Off-the-Shelf Implant to Bridge a Urethral Defect: Multicenter 8-Year Journey From Bench to Bed

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OBJECTIVE To engineer an acellular mesh to reconstruct the urethra to replace the current surgical practice of using autologous tissue grafts. Cell based approaches have shown progress. However, these have been associated with high costs and logistical challenges.

MATERIALS AND METHODS Acellular meshes were engineered using liquid collagen. They underwent in vitro, mechanical and bench testing by surgeons. Sixty-nine male New Zealand rabbits were used to refine the design. The final prototype based on the TissueSpan patented technology was then implanted again in a 2 cm long urethral defect in 9 rabbits and in a 4 cm long defect in 6 dogs.

RESULTS The TissueSpan technology platform allows for the manufacturing of tubular and rectangular meshes in different diameters and thicknesses. The tubular mesh acted as physical conduit to gap the urethral defect with a patent urethra demonstrated after 1 month in both animal models. The mesh was absorbed within 1-3 months. Spontaneous urothelial coverage of the mesh and smooth muscle cell migration into the surgical area was demonstrated even in a 4 cm long urethral defect. A first in man clinical trial was subsequently initiated.

CONCLUSION The acellular mesh may have the potential to be an off-the-shelf product for substitution urethroplasty. Its mechanical properties allow surgeons to easily create a physical conduit while its material properties favor tissue remodeling. A large-scale clinical trial is still required to further confirm the safety, performance, and patient benefit of this new medical device. UROLOGY 196: 294–299, 2025.

A surgically efficient and cost-effective tissue engineered implant to reconstruct the urethra has not yet replaced current surgical practice of using autologous tissue grafts. Acellular, cellular, and growth factor incorporation into existing biomaterials as urethral implants have been attempted, but with limitations; acellular grafts have managed to be utilized only in short urethral defects as an on-lay. To date cell-based grafts have shown better functional results in large preclinical animal models and in subsequent clinical trials. However, the final urethral graft's regenerative potential is dependent on the quality of the cells cultured and seeded, which can be variable.[1] The complex and time intensive manufacturing processes required to engineer these grafts prior to implantation in the patient, and the logistics involved, increase the final cost of production. It is unlikely therefore that this approach will become standard clinical practice or will be useful for patients with limited financial resources. Though previously attempted for clinical translation by others we believe an acellular mesh has the best potential for wide clinical application in urethral reconstructive surgery. This has been our motivation since 2014 using collagen, a natural biomaterial, and the most predominant protein in the human extracellular matrix to achieve this goal.[2]

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Submitted: October 14, 2024, accepted (with revisions): December 9, 2024

 https://doi.org/10.1016/j.urology.2024.12.016
0090-4295

Multiple meshes were developed over a period of 8 years all using animal derived collagen as the base material. We have prior published our experience with the earlier engineered meshes and had utilized a total of 69 male New Zealand rabbits in the implant design refinement process.[3,4] The data collected led to the development of the TissueSpan patented technology platform (EP 3 442 608 B1, US 11.266.764 B2 and MY-196309-A). The aim of this study is to evaluate the utility and effectiveness of the developed TissueSpan mesh across two animal models, leading the way toward future human applications.

MATERIALS AND METHODS

Tubular collagen meshes based on the TissueSpan patented technology platform were manufactured using liquid bovine collagen. The meshes underwent in vitro testing, mechanical analyses, and bench testing by surgeons.

Animal Studies

Rabbits: Nine male New Zealand rabbits were utilized to study the tubular TissueSpan meshes, and surgical implantation on a 2 cm urethral defect was done as we have previously described.[3,4] Following approval by the Animal Ethics Committees of the Canton of Vaud (Authorization number: VD-2740), Switzerland and of the Faculty of Medicine of the University of Malaya (Authorization number: 2013-07-19/SUR/R/TCR), Malaysia, rabbits were evaluated at 1-, 3-, and 6-month postsurgery using visual examination and contrast voiding cysto-urethrography. One rabbit in the 1 month group and 4 each in the 3- and 6-month group. Biopsies of implantation sites from euthanized rabbits were subjected to histology and immunohistochemistry. No catheter was used during postop follow-up care in this rabbit study.

