Urol Res (1997) 25:137–142

ORIGINAL PAPER

G. Italiano · G. Abatangelo Jr. · A. Calabro'
G. Abatangelo Sr. · R. Zanoni · M. O'Regan
G. Passerini Glazel

Reconstructive surgery of the urethra: a pilot study in the rabbit on the use of hyaluronan benzyl ester (Hyaff-11) biodegradable grafts

Abstract We investigated the outcome of reconstructive surgery of the urethra through guides composed of a novel biodegradable and highly biocompatible polymer, Hyaff-11. A tract of about 1.5 cm of the rabbit pendulous urethra was totally resected and replaced by a Hyaff-11 tubular graft. Eleven animals were analysed at each of the time points ranging from 7 days to 4 weeks following surgery. Histological and radiological evaluation showed a satisfactory remodelling of the neo-urethra around the implant. The regenerated connective tissue connected both urethral stumps within the first 7 days. On postoperative week 3, the Hyaff-11 guide had disappeared. At the 4-week time point the retrograde urethrogram showed a good distensibility of the neo-urethra. The regenerated stroma consisted of fibroblastic cells, and collagenous and elastic fibres. The neo-epithelium was pluristratified and exhibited cells of the cuboidal type.

Key words Hyaluronic acid · Urethral · Guided tissue regeneration · Rabbits

Introduction

The reconstruction of the urethra for the treatment of strictures and traumatic defects still represents a major urological problem. A variety of operative techniques has been described for the surgical management of urethral reconstruction, including the use of preputial, penile and scrotal skin and buccal and bladder mucosal grafts [2, 9, 14, 16, 17, 20]. However, there is no method that guarantees good results, and complications such as fistula formation and urethral stenosis have been reported. In the search for alternative surgical treatments of hypospadias and cases of trauma and stenosis of the urethra, which would avoid the risks associated with tissue consumption and multistage surgery, attempts to approach surgical therapy by means of guided regeneration of the urethra are currently underway [7, 10, 11, 19]. The purpose of the present investigation was to explore such an approach by using guides composed of hyaluronan derivatives. Hyaluronan (HA) is a naturally occurring polysaccharide which is widely distributed in the extracellular matrix of vertebrate soft connective tissues [13]. A series of biodegradable ester derivatives of HA have been developed and transformed into a range of biomaterials [3]. Due to the potential advantages of biodegradable materials in this type of surgery and the excellent performance of these HA derivatives in other therapeutic sectors [1, 3, 8, 12, 13], an evaluation of their performance in urological reconstructive surgery was considered worthwhile.

In this study we evaluated the potential of a bioresorbable tube composed of the benzyl ester of HA (Hyaff-11), in guiding urethral regeneration. Accordingly, we tested the outcome in animals undergoing replacement of a 1.5-cm gap of the urethra with Hyaff-11 tubular grafts. Additional experiments were carried out by implanting silicone tubular guides.

G. Abatangelo Jr. · A. Calabro' · G. Passerini Glazel
Institute of Urology, University of Padua, Padua, Italy

G. Abatangelo Sr.
Institute of Histology, University of Padua, Padua, Italy

M. O'Regan
Fidia Advanced Biopolymers, Abano Terme, Padua, Italy

G. Italiano (✉) · R. Zanoni
Fidia Research Laboratories, Via Ponte della Fabbrica 3/A, I-35013, Abano Terme, Padua, Italy

Materials and methods

Eighteen male New Zealand rabbits weighing about 3 kg entered the study. Group 1 ($n = 4$) consisted of age-matched rabbits that were sacrificed with no surgery. Group 2 animals ($n = 11$) underwent surgery for urethral reconstruction using Hyaff-11 guides, and were sacrificed at postoperative weeks 1 ($n = 2$), 2 ($n = 2$), 3 ($n = 3$) and 4 ($n = 4$). Group 3 ($n = 3$) was identical to group 2, except that silicone guides were implanted. Group 3 rabbits were sacrificed at postoperative week 2.

