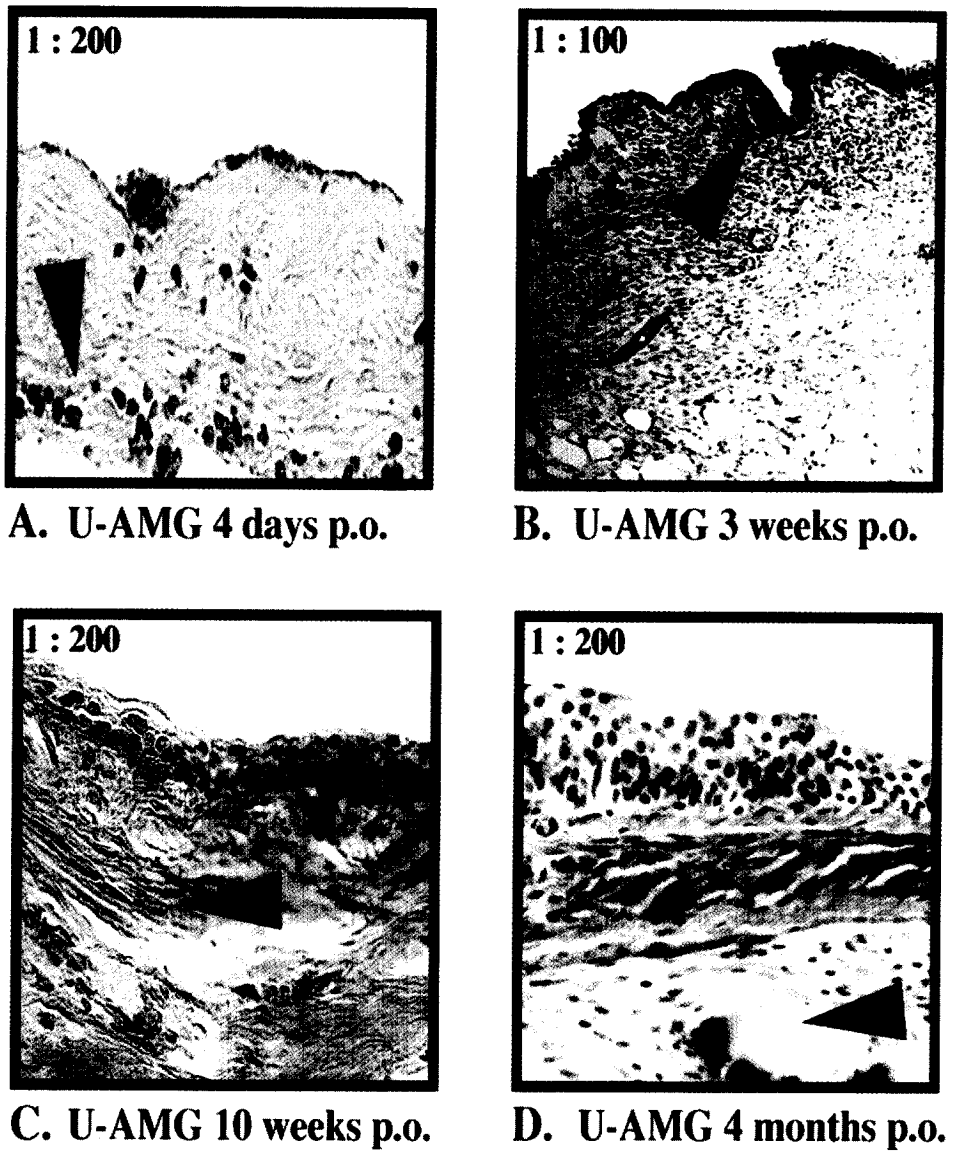


FIGURE 5. *Step-by-step regeneration of ureteral wall components (Masson's trichrome stain, original magnification ×200). After infiltration of erythrocytes and mononuclear cells (arrow) (A), urothelium (arrow) begins to develop in the first weeks (B). At 10 weeks (C), the first few muscle fibers are seen (arrow), and after 4 months (D), a complete regeneration of urothelium and muscularization can be observed (arrow marks borderline [suture] between the matrix graft [left] and the host ureter [right]) (U-AMG, ureteral acellular matrix graft).*

teral matrix surface and confirmed the scaffold-like structure of the graft without evidence of cellular elements (Fig. 3B, C).