Dogs: Implantations were performed on 6 male dogs. The animal experiment was approved by the Animal Ethics Committees of the Faculty of Veterinary Medicine of University Putra Malaysia, Kuala Lumpur (Authorization number: UPM/IACUC/AUP-RO51/2016), Malaysia. A 6 cm long skin incision was done proximal to the meatal opening on the penis in the dog. Subcutaneous tissue was dissected. The urethra within the muscular corpora was identified. Stay sutures were placed in the proximal and distal urethra and the middle third of the urethra along with the corpus spongiosum was excised from the corpus cavernosum to create a critical size 4 cm long urethral defect. Assisted by a catheter, the tubular meshes was positioned in the urethral defect. The mesh was anastomosed on both ends of the native urethra. On post op day 7, the catheter was removed under local anesthesia. The dogs were examined by the ability to micturate postsurgery and histology up to 16 months postsurgery.

Histological Analysis

At the determined time of euthanasia, the animals received a lethal intravenous pentobarbital injection (Esconarkon ad.us VET Streuli). Thereafter, the entire penis was harvested and fixed in 4% Formalin (PFA). For histological work-up, the specimen was embedded in paraffin, and 8 μm thick sections were prepared. Hematoxylin & Eosin (H&E) staining and immunohistochemistry was done using Anti-uroplakin 2 antibodies, anti-alpha smooth muscle actin (SMA) antibodies, and DAPI.

Ethical Considerations

All animal experiments were approved by relevant authorities as described.

RESULTS

Engineering of an Acellular Collagen Urethral Mesh

The mesh manufacturing was done in an aseptic process carried out in a cleanroom environment. The TissueSpan technology platform allows for the manufacturing of tubular shaped meshes used for the animal experiments and rectangular meshes which would be required for substitution urethroplasty in future clinical trials in different diameters and thicknesses (Fig. 1). Once fabricated the tubular collagen meshes were kept in phosphate buffered saline (D-PBS) and stored at 4 °C until implantation. The mesh can be used directly from the storage package during surgery without any other modifications or additional steps. Meshes for the rabbit

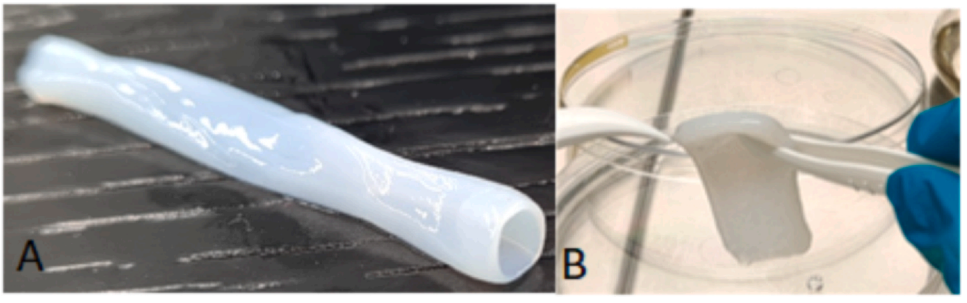

Figure 1. (A, B) Off-the-shelf available meshes manufactured using TissueSpan technology in different shape and dimension for urethral reconstruction.

