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# The use of small intestinal submucosa graft for hypospadias repair: Pilot study

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**KEYWORDS**

Small intestinal submucosa graft;
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Urethral regeneration;
Acellular matrix

**ABBREVIATIONS**

SIS, small intestinal submucosa;
TIP, tubularised-incised plate;
PVR, postvoid residual urine volume

**Abstract** ***Objective:*** To evaluate the outcome of using commercially available (SIS) grafts for repairing hypospadias. Collagen-based acellular matrices, including SIS and bladder submucosa matrix, have been used to repair urethral strictures, with varying success, and patients with hypospadias and with inadequate or no genital skin need a substitute tissue for urethroplasty.

***Patients and methods:*** This pilot study included 12 patients (mean age 8 years, range 1.5–15) with hypospadias (distal in six, mid-shaft in four and proximal in two). They underwent a repair with four layers of prefabricated SIS as an onlay graft. The outcome was assessed for cosmetic appearance, urinary stream and the postvoid residual volume. The chi-squared and Mann–Whitney *U*-tests were used to assess the relationship between preoperative factors and the outcome of the repair with SIS grafting.

***Results:*** The mean (range) follow-up was 23 (6–36) months. Nine patients ultimately voided normally, with a good cosmetic appearance and no postvoid residual urine. Six patients had a successful repair with no further intervention, whilst three had small fistulae that were treated by simple closure. In three patients the graft failed, by complete disruption or stricture. Graft infection adversely affected the outcome of SIS grafting.

***Conclusions:*** The prefabricated SIS graft can be used as an alternative substitute for urethral reconstruction when genital skin is insufficient or lacking, as in

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circumcised patients or a repeat hypospadias repair. Graft infection is the chief reason for graft failure and should be prevented. Further studies with more patients are needed to confirm these preliminary results.



## Introduction

Reconstruction of the male anterior urethra in cases of hypospadias is one of the great surgical challenges for urologists. Many reconstructive methods have been described for hypospadias repair, most of which use preputial skin as vascularised flaps [1]. For patients with insufficient preputial skin, due to unsuccessful surgical repairs or previous circumcision, many other sources have been used for the graft. Currently, buccal mucosa is considered the best tissue for urethral substitution [2]. However, the use of a commercial material for urethral replacement avoids donor-site morbidity and the time-consuming harvesting of flaps or grafts.

Naturally occurring extracellular matrices have received significant attention for their potential therapeutic applications as biological scaffolds. Many collagen matrices have been applied for urethroplasty. They can promote urethral regeneration but with the potential risks of infection [3]. Porcine-derived small intestinal submucosa (SIS) is one of the most widely studied extracellular matrix scaffolds [4]. It had promising results when used as a urethral substitute material in animals [5]. Many studies reported on the use of SIS in the repair of anterior urethral strictures, with varying results [6,7]. In the present pilot study we evaluated the outcome of the use of SIS biomaterial as an onlay graft for repairing hypospadias.

## Patients and methods

The study was conducted after obtaining the approval of the institutional ethics committee, and informed consent from all the patients' parents or guardians. We prospectively evaluated the outcome of hypospadias repair after using four layers of SIS (Surgisis Biodesign™, Cook Medical, Bloomington, IN, USA) as an onlay graft (Fig. 1A) in 12 patients (mean age 8 years, range 1.5–15). The hypospadias was distal in six, mid-shaft in four, and proximal in two. Three of them had a failed previous repair by tubularised-incised plate (TIP) urethroplasty and urethral advancement. The mean (range) urethral plate width was 5.5 (3–8) mm. Three of the 12 patients were circumcised. Penile curvature was moderate in one patient, mild in four and absent in seven. The patients' characteristics and results are summarised in Table 1.

All the patients were operated under general anaesthesia, and with caudal analgesia. An intravenous broad-spectrum antimicrobial agent was given to all patients 30 min before surgery and continued for 2 days, to be replaced by an oral antibiotic until the urethral stent was removed.

The penile skin was degloved through a subcoronal incision, preserving the urethral plate. Glanular wings were developed lateral to the edges of the urethral plate. The SIS graft was rinsed in isotonic saline solution for 10 min to re-hydrate it. It was then trimmed to a size suitable for each patient, considering the possibility of graft shrinkage (i.e., the size of the graft was increased by 20–30%). The graft was positioned so that the smooth intraluminal side faced the urethral lumen. The trimmed SIS was fixed to the urethral plate in an onlay fashion with a continuous 6–0 polyglactin suture. A dorsal penile dartos flap was mobilised and split longitudinally into two halves, each being mobilised ventrally to be overlapped to give a two-layer coverage of the graft. The glans was then closed in two layers. In those patients who were not circumcised, the redundant dorsal skin was removed. The skin coverage was then completed by suturing the subcoronal line (Fig. 1B–F). A Nelaton urethral catheter was left for 14 days in all patients, except for one who developed acute epididymo-orchitis after 10 days and had his catheter removed.