Macroscopic Appearance of Explanted Grafts

Moderate adhesions were noted to the surrounding retroperitoneal fat. The graft remained in its original position in all animals, without evidence of incrustation or infection (Fig. 4), whereas the polyethylene tube migrated to the distal ureter. For this reason, gross examination of all surgical specimens revealed varying degrees of hydroureteronephrosis to the level of the graft. There was no evidence of postoperative urinary leakage in any of the animals at death.

Light Microscopy

Histologic examination of all graft-regenerated ureters showed step-by-step regeneration of urothelium and smooth muscle fibers (Fig. 5). At 4 days, the graft showed an infiltration of erythrocytes and mononuclear cells, and the urothelial lining appeared to begin to bridge the matrix graft. At 10 weeks, the graft was composed of several layers of urothelium and some capillaries, and characteristic arrangements of smooth muscle fibers (Fig. 6) were first observed. The number of vascular elements (capillaries) had increased. At 12 weeks, nerve regeneration was first detected by PGP 9.5-positive staining (Fig. 7). At 4 months, neomuscularization was well developed. The

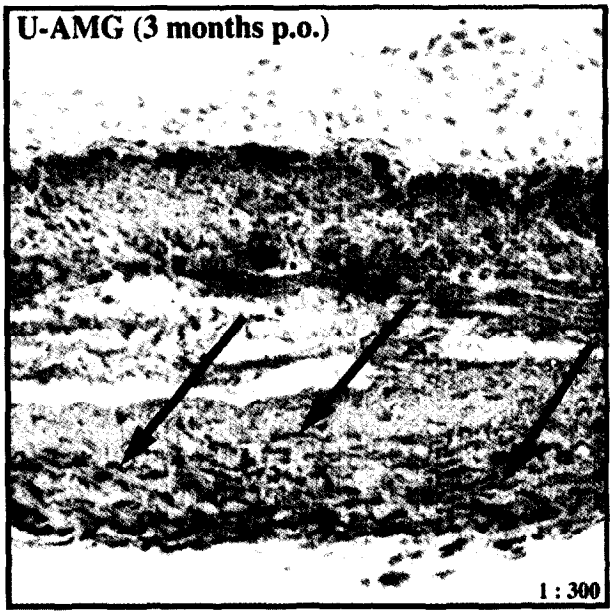


FIGURE 6. *Histologic appearance of the ureteral acellular matrix graft (U-AMG) 3 months after grafting. Smooth muscle cell differentiation is confirmed by localization of alpha-actin (original magnification ×300). Note the opaquely stained thick strands, which represent alpha-actin smooth muscle fibers (arrow).*

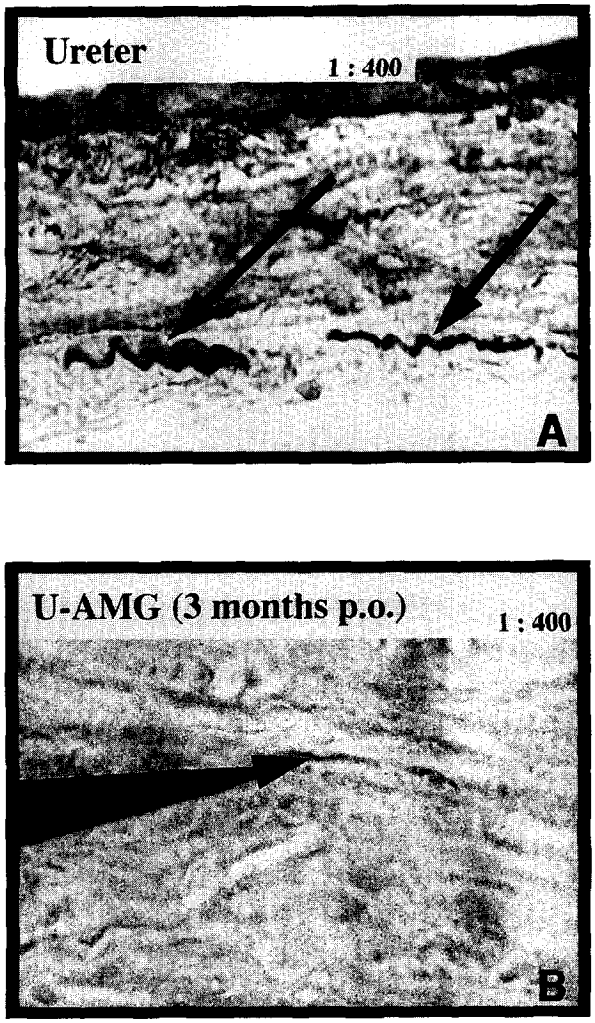


FIGURE 7. *PGP 9.5 staining of normal ureter (A) and graft 3 months postoperatively (B). Nerve regeneration is confirmed (original magnification ×400). Note the lower density of PGP 9.5-positive nerve staining in the graft (arrow) (U-AMG, ureteral acellular matrix graft).*