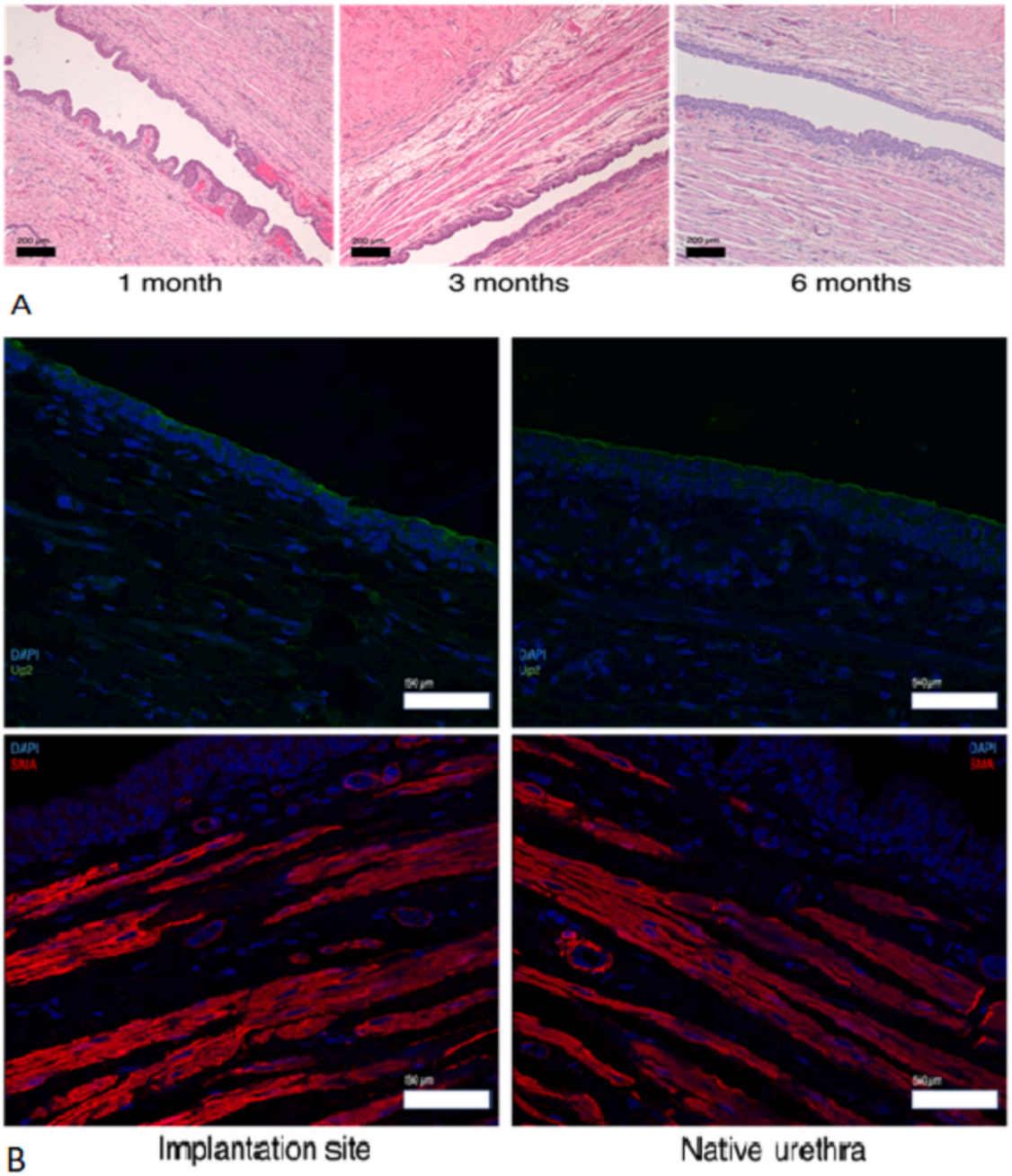

Figure 2. **(A)** H&E-stained tissue sections from the implantation site in a rabbit urethra 1, 3- and 6-month postimplantation. Scale bars represent 200 μm. **(B)** Immunohistochemistry on tissue sections from the implantation site in a rabbit urethra 6 months postimplantation and native urethra. Anti-uroplakin 2 antibodies (green) and anti-alpha smooth muscle actin antibodies (red) were used. Cell nuclei were counter stained with DAPI (blue). Scale bars represent 50 μm.

and dog implantation study had been shipped across the globe from Switzerland to Malaysia. Though no specific studies were done at that time on shelf life and transportation, the implants showed no changes in their physical properties when implanted 6 months post fabrication in the animals.

Rabbit Urethra Model

In all 9 rabbits no macroscopic complications were seen and all the animals demonstrated normal micturating cystourethrography. The rabbits in the 6 months study group also participated in the in house animal facility breeding program and were able to mate and produce offspring, with a female partner thereby demonstrating full functionality of the operated urethra.