Rabbits assigned to groups 2 and 3 were anaesthetized by an injection of sodium pentobarbital in the ear vein (35 mg/kg). Surgical anaesthesia was maintained with 1-ml boli of pentobarbital (20 mg/ml i.v.). The abdomen, prepared and draped with sterile towels, was opened by a midline, suprapubic incision to expose the bladder. Animals underwent cystostomy to divert the bladder drainage, thus avoiding urine extravasation with possible formation of urethral strictures [22]. Vesicostomy was achieved by securing a 4–0 silk ligature around the bladder apex. The abdomen was then closed following the standard surgical technique. Subsequently, the pendulous urethra was exposed and mobilized from the corpora cavernosa. A 1.5-cm segment was totally excised, and simple interrupted 5–0 catgut sutures were used to anastomose the guide, bridging the urethral defect in an end-to-end fashion (Fig. 1). The guide consisted of a 2-cm-long 7-F Hyaff-11 fibre-mesh tube, with an internal diameter of 1.5 mm (manufactured by Fidia Advanced Biopolymers, Abano Terme, Padua, Italy). Grafts for group 3 animals were obtained from full-silicone 7-F Silkomed catheters (Rusch, Kernen, Germany). Following anastomosis, the wound and skin were closed in layers with 5–0 catgut and 4–0 silk sutures. Enrofloxacin was given 12 h before surgery and for 5 days postoperatively.

All animals implanted with the urethral prosthesis (group 2 and 3, $n = 14$) underwent anterograde urethrography prior to surgery. This was done to ensure that a normal anatomy was present. Rabbits were transported to the X-ray room and placed in a prone position. A 10-ml syringe filled with contrast material was connected to a 12-F Foley catheter, which was inserted throughout the vesicostomy and into the posterior urethra. The anterior urethra was filled with contrast material, and a complete film of the pelvis was taken. Prior to sacrifice, both normal and operated animals ($n = 18$) underwent urethrographic analysis, as described above.

Histological examinations of the urethra were carried out on Bouin's-fixed specimens, dehydrated through graded ethanols and embedded in paraffin. The urethra was sliced crosswise or longitudinally at 1-mm and 250-μm intervals, respectively, and 5-μm-thick cross-sections were stained with the standard haematoxylin-eosin protocol, by Gomori's method for parting of smooth muscle fibres and by Weigert's method for delineation of elastic fibres, and examined by light microscopy.

Experiments were performed according to the Federation of European Laboratory Animal Science Association guidelines on the care and use of laboratory animals.

Results

Control animals (group 1)

The connective tissue of the pendulous urethra was found to contain numerous longitudinally oriented bundles of smooth muscle, scattered in a matrix composed of collagen and elastic fibres (Fig. 2A, B). The urethral lumen was lined with a stratified columnar epithelium whose cells were prismatic and cuboidal in form (Fig. 2C).

Hyaff-11, different periods of observation (group 2)

Postoperative week 1

Stroma continuity had been re-established along the transmural defect with a normal and regular luminal diameter, as revealed by urethrography. At this stage the guide was present, and contrast medium extravasation was not seen. Postmortem histological analysis indicated that the connective tissue was composed of an abundant