smooth muscle cells were arranged in parallel rows in the longitudinal direction. The thickness of these muscle bundles seemed to decrease in the central part of the graft. There was no sign of degenerative change such as calcification or necrosis of the smooth muscle layer. The urothelial lining, differentiated muscularization, and surrounding fibrous adventitia appeared qualitatively similar to normal ureteral wall components (see below). In contrast, we noted that the number of nerve fibers was less than in the normal ureter.

Electron Microscopy

Smooth muscle regeneration in the grafted ureteral matrix was confirmed by transmission electron microscopy (Fig. 8). At 3 months, specimens demonstrated a lower density of myofilaments in the graft than in the normal rat ureter. At 4 months, the number of myofilaments was significantly increased. These observations corresponded to the light microscopic findings at the same time. Nerve regeneration was confirmed in 4-month specimens studied by electron microscopy (Fig. 9). Although the number of nerve fibers in the matrix graft was notably less than in the normal ureter, their morphologic characteristics were similar.

COMMENT

Ureteral replacement has long been a challenge for urologists. In other specialties, the success of acellular matrix grafts for organ substitution has been reported (eg, replacement of heart valves, coronary artery bypass, and, in particular, skin).[11–14] Although the concept of free grafts to treat ureteral diseases is not new, none of the materials previously reported has been able to satisfy all the criteria for the ideal substitute (Table I).[15–24] In the present study, complete epithelialization, angiogenesis, and regeneration of smooth muscle fibers and nerves were observed with no signs of rejection.

The reasons for the improved acceptance of the ureteral acellular matrix graft over that of other free ureteral transplants are still unknown. In accordance with our previous experience, we used extremely fine nonabsorbable suture material to minimize immediate postoperative inflammatory reactions, which can result in incrustation or stone formation. Rapid epithelialization, progressive