The meshes could successfully support the urethra mechanically without a need of a catheter and all the implant location could be analyzed at the selected harvesting timepoints. All harvested samples were stored in formalin and embedded in parafin for histological analysis. H&E staining was done for all samples (Fig. 2) at various time points. At 1 month, urothelial cell ingrowth was demonstrated in the

grafted section of the urethra. However, at this point in time, there was limited smooth muscle cell remodeling of the collagen mesh. At 3 months, the presence of collagen mesh was no longer seen in any of the histological sections. The grafted region was covered with multilayered urothelial cells with ingrowth of smooth muscle cells seen into the implanted section. By 6 months, cell infiltration had resulted in complete tissue remodeling at the implantation site.

Immunohistochemistry showed similar features in the 6 months implantation site when compared to the native urethra demonstrating a picture of complete remodeling of the grafted region (Fig. 2). Uroplakin 2, a specific antibody for terminal urothelial differentiation was seen expressed throughout the implantation site as similar to native urethra. Alpha SMA, a specific marker for smooth muscle cells seen in red, confirmed smooth muscle formation inside the mesh as per native urethral architecture following 6 months of implantation. Smooth muscle cells of the vascular architecture were also demonstrated by the same marker in the 6 months biopsy.

Dog Urethral Model

In total 6 dogs underwent implantation with tubular meshes (Table 1). Catheter removal was done on postoperative day 7 and micturition was observed. The mesh was successful in mechanically supporting the urethral graft bed after the 7-day catheter removal and throughout the different time points of the study, all the animals were able to micturate postsurgery with no fistula or stricture seen macroscopically. This observation was confirmed with the H&E-stained biopsy samples showing an ingrowth of multilayered urothelial and smooth muscle cells at the implantation site 12 months postsurgery (Fig. 3).

DISCUSSION

Tissue engineering has long been hoped to provide an alternative material for the use in urethral reconstructive surgery.[5] This is particularly beneficial in patients with long urethral strictures that need to be bridged by a buccal mucosa graft. The harvesting of these grafts is associated with complications at the donor site.[6] There are two approaches in tissue engineering, one is to utilize an acellular graft to replace the defect and allow cell infiltration to occur in vivo and the other involves the seeding of the graft with the specific cells of the area of interest prior to implantation.

Cell based approaches have shown progress and cell seeded urethral grafts are now available commercially in certain countries, thus avoiding complications related to harvesting long segments of oral mucosa.[7,8] Indeed, this novel technology has revolutionized the field of urethral tissue engineering. Nevertheless, there remains some limitations for the widespread utilization of this technology platform. The patient still needs to have a small sample of their buccal mucosa biopsied, to be sent to the lab for cell multiplication before being seeded on the graft and sent back to the urologist for implantation. The cost involved and limitation in commercial up scaling of the production in a safe and regulated framework for clinical use will hinder the widespread availability of this technology.[9] An acellular, off-the-shelf graft with good regenerative potential is thus still more likely to be used in clinical practice than a cellular implant.

The utilization of stents or nonabsorbable acellular implants has evolved from passive mechanical support to absorbable implant approaches that truly harness and direct endogenous remodeling and cell infiltration processes by having a favorable absorption rate of the implants. Commercial products based on porcine small intestinal submucosa are used clinically to augment soft tissue repair, and their mechanism of action is being elucidated.[10] Though previously attempted, acellular grafts have not shown efficacy to bridge large urethral defects and thus have failed to be adopted into standard clinical practice.[11] The envisioned engineered biomaterial for urethral reconstruction ideally should be able to provide a wide range of products that differ in dimension and shape for adaptation in different indications for urethroplasty.[12] We describe here our journey to achieve that objective.