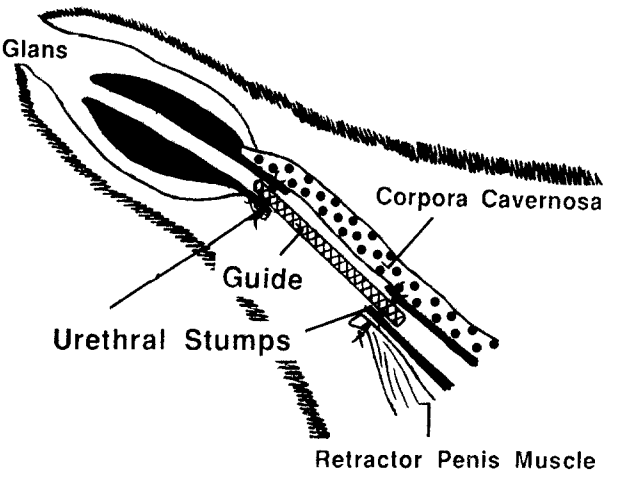


Fig. 1 End-to-end implant of the urethral guide

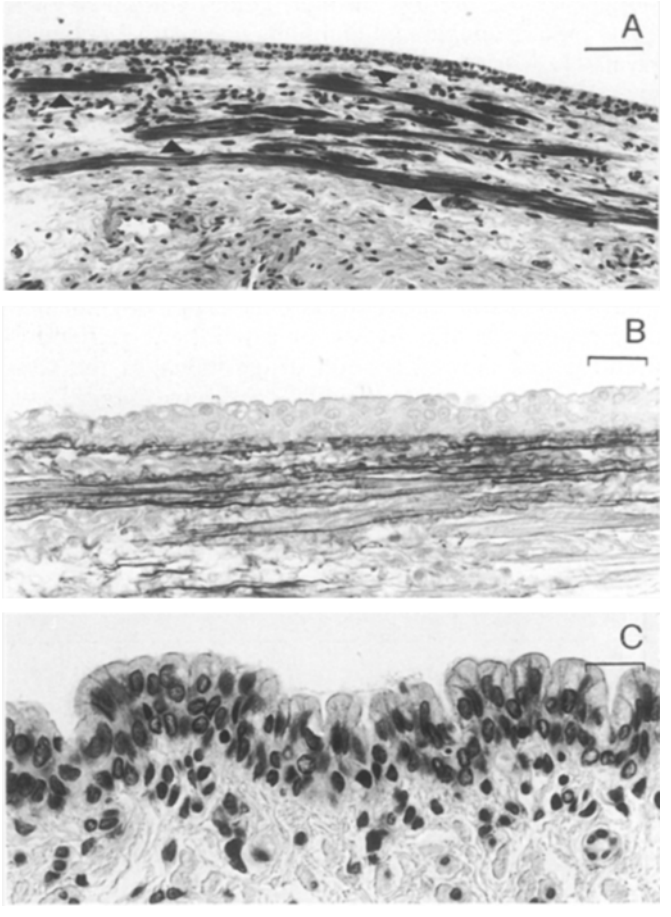


Fig. 2A–C Control urethra. **A** Small compact muscular fascicles (*headarrows*) interspersed with collagen fibres characterize the normal urethral stroma. The spaces between the smooth muscle fibres are filled with a network of collagenous fibres and a few fibroblasts. The fascicular arrangement of the muscle fibres along the lumen is consistent with a propulsive action on the intraurethral bolus. Longitudinal section stained by Gomori's method. *Length of calibration bar* 57 μm. **B** Elastic network on a longitudinal section stained by Weigert's method. *Bar* 28 μm. **C** Typical stratified epithelium from a cross-section of the normal pendulous urethra. Haematoxylin and eosin. *Bar* 28 μm

collagen matrix with elastic fibrils, fibroblastic cells and a few inflammatory cells. Collagen organization was poor, with slender networks of branching fibres among the fibroblastic cells. Cells were not aligned and were packed very loosely. Neovascularization was marked. The luminal surface was covered by neo-epithelial cells, which were arranged in a single or double layer. In some tracts the neo-epithelium exhibited a superficial layer of flattened plate-like cells. At this stage of regeneration, epithelial cells showed intense nuclear activity, as revealed by the strongly basophile cytoplasm when stained with the haematoxylin and eosin method. A modest inflammatory response was observed with a few inflammatory cells infiltrating the epithelium and subepithelial layers. Granulocytes were found adhering to the guide surface. Periurethral fistulas were observed at the sites of needle holes along suture lines, possibly due to transepithelial closure.

Postoperative week 2

By this time the luminal surface was completely covered by a multilayered epithelium, the thickness of which was still not uniform. The guide was still present (Fig. 3B). Histopathological examination showed small epithelial areas with intense cellular proliferation. The typical morphology of the regenerated epithelium is shown in Fig. 3A. Infiltration of inflammatory cells into the epithelial lining and subepithelial space was observed.