All patients were followed up at 4 weeks, 3, 6 and 12 months, and thereafter once yearly. The cosmetic appearance was evaluated by both the parents and the surgeon at each follow-up visit. The voiding pattern was documented by photographs taken from a lateral view. Any symptoms of voiding difficulty were self-assessed by the older boys or by the parents of infants. The postvoid residual urine volume (PVR) was assessed by abdominal ultrasonography. The uroflow was measured after 1 year in toilet-trained patients who were willing to co-operate. Any UTIs were assessed by culturing a urine sample.

The chi-squared and Mann–Whitney *U*-tests were used to assess any factors before and after surgery that might affect the outcome of the hypospadias repair with a SIS graft.

## Results

All the patients had an uneventful recovery, with no major complications. The operative duration was 112–183 min, and the mean (range) graft length, width and surface area were 2.7 (1.5–5) cm, 1.28 (0.9–2) cm and 3.69 (1.35–10) $cm^2$, respectively. Early complications

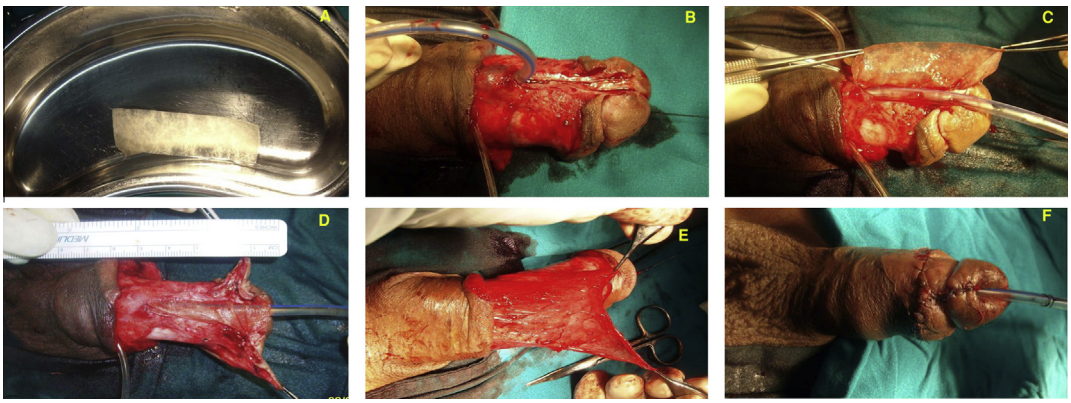


**Figure 1** (A) The gross appearance of the SIS graft; (B) penile skin degloving through a subcoronal incision, preserving the urethral plate; (C) suturing of SIS graft to the urethral plate in an onlay fashion; (D) completed onlay SIS graft suturing; (E) splitting of the dartos flap into two halves to form the second layer of coverage for the graft; (F) the final postoperative appearance.

**Table 1** The patients' characteristics, graft characters and outcome.

| Patient/age (years) | Meatal location | curvature | UP width (mm) | Previous repair | Graft size (cm) | Graft area ($cm^2$) | Postoperative complications | Final outcome |
|---|---|---|---|---|---|---|---|---|
| 1/12 | Distal | – | 5 | – | $1.6 \times 1.2$ | 1.92 | Epididymo-orchitis | Good result |
| 2/14 | Distal | – | 8 | UA | $3.5 \times 1.5$ | 5.25 | Fistula | Fistula closed |
| 3/3 | Mid | Mild | 4 | – | $2.5 \times 1$ | 2.5 | Small dorsal Haematoma and Infected fistula | Fistula closed |
| 4/9 | Distal | – | 5 | – | $2.3 \times 1.2$ | 2.76 | – | Good result |
| 5/15 | Distal | – | 6 | – | $2 \times 1.2$ | 2.4 | Sloughing of Ventral skin | Large fistula with Obliterated distal Segment |
| 6/2 | Mid | Mild | 8 | – | $2 \times 1.2$ | 2.4 | – | Good result |
| 7/7 | Proximal | Moderate | 5 | – | $3.8 \times 1.5$ | 5.7 | Fistula | Fistula closed |
| 8/7 | Distal | – | 5 | TIP | $1.7 \times 1.3$ | 2.21 | – | Good result |
| 9/5 | Mid | Mild | 3 | – | $3.5 \times 1.2$ | 4.2 | – | Good result |
| 10/6 | Distal | – | 4 | – | $1.5 \times 0.9$ | 1.35 | Graft infection | Complete disruption |
| 11/15 | Mid | – | 8 | TIP | $5 \times 2$ | 10 | – | Good result |
| 12/1.5 | Proximal | Mild | 5 | – | $3 \times 1.2$ | 3.6 | Graft infection | Complete disruption |

UP, urethral plate; UA, urethral advancement.

after surgery included epididymo-orchitis in one patient, penile haematoma in one, sloughing of the penile skin in one and graft infection in three. The epididymo-orchitis was relieved with catheter removal, while the penile haematoma and skin sloughing were treated conservatively, with no additional surgical measures.