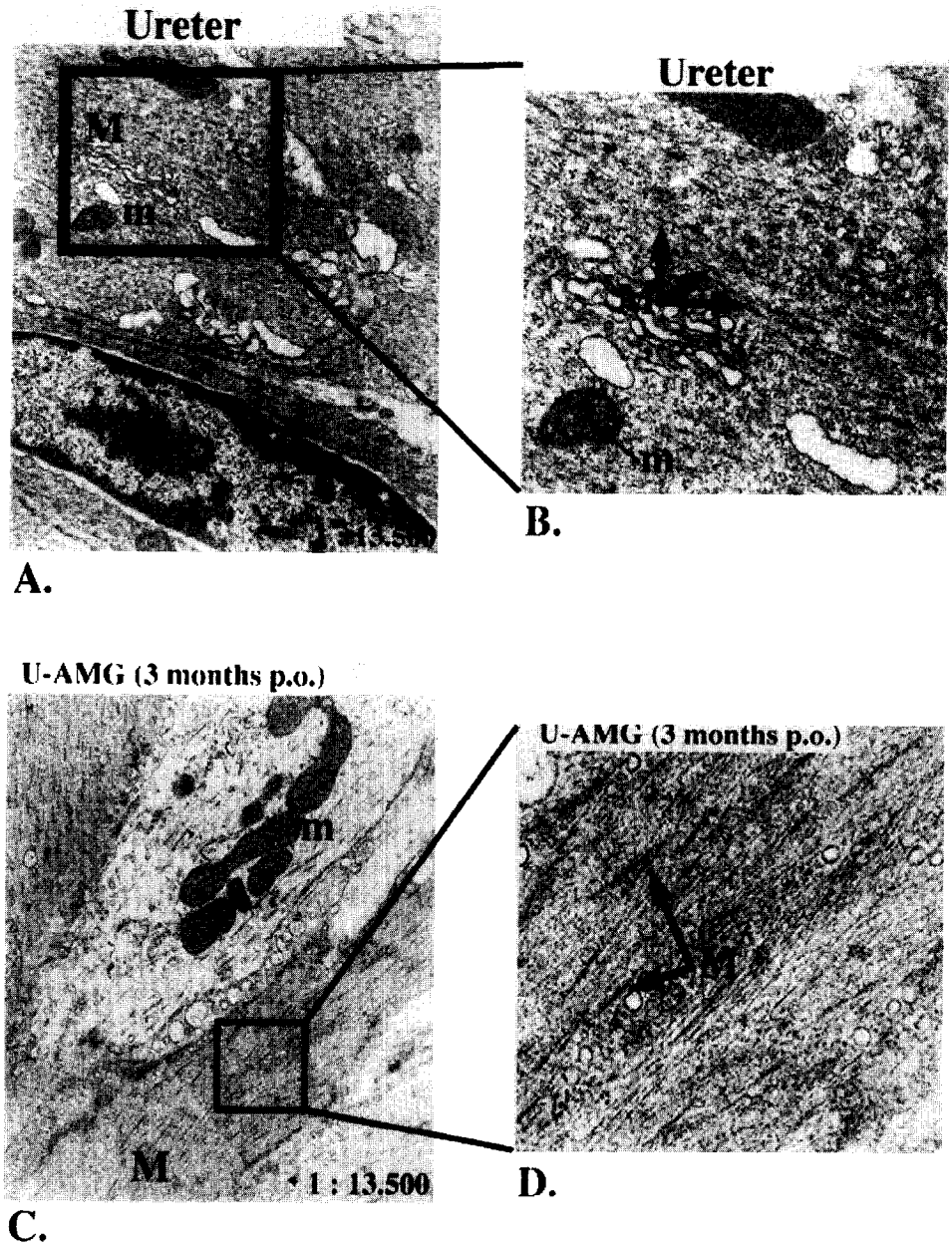
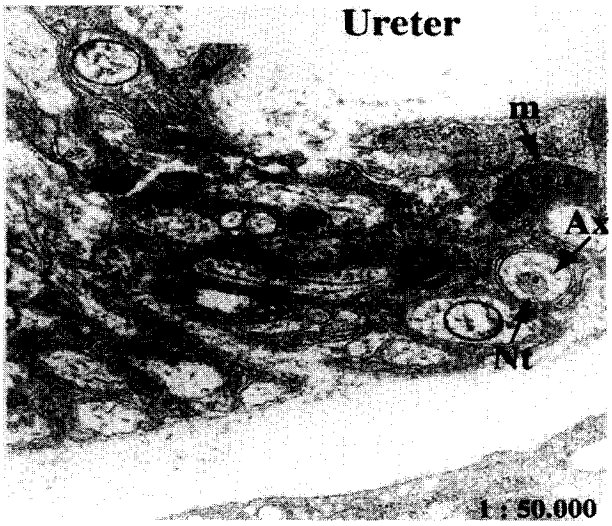


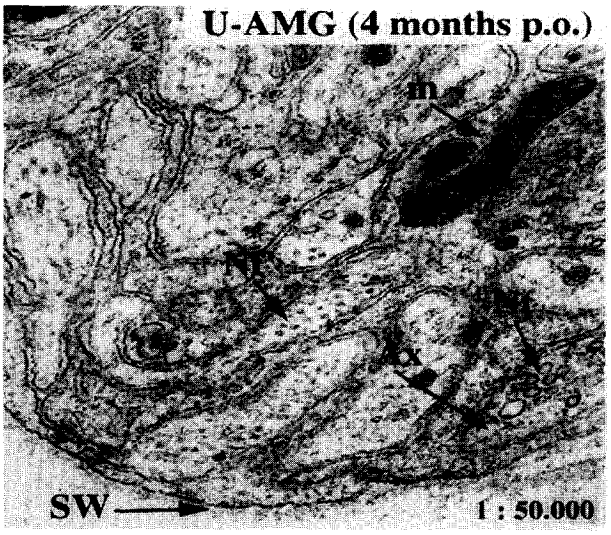
FIGURE 8. *Transmission electron microscopy of normal ureter (A and B) and graft 3 months postoperatively (C and D) (original magnification ×13,500). Note lower density of myofilaments (M) in the graft-regenerated rat ureter than in the normal ureter. Mitochondria (m) are also seen (U-AMG, ureteral acellular matrix graft).*

neoangiogenesis, and continuous smooth muscle regeneration, apparently arising from the adjacent edges of the host ureter, are possible factors responsible. Expression of growth factors seems to play a key role in the mechanisms controlling epithelialization.[25] In our research, studies of transforming growth factor (TGF) expression in the rat bladder acellular matrix graft showed variable induction of TGFα, TGFβ_1, TGFβ_2, and TGFβ_3 transcription, with prominent mRNA expression of TGFα and TGFβ_1 (preliminary results).