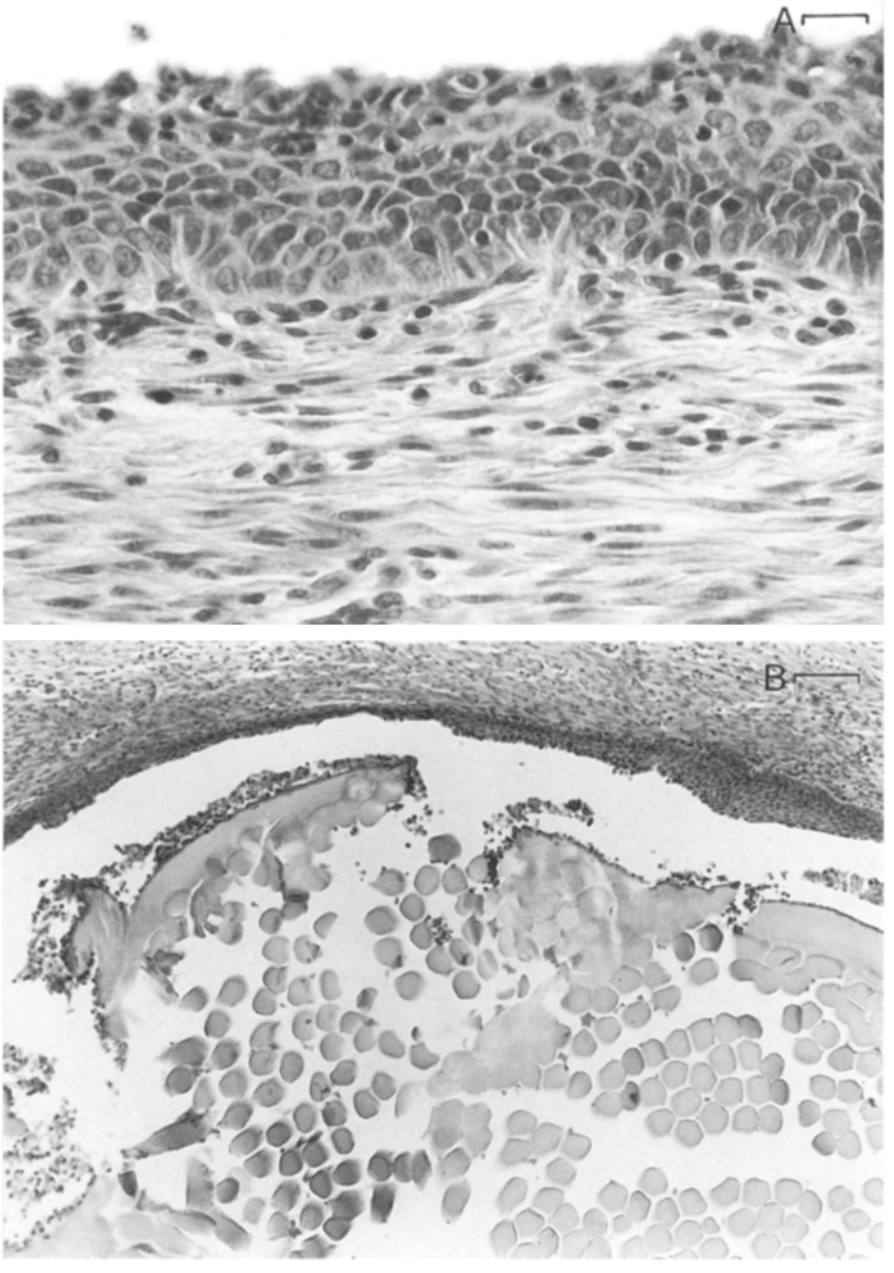


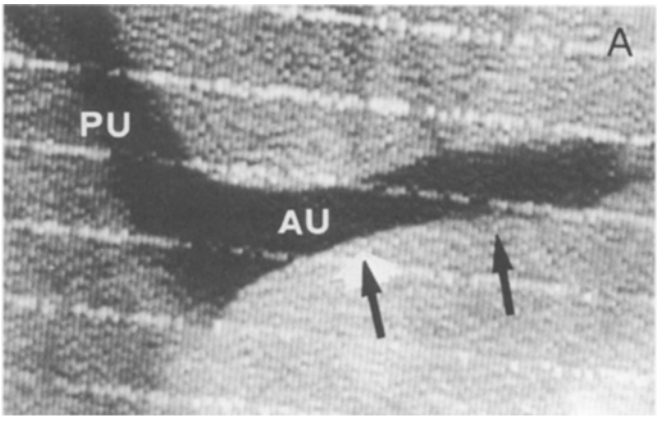
Fig. 3A,B Cross-section at the midpoint of the regenerated urethra, 2 weeks after Hyaff-11 guide implant. **A** The neo-epithelial cells still exhibited an intensely basophile cytoplasm. In the connective tissue, fibroblasts appear as eosinophilic fusiform elements, with long tapering processes. Haematoxylin and eosin. *Bar* 15 μm **B** A detail of granulocytes attacking the surface of the guide. Haematoxylin and eosin. *Bar* 83 μm.

Postoperative week 3

At this stage, the implanted guide had disappeared. The regenerative pattern was more advanced than that of postoperative week 2.