The mean (range) follow-up was 23 (6–36) months. Nine patients eventually voided normally and had a wide urethra with a good cosmetic appearance and no PVR. Six patients had a completely successful repair with no auxiliary procedures. They had a cosmetically acceptable appearance with an orthotopic external urethral meatus, wide-calibre urethra and a satisfactory urinary stream. Their hypospadias was distal in three and mid-penile in three. Two of them were repeat repairs. The mean length, width and surface area of the graft was 2.68, 1.35 cm and 3.92 $cm^2$, respectively. Uroflowmetry results were available for three patients. The 15- and 7-year-old patient had a normal bell-shaped curve with a normal maximum flow rate, while the 5-year-old patient had a borderline curve with a maximum flow rate between the fifth and 10th percentiles.

Three patients had a persistent urethrocutaneous fistula, and one also had a small dorsal penile haematoma that was managed conservatively and resolved within 10 days after surgery. They had simple anatomical closure of their fistulae 1–2 years later, with a good cosmetic and functional outcome. The hypospadias was distal in one, mid-penile in another, and proximal in the third patient. One of them was a repeat repair. The mean graft length, width and surface area were 3.27, 1.33 cm and 4.48 $cm^2$, respectively.

Three patients had a complete failure in the form of complete disruption in two and a large fistula with a stenosed distal segment in one. All of them had graft infection soon after surgery, and the urine culture showed infection with *Staphylococcus aureus*. The degree of hypospadias was distal in two and proximal in the third. None of them had had a previous repair. The mean graft length, width and area were 2.17, 1.1 and 2.45 $cm^2$, respectively. They later had a one-stage successful repair.

The only statistically significant factor that affected the outcome was the presence of graft infection ($P = 0.002$; Tables 2 and 3). The absence of graft infection was associated with a good result, while the presence of graft infection was associated with failure of the graft to take.

**Table 2** The factors affecting outcome of hypospadias repair with SIS, as assessed by a chi-squared analysis.

| Factor | Success (6) | Fistula (3) | Complete failure (3) | Total (12) | *P* |
|---|---|---|---|---|---|
| *Type* | | | | | |
| Distal | 3 | 1 | 2 | 6 | 0.429 |
| Mid | 3 | 1 | 0 | 4 | |
| Proximal | 0 | 1 | 1 | 2 | |
| *Penile curvature* | | | | | |
| Absent | 4 | 1 | 2 | 7 | 0.489 |
| Mild | 2 | 1 | 1 | 4 | |
| Moderate | 0 | 1 | 0 | 1 | |
| *Previous repair* | | | | | |
| Yes | 2 | 1 | 0 | 3 | 0.513 |
| No | 4 | 2 | 3 | 9 | |
| *Graft infection* | | | | | |
| Yes | 0 | 0 | 3 | 3 | 0.002 |
| No | 6 | 3 | 0 | 9 | |

**Table 3** The effect of patient age, urethral plate, penile length and graft area on the outcome of hypospadias repair with SIS graft, as assessed by the Mann–Whitney *U*-test.

| Factor and outcome | N | Mean (SD) | Mean rank | *P* |
|---|---|---|---|---|
| *Age* | | | | |
| Success | 6 | 8.3 (4.7) | 6.83 | 0.925 |
| Fistula | 3 | 8.0 (5.6) | 6.5 | |
| Complete failure | 3 | 7.5 (6.9) | 5.83 | |
| *Urethral plate* | | | | |
| Success | 6 | 5.7 (1.9) | 6.83 | 0.919 |
| Fistula | 3 | 5.7 (2.1) | 6.5 | |
| Complete failure | 3 | 5.0 (1.0) | 5.83 | |
| *Penile length* | | | | |
| Success | 6 | 6.9 (5.3) | 7 | 0.836 |
| Fistula | 3 | 4.2 (2.5) | 5.5 | |
| Complete failure | 3 | 5.3 (3.3) | 6.5 | |
| *Graft area* | | | | |
| Success | 6 | 3.9 (3.1) | 6.25 | 0.301 |
| Fistula | 3 | 4.5 (1.7) | 9 | |
| Complete failure | 3 | 2.5 (1.1) | 4.5 | |

## Discussion

Hypospadias is a common congenital anomaly with an incidence of 1 in 300–400 male neonates [8]. In a review of 5882 neonatal circumcisions, 35% of boys with hypospadias were circumcised [9]. The type and availability of the substitute material for grafting remain the most challenging dilemma in urethral replacement surgery, with buccal mucosa currently being the standard tissue for urethral replacement. The donor-site morbidity associated with the harvesting of flaps or grafts, the additional operative time needed for harvesting, and the unavailability of enough tissue for repair, are challenges faced when using autologous tissues for repairing urethral defects. Hence, a new prefabricated source of tissue is needed to overcome these problems, especially in patients with hypospadias who are circumcised, or in repeat repairs or for 'crippled' cases.