In time, early smooth muscle cells matured into normal-appearing smooth muscle bundles. Because smooth muscle production has been reported from pericytes after capillary neovascularization,[26] we hypothesize on the basis of our studies that the source of smooth muscle fibers in the acellular matrix graft is the adjacent edges of the host ureter. Regulating mechanisms of smooth muscle differentation are poorly understood. Besides growth factor expression (as just mentioned), epithelial-mesenchymal interactions are thought to be important for smooth muscle regeneration. In 1961, Taderera[27] demonstrated that the absence of lung epithelium causes failure of both smooth muscle and cartilage differentation. Consistent with these results, Baskin *et al.*[28] reported in 1996 that intact bladder as well as isolated blad-



A.



B.

FIGURE 9. *Transmission electron microscopy of normal ureter (A) and graft 4 months postoperatively (B) (original magnification ×50,000). The graft-regenerated rat ureter shows morphologically intact nerves with axons (Ax), mitochondria (m), neurotubules (Nt), neurofilaments (Nf), and Schwann cell sheath (SW) (U-AMG, ureteral acellular matrix graft).*

der mesenchyme recombined with bladder urothelium from rat fetuses, when grafted under the renal capsule of adult rats, demonstrated expression of smooth muscle differentiation; however, grafts of bladder mesenchyme alone failed to induce smooth muscle differentiation. Therefore, early epithelialization in the graft may be highly important for smooth muscle differentation.

The observation that the overall (PGP 9.5-positive) density of the reinnervation of the ureteral acellular matrix graft was significantly lower than that of the normal ureter may indicate that cell-derived factors are needed to achieve normal levels of innervation. A similar experience has been described by Gavazzi *et al.*[29] They showed that, after grafting of frozen and thawed acellular cerebral blood vessels in oculo, the reinnervation of the graft was less than that of the control, and PGP 9.5-positive nerves appeared less dense on the transplants as well. However, in the present study regeneration of nerves was confirmed by light and electron microscopy and may increase over a longer period of time to achieve functional capacities.

TABLE I. *Criteria for the ideal ureteral substitute

Close histologic resemblance to normal ureter
Peristaltic activity synchronous with host ureter
Adequate blood supply
Free transport of urine
Impermeable, nonabsorptive lining
No immunologic reaction
No stone formation
Technical ease
No stricture formation at the anastomotic site
Normal innervation and adequate pharmacologic response

* *Summarized from Baum* et al.[2]

In conclusion, the ureteral acellular matrix graft appears to promote the regeneration of all ureteral wall components. These results may indicate that the graft has a potential for functional neomuscularization that will result in its maintenance as a physiologic ureteral wall. Tube migration with consequent obstruction at the level of the stent, causing hydroureteronephrosis, was not avoidable. It would thus be reasonable to undertake further study in a larger animal model. Detailed functional and molecular biologic experiments are needed to evaluate whether the ureteral acellular matrix graft as a ureteral substitute can conduct peristaltic activity in coordination with the host components to preserve the functional integrity of the renal parenchyma. The findings of regenerated ureteral wall components are encouraging and support the clinical potential of the acellular matrix graft in genitourinary tract reconstruction.

REFERENCES

1. Hovanian AP, Javadpour N, and Gruhn JG: Reconstruction of the ureter by free autologous bladder mucosa graft. Part I. A preliminary report. J Urol 93: 455–461, 1965.

2. Baum N, Mobley DF, and Carlton CE: Ureteral replacements. Urology 5: 165–171, 1975.

3. Vistnes LM, and Lilla JA: Free grafts of autologous and homologous ureter. Invest Urol 13: 255–258, 1976.

4. Becht E, Derouet H, and Ziegler M: Alloplastic ureter substitution and limitations in clinical application. A status analysis. Urologe A 30: 299–301, 1991.

5. Kropp BP, Sawyer BD, Shannon HE, Rippy MK, Badylak SF, Adams MS, Keating MA, Rink RC, and Thor KB: Characterization of small intestinal submucosa regenerated canine detrusor: assessment of reinnervation, in vitro compliance and contractility. J Urol **156**: 599–607, 1996.

6. Sutherland RS, Baskin LS, Hayward SW, and Cunha GR: Regeneration of bladder urothelium, smooth muscle, blood vessels and nerves into an acellular tissue matrix. J Urol **156**: 571–577, 1996.