Postoperative week 4

Urethrographies obtained in postoperative week 4 showed no fistulas and no obstructions of the urethra (Fig. 4A). Furthermore, radiological evaluation revealed satisfactory functional results, as the neo-urethra was distended by the radiopaque fluid and the boli of fluid were promptly expelled from the lumen. Placing under pressure the anterior urethra by closing the urethral meatus before a second injection of the radiopaque fluid, a normal distension of the neo-urethra was observed similarly to in controls. Histological analysis established that the regenerated connective tissue consisted of collagen and elastic fibres showing early organization (Fig. 5A, B). The tissue appeared to have tightened up, with the density of collagen fibrils and fibroblastic cells being greater than in previous subgroups. In cross-sectioned specimens, a circumferential rearrangement of collagen fibrils into parallel wavy bundles of appreciable thickness was seen around the lumen. At this stage the neo-epithelium assumed a uniform thickness (Fig. 4B). Epithelial hyperplasia was observed in some areas. No signs of an inflammatory reaction were observed. No polymer material was identifiable using light microscopy.



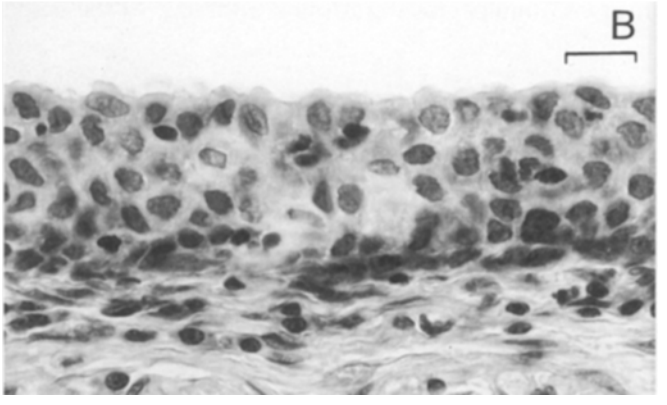
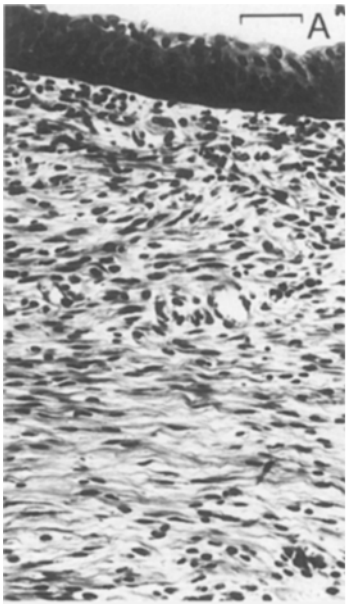


Fig. 4A,B Four weeks from Hyaff-11 guide implant. **A** Urethrography imaging obtained 4 weeks after Hyaff-11 guide implant, showing a satisfactory remodelling of the neo-urethra. Grafted area *at arrows*. *PU* posterior urethra, *AU* anterior (pendulous) urethra. **B** Regenerated epithelium exhibiting pluristratified neo-epithelium with plump cuboidal cells. Cross-section. Haematoxylin and eosin. *Bar* 14 μm

Silicone, 2-week period of observation (group 3)

The superficial layers of the epithelium were composed of thin squamous cells. The process of keratinization involved part of the regenerated epithelium, whose cells