An important component for successful clinical translation was the need for early establishment of a working collaboration between the bioengineers and urologist to design a clinically relevant mesh. The multidisciplinary team here formed in 2014 was from different institutions based in Switzerland and Malaysia. Urethral meshes were developed over a period of 8 years all using collagen as the base material. The final technology platform was able to be versatile to manufacture meshes of various lengths and shapes as per clinical requirement. The versatility and durability of the meshes were tested at preclinical development stages by allowing them to be shipped and utilized by different urologist in different centers and countries thus mirroring a basic requirement of a medical device. Sixty-nine male New Zealand rabbits were utilized to refine the final design. The earlier developed collagen based tubular urethral meshes were able to regenerate a 2 cm long urethral defect in the rabbit model, challenging the consensus that defects of such length were unable to be bridged by an acellular tubular mesh.[13,14] Data that was obtained from the prior mesh generations allowed us to improve our engineering design further to the final prototype. The final prototype was again implanted in the same animal model for comparison. The implanted meshes were shown to mechanically support the urethral graft bed without a catheter (in rabbits) or with a short catheter usage (1 week in dogs). There was no demonstrable macroscopic abnormality in the rabbit penis or narrowing at the time of micturating cystourethrography.

Table 1. Clinical outcome at different time points in the dog urethral model.

	Dog Urethra: 4 cm Defect				
Sacrifice time (mo)	1	5	10	12	16
Number of animals	2	1	1	1	1
Micturition and patency	Normal micturition and patent urethra				

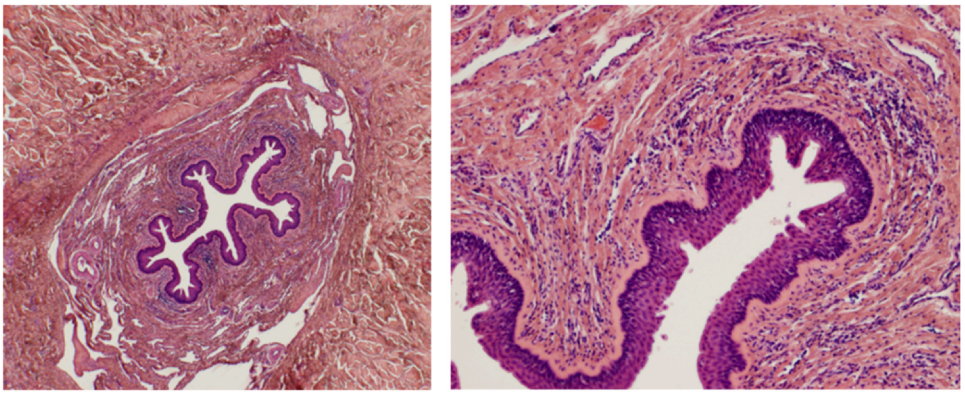

Figure 3. H&E-stained tissue sections from the implantation site at 12 months postimplantation in the dog model.

The ability of the rabbits in the 6 months group to have successfully mated with female rabbits showed that both voiding and sexual function in the rabbit had recovered postsurgery. This was possible even after 2 cm of the native urethra was excised, a significant length in this animal model with the average urethral length being between 2.7 to 3 cm.[15] Histology and immunohistochemistry further validated the superiority of the final prototype to its predecessor as demonstrated by earlier urothelial and smooth muscle cells infiltration into the grafted area. Urothelium, a multilayered tissue that functions primarily as a first line barrier against pathological bacteria and urine, exhibits important signaling properties that potentially attract the ingrowth of smooth muscle cells. This could explain the ingrowth of smooth muscle cells into the mesh that was seen at 3 months in coordination with the development of a complete urothelial layer.[16,17] Cellular vascularity was not examined by specific immunohistochemistry however SMA did stain the smooth muscle on the vessels at 6 months in directly demonstrating vascular ingrowth. The meshes were regarded as absorbed within 1-3 months. The ingrowth of native cells was seen as early as 1 month, and further complete remodeling was achieved by 6 months.