7. Probst M, Dahiya R, Carrier S, Lipkin D, and Tanagho EA: Reproduction of functional smooth muscle and partial bladder replacement. Br J Urol **79**: 505–515, 1997.

8. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillesen D, and Gabbiani G: A monoclonal antibody against α-smooth muscle actin: a new probe for smooth muscle differentation. J Cell Biol **103**: 2787–2796, 1986.

9. Gulbenkian S, Wharton J, and Polak JM: The visualization of cardiovascular innervation in the guinea pig using an antiserum to protein gene product 9.5 (PGP 9.5). J Auton Nerv Syst **18**: 235–247, 1987.

10. Dalsgaard CJ, Rydh M, and Haegerstrand A: Cutaneous innervation in man visualized with protein gene product 9.5 (PGP 9.5) antibodies. Histochemistry **92**: 385–390, 1989.

11. Wilson GJ, Courtman DW, Klement P, Lee JM, and Yeger H: Acellular matrix: a biomaterials approach for coronary artery bypass and heart valve replacement. Ann Thorac Surg **60**(suppl): S353–S358, 1995.

12. Lievesey SA, Herndon DN, Hollyoak MA, Atkinson YH, and Nag A: Transplanted acellular allograft dermal matrix. Transplantation **60**: 1–9, 1995.

13. Takami Y, Matsuda T, Hanumadass M, and Walter RJ: Dispase/detergent treated dermal matrix as a dermal substitute. Burns **22**: 182–190, 1996.

14. Wainwright D, Madden M, Luterman A, Hunt J, Monafo W, Heimbach D, Kagan R, Sittig K, Dimick A, and Herndon D: Clinical evaluation of an acellular allograft dermal matrix in full-thickness burns. J Burn Care Rehabil **17**: 124–136, 1996.

15. Lord JW, Stefko PL, and Stevens AR: On bridging a gap in the ureter by means of a free fascial transplant over a straight vittalium tube: an experimental tube. J Urol **49**: 249–254, 1943.

16. Rosenberg ML, and Dahlen GA: Autogenous vein grafts and venous valves in ureteral surgery: an experimental study. J Urol **70**: 434–446, 1953.

17. Sanders AR, Schein CJ, and Hurwitt ES: The bridging of long ureteral defects by fresh autogenous arterial grafts embedded in submucosal intestinal tunnels: an experimental study. Br J Urol **27**: 245–253, 1955.

18. Davis D: Intubated ureterotomy. J Urol **66**: 77–84, 1951.

19. Horton CE, and Politano V: Ureteral reconstruction with split skin grafts: an experimental study. Plast Reconstr Surg **15**: 261–273, 1955.

20. Harvard BM, Camilleri JA, Nadig PW, and Glenn JF: Experimental transplantation of freeze-dried homologous and autologous ureteral segments. J Urol **86**: 385–389, 1961.

21. Reid RE, Levitt S, and Newman HR: Replacement of upper one-third ureteral defects with free autologous full thickness tubular grafts of bladder. Invest Urol **6**: 170–178, 1968.

22. Ivancevic LD, Hohenfellner R, and Wulff HD: Total replacement of the ureter using a bladder flap and cinematographic studies on the newly constructed ureter. J Urol **107**: 576–579, 1972.

23. Greenberg R, Coleman JW, Quiguyan CC, Marion D, Albanese C, O'Dell G, and McGovern JH: Bladder mucosal grafts: experimental use as a ureteral substitute and observation of certain physical properties. J Urol **129**: 634–636, 1983.

24. Urban DA, Kerbl K, Clayman RV, and McDougal E: Endo-ureteroplasty with a free urothelial graft. J Urol **152**: 910–915, 1994.

25. de Boer WI, Schuller AG, Vermey M, and van der Kwast TH: Expression of growth factors and receptors during specific phases in regenerating urothelium after injury in vivo. Am J Pathol **145**: 1199–1207, 1994.

26. Tilton RG: Capillary pericytes: perspectives and future trends. J Electron Microsc Technol **19**: 327–344, 1991.

27. Taderera JV: Control of lung differentation in vitro. Dev Biol **16**: 489–512, 1961.

28. Baskin LS, Hayward SW, Young P, and Cunha GR: Role of mesenchymal-epithelial interactions in normal bladder development. J Urol **156**: 1820–1827, 1996.

29. Gavazzi I, Boyle KS, and Gowen T: Extracellular matrix molecules influence innervation density in rat cerebral blood vessels. Brain Res **734**: 167–174, 1996.