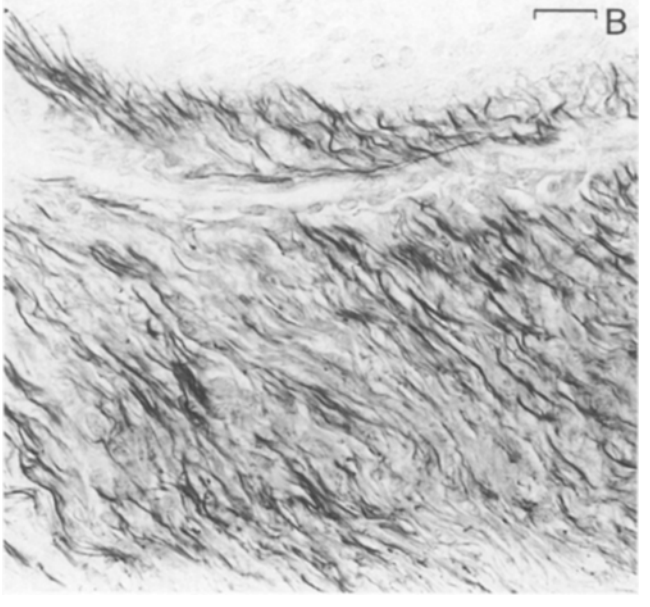


Fig. 5A,B Four weeks from Hyaff-11 guide implant. **A** Regenerated connective tissue. Collagen fibres were found to be oriented parallel to one another. Notice the scattered fibroblastic cells. Longitudinal section stained by Gomori's method. *Bar* 22 μm. **B** Elastic fibres are detectable on the regenerated connective tissue. Longitudinal section stained by Weigert's method. *Bar* 22 μm

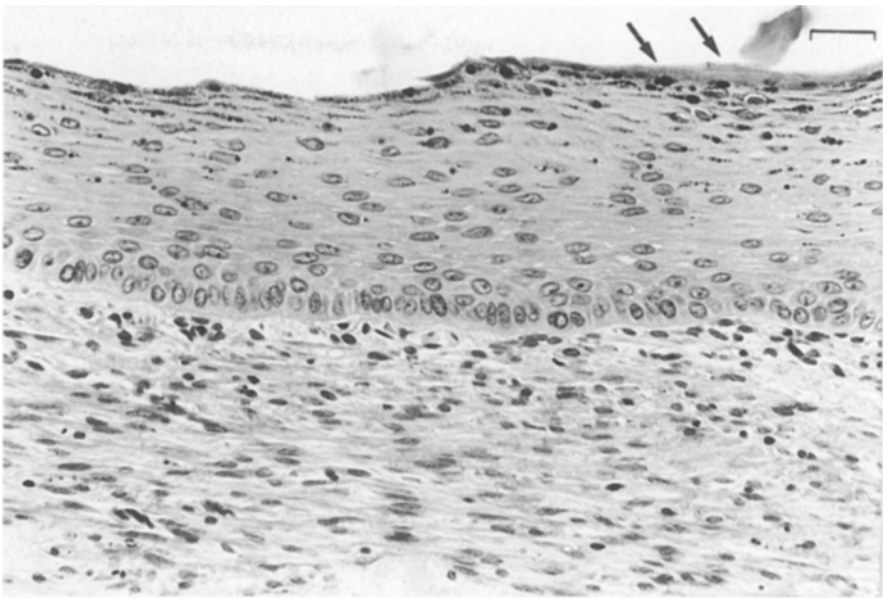


Fig. 6 Two weeks after silicone guide implant. Basophile granules are visible within the neo-epithelial cell. Note the partial keratinization of the epithelium (*arrows*). Haematoxylin and eosin. *Bar* 29 μm

exhibited clear signs of regression, i.e. pale nuclei and basophile granules within the cytoplasm (Fig. 6). Portions of the squamous neo-epithelium appeared keratinized in two out of three animals. One of the three rabbits showed a granulation tissue capsule that walled off the silicone implant, with an extensive inflammatory response. In contrast to the epithelium, the connective tissue of the neo-urethra showed histological features similar to those of animals implanted with Hyaff-11 guides.

Discussion

Guided tissue regeneration is a new experimental approach in surgical therapy. The advantages of this approach are that reconstruction requires less surgery time and can be completed with only one intervention. Implants used for reconstructive surgery must be inert to ensure minimal interfacial reaction. Biocompatibility and functionality of materials used for tissue replacement have always been questions at the forefront of the development of surgical implant devices. Previous investigations, performed in dogs and rabbits, report experience of partial or total replacement of the urethra by polytetrafluoroethylene and polyglycolic acid alone or coated with polyhydroxybutyric acid [7, 10, 19]. We used a device manufactured with Hyaff-11, a semi-synthetic derivative of HA in which the carboxylic groups of the polymer are esterified with benzyl alcohol. HA is a naturally occurring polysaccharide, widely distributed in the extracellular matrix of mammalian connective tissues. The choice of the polymer employed in the present study is based upon its inertness [1, 4, 8], the possibility of producing implantable devices with varying rates of biodegradation [4], its favourable profile of toxicity and biocompatibility [1, 4, 6, 18], the well-known properties of HA in protecting delicate tissues, for instance the corneal endothelium, the tympanic membrane and articular tissues [13], and the potential of Hyaff in wound healing [13]. It is particularly relevant that these HA esters were found to be suitable for fibroblast spreading and growth [8]. In addition, preliminary investigations have shown that isolated urothelial cells attach and grow on Hyaff-11 membranes (unpublished data).