The adequate smooth muscle cell infiltration from the surrounding native tissue within the mesh at the 6 months' time point is a finding that seems not to have been described yet. Early smooth muscle regeneration is an advantage, as the primary pathology in urethral stricture is fibrosis of the muscular component of the urethra leading to narrowing of the urethral lumen.[18]

The maximum urethral length that can be replaced in the rabbit in our experience is 2 cm which is a limitation of this model to study for long urethral regeneration. Furthermore, for clinical translation, in vitro *testing* in at least 2 animal models is deemed necessary.[19] Therefore, the dog urethral model was utilized for the replacement of a clinically relevant urethral segment with the final prototype. A longer and larger tubular mesh was engineered for this purpose and implantation was done onto a totally excised urethral tissue requiring full circumferential replacement. This varies from clinical practice where only partial circumferential replacement is performed using rectangular sheets. Prior studies using this animal model for 3 cm long resection in literature had demonstrated that an acellular implant can be only applied as an on-lay and not as a tubular graft. The conclusion was that the presence of a urethral bed was essential for the successful regeneration of a long urethral defect, and therefore long tubular acellular implants should be avoided in favor for cell seeded grafts.[20,21] The ability to regenerate a 4 cm long urethral defect using a tubular acellular mesh as shown by the final prototype in the dog model has yet to be described in literature. This new preclinical data further convinced the collaborating urologist to proceed with an application for a first in man clinical trial.

A limitation of our preclinical data was that the animal models utilized had an artificially created urethral defect, with healthy urethral tissue on the edges of the defect. We did not attempt to create a stricture model as we had not done it in our prior studies and thus would not be able to compare the final engineered mesh with its predecessors. In urethral stricture patients, the underlying problem is ischemia of the corpus spongiosum, leading to pathological tissues. Challenging the potential of the mesh on an unhealthy urethral bed would be ideal. However, a standardized animal model for this purpose has not yet been described in literature. To circumvent this limitation in our animal model we had utilized tubular acellular meshes rather than rectangular sheets as per clinical practice to test the meshes remodeling and absorption potential in a circumferential completely excised urethral defect of significant defect in the animal models. We have now initiated a 5 patient first in man study in urethral stricture patients using rectangular meshes manufactured using this technology. The transition from a research product developed in an academic setting to a clinical-grade product developed and produced according to regulatory standards has been successfully achieved. This study however needs to be completed before any conclusion can be done with regards to the safety and efficacy of this new mesh in urethral reconstructive surgery. The development of an innovative medical device technology for clinical practice is challenging with multiple hurdles but early collaboration between engineers and clinician will help to bridge the translation gap between bench and bed.

CONCLUSION

The acellular engineered mesh based on the TissueSpan patented technology may have the potential to be an off-the-shelf product rendering substitution urethroplasty less harmful for patients and shortening operation times. Its mechanical properties will allow surgeons to easily recreate a physical conduit while its material properties favor tissue remodeling, and mesh absorption as seen in the in vivo model. A large-scale clinical trial however is required to further confirm safety, performance, and patient benefit.

Disclosures

Swiss Federal Commission for Innovation and Technology. CTI Project No 16627.1 PFLS-LS. Project title: Engineering of smart collagen-based tubular matrices for regenerative medicine.

Ethical Declaration

The animal studies were approved by the Animal Ethics Committees of the Canton of Vaud (authorization number: VD-2740), the Animal Ethics Committees of the Faculty of Medicine of the University of Malaya, Malaysia (ethics approval number: 2013-07-19/SUR/R/TCR) and the Animal Ethics Committees of the Faculty of Veterinary Medicine of University Putra Malaysia, Malaysia (ethics approval number: UPM/IACUC/AUP-RO51/2016).

Declaration of Competing Interest

Dr Ganesh Vythilingam: Patent Inventor for Mentioned Product/Stockholder for Mentioned Product/Company. Hans M. Larsson: Patent Inventor for Mentioned Product/Stockholder for Mentioned Product/Company Board Membership with Sponsor. Eva-Maria Engelhardt: Patent Inventor for Mentioned Product/Stockholder for Mentioned Product/Company Board Membership with Sponsor. Kalitha Pinnagoda: Patent Inventor for Mentioned Product/Stockholder for Mentioned Product/Company/Board Membership with Sponsor. Jeffrey A. Hubbell: Patent Inventor for Mentioned Product/Stockholder for Mentioned Product/Company. Peter Frey: Patent Inventor for Mentioned Product/Stockholder for Mentioned Product. The other authors have no conflict of interest to declare.

Appendix A. Supporting Information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.urology.2024.12.016.

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