The results of the present study indicate that Hyaff-11 tubular guides represent promising devices for the management of short urethral gaps. Furthermore, favourable tissue compatibility of the polymer employed provides support for the possible use of Hyaff-11 nonwoven meshes as scaffolds in the correction of strictures or defects requiring partial urethrotomy. Hyaff-11 prostheses appeared to promote the proliferation of epithelial cells of the cuboidal type, with no keratinization of the epithelium. At 3 and 4 weeks from the implantation of Hyaff-11 prosthesis, the neo-epithelium appeared as stratified-cuboidal (Fig. 4B). The data obtained in this study with silicone guides confirm previous observations [10] that silicone guides, in contrast to Hyaff-11, lead to the proliferation of squamous epithelium covering the urethral lumen (Fig. 6). As squamous epithelium is more adapted for protection of the organ against mechanical damage than the cuboidal epithelium [5], it may be argued that, although chemically inert, the silicone guide did not prove to be as acceptable a material as the Hyaff-11 guide. The fact that a persistent irritative stimulus is associated with morphological changes in the urethral epithelium from transitional to squamous is well known, as pointed out by previous reports [22, 23]. The reason for the superiority of Hyaff-11 guides in promoting the development of cuboidal epithelium is unclear but may be related to its physicochemical characteristics, e.g. hydration [15] as well as to its biological properties.

Urethrographic analyses indicated the good distensibility of the neo-urethra, the absence of urethrocutaneous fistulae and the constant caliber of the lumen with

absence of strictures or obstructions (Fig. 4A). As revealed by light microscopy, remodelling of the urethral canal on the guide did not lead to folding of the urethral tissue. We did not observe phenomena such as cicatrizial retraction and fibrosis of the neo-urethra, which might affect the elastic functionality during penile erection.

Our experiments indicate that this inert Hyaff-11 guide leads to healing with minimal inflammation. The material seems to be well tolerated, as no sign of chronic inflammation or local interaction between the host and the implant was observed in postoperative week 4. It is noteworthy that 3 weeks following implantation Hyaff-11 guides had disappeared. It is reasonable to assume that, by this time, the material has been partially biodegraded and expelled from the urethra. This hypothesis is based upon three elements. Firstly, histological examination showed that granulocytes were attacking the surface of Hyaff-11 grafts at the 1- and 2-week time points, but no substantial damage to the structure of the prosthesis was observed (Fig. 3B). Secondly, 1 week later (3-week time point) neither the prosthesis nor residue of the prosthesis itself was traceable within the urethral lumen. Thirdly, the biodegradability of Hyaff-11 was assessed by using films of the polymer implanted in the subcutaneous and peritoneal tissues of the rat [4]. The material was still present at 90 days. Taken together these findings suggest that total biodegradation of the prosthesis from postoperative week 2 to week 3 is improbable. Possibly, the partial biodegradation of Hyaff-11 caused a disjunction of the tubular graft from the anchoring sutures, with expulsion of the tube from the urethra.

With respect to the technique for implanting the guide, we observed the fact that transepithelial suture for anchoring the stent appears to generate periurethral microfistulas. This phenomenon should be taken into account, as it might then increase the risk of urethro-cutaneous fistulization. As emphasized by Scherz et al. [21], parafistula formation should be limited by using a low-sized needle.

Sanitary measures to maintain an incontinent animal (i.e. the vesicostomized rabbit) clean for long periods with high-density urine were not successful. Due to the difficult long-term management of the vesicostomized rabbit, the observation time was short for a final evaluation. Thus, 4 weeks following the implant no elements of the urethral stroma having the features of differentiated mature smooth muscle cells were observed, and the possibility that smooth muscle cells may be formed anew from fibroblasts remains to be established.

This pilot study encourages further experiments with extended follow-up, to evaluate long-term outcome, and on animal models providing the closest approximation to the human urinary tract (i.e. the pig). Future studies might also consider devices (tubular grafts, meshes) manufactured with HA ester derivatives having a biodegradation time different to that of Hyaff-11.

Acknowledgements We are grateful to Dr. Marco Prosdocimi for helpful discussion and advice, and to Dr. Fabio Fante for the veterinary care. We are indebted to Mr. Mario Finesso for the urethrographic analyses.

References

1. Andreassi L, Casini L, Trabucchi E, Diamantini S, Donati L, Tenchini ML, Malcovati M (1991) Human keratinocytes cultured on membranes composed of benzyl ester of hyaluronic acid suitable for grafting. Wounds 3:116
2. Baskin LS, Duckett JW, Uloka K, Seibold J, Snyder HM (1994) Changing concepts of hypospadias curvature lead to more onlay island flap procedures. J Urol 151:191
3. Benedetti L (1994) New biomaterials from hyaluronic acid. Med Dev Technol 5:32
4. Benedetti L, Cortivo R, Berti T, Berti A, Pea F, Mazzo M, Moras M, Abatangelo G (1993) Biocompatibility and biodegradation of different hyaluronan derivatives (Hyaff) implanted in rats. Biomaterials 14:1154
5. Bloom W, Fawcett DW (1975) Epithelium. In: Bloom W, Fawcett DW(eds). A textbook of histology. W.B. Saunders, Philadelphia, pp 83
6. Campoccia D, Hunt JA, Doherty PJ, Zhong SP, O'Regan M, Benedetti L (1996) Quantitative assessment of the tissue response to films of hyaluronan derivatives. Biomaterials 17: 963
7. Cilento BG, Retik AB, Atala A (1995) Urethral reconstruction using a polymer mesh. J Urol 153 (Suppl): 371A (abstract 571)
8. Cortivo E, Brun P, Rastelli A, Abatangelo G (1991) In vitro studies on biocompatibility of hyaluronic acid esters. Biomaterials 12:727
9. Dessanti A, Rigamonti W, Merulla V, Falchetti D, Caccia G (1992) Autologous buccal mucosa graft for hypospadias repair: an initial report. J Urol 147:1081
10. Dreikorn K, Lobelenz J, Horsch R, Rohl L (1979) Alloplastic replacement of the partially resected canine urethra by expanded polytetrafluoroethylene grafts. Urol Res 7:19
11. Fairbanks JL, Sheldon CA, Khoury AE, Gilbert A, Bove KE (1992) Free bladder mucosal graft biology: unique engraftment characteristics in rabbits. J Urol 148:663
12. Favaro G, Bortolami MC, Cereser S, Dona' M, Pastorello A, Callegaro L, Fiori MG (1990) Peripheral nerve regeneration through a novel bioresorbable nerve guide. Trans Am Soc Artif Intern Organs 36:1
13. Goa KL, Benfield P (1994) Hyaluronic acid: a review of its pharmacology and use as a surgical aid in ophthalmology, and its therapeutic potential in joint disease and wound healing. Drugs 47:536
14. Kinkead TM, Borzi PA, Duffy PG, Ransley PG (1994) Long-term followup of bladder mucosa graft for male urethral reconstruction. J Urol 151:1056
15. Laurent TC, Fraser JRE (1992) Hyaluronan. FASEB J 6:2397
16. McAninch JW (1993) Reconstruction of extensive urethral strictures: circular fasciocutaneous penile flaps. J Urol 149:488
17. Noll F, Schreiter F (1990) Meshgraft urethroplasty using split-thickness skin graft. Urol Int 45:44
18. Olivi A, Kim KD, Benedetti L, Epstein JI, Pinn ML, Callegaro L, Brem H (1995) Ocular and brain biocompatibility of polymeric delivery materials prepared from ester derivatives of hyaluronic acid. Clin Materials (in press)
19. Olsen L, Bowald S, Busch C, Carlsten J, Eriksson I (1992) Urethral reconstruction with a new synthetic absorbable device. Scand. J Urol Nephrol 26:323
20. Provet JA, Surya BV, Grunberger I, Johanson KE, Brown J (1989) Scrotal island flap urethroplasty in the management of bulbar urethral strictures. J Urol 142:1455
21. Scherz HC, Kaplan GW, Boychuk D, Landa, HM, Haghighi P (1992) Urethral healing in rabbits. J Urol 148:708
22. Singh M, Blandy JP (1976) The pathology of urethral stricture. J Urol 115:673
23. Singh M, Scott TM (1976) The ultrastructure of human male urethral stricture. Br J Urol 47:871