Tissue engineering has emerged as a new option for organ and tissue replacement; this approach includes the use of acellular matrices and of cell-seeded matrices. In urethral reconstruction, cell-seeded matrices have been used successfully both experimentally in a large-animal model [10] and in clinical trials [11]. In the latter study, autologous urothelial cells from bladder washes of six children were isolated and seeded on acellular dermis to form cell-seeded constructs; these were used to repair severe hypospadias defects in six children. Ultimately, five patients were able to void while standing, and the last patient developed a stricture that was managed successfully with internal urethrotomy. However, the cell-seeded construct should be preserved for complicated hypospadias repairs, like 'cripple hypospadias', bladder exstrophy and complex urethral strictures.

Acellular matrices function as scaffolds to guide the regeneration of urothelial and connective tissue. An acellular graft can be used only when there is a healthy part of the urethral plate, as tissue regenerates from its edges to complete the urethral lumen. Two human studies have used cadaveric bladder submucosa, one in patients with a urethral stricture and one in hypospadias repair [12,13]. In the latter study, a collagen matrix was used as an onlay graft to repair hypospadias in four boys. The created neo-urethras were 5–15 cm long, and the outcome was successful in cosmetic appearance and function. However, cadaveric bladder submucosa has the problem of a shortage of donors.

SIS is a readily available acellular matrix with long-term safety and efficacy, and has been used in substitution urethroplasty for urethral stricture in human studies, with relatively good results. The success of a bladder submucosa matrix in hypospadias surgery encouraged us to use it for urethral replacement in hypospadias repair. We used four layers of SIS for the urethral repair (instead of one), similar to the graft used in SIS urethroplasty studies [6,7,13].

In the present study, SIS grafting was ultimately successful in nine of the 12 patients; six required no further procedures, while three developed small fistulae that were repaired with simple closure. The fistulae were probably a result of inexperience with this new method, and were easy to repair. The SIS graft supported the regeneration of the urethral tissue, as it has many favourable physical and biological characteristics. It is thin but strong, inelastic, easy to handle, and immediately ready for grafting. There is no limit to the graft length that can be used, as it is manufactured in different sizes [11]. Also, SIS is primarily composed of collagen type I, with smaller amounts of collagen types III, IV, V and VI [14]. SIS is rich in growth factors, that include fibroblast growth factor, TGF-β and vascular endothelial growth factor, all of which are known to be important for cellular growth [15]. In animal studies, SIS grafts encouraged the regeneration of normal rabbit epithelium, supported by collagen and smooth muscle tissue [4]. SIS has been used in urological diseases in experimental and human studies, e.g., neurogenic urinary incontinence and female stress incontinence [16]. In penile surgery, SIS has been used successfully in bulbar urethroplasty [6,7] and for correcting penile curvature [17]. When used for corporal body grafting for severe curvature with hypospadias, in several studies one layer of SIS was preferred as the commercial material for successfully correcting the curvature.

In the present study the repair failed in three of the 12 patients, the only factor related to graft failure being graft infection ($P = 0.002$). The infection leads to breakdown, by failure in the tensile strength and stimulation of the T1 immune response, both of which result in graft failure. Other possible causes of SIS failure include the early removal of the urethral catheter, and residual porcine DNA after the preparation process [18,19]. All of these factors lead to an acute inflammatory reaction that causes graft rejection. The implanted SIS scaffold mostly elicits an immune lymphocytic response that is predominantly Th2-like. Activation of the Th2 pathway promotes effective tissue remodelling, structural repair and functional recovery of the injured tissue after graft acceptance. However, activation of the alternative lymphocytic pathway (Th1) produces an acute inflammatory reaction. This host-derived inflammatory response ultimately leads to xenogenic graft rejection [20].

The repair of a failed graft was uneventful and successful in a one-stage procedure. This indicates that SIS did not complicate the future repair in failed cases.

Although SIS grafting was used in three patients as a repeat repair, the previous operations did not affect its success. It is expected that SIS grafts could work better after failure of TIP urethroplasty or urethral advancement repairs, as there is minimal surgical trauma to the urethral plate during these procedures.

The present high complication rate (fistula or complete disruption) can be attributed to initial inexperience with the new technique, and to graft infection. To improve the outcome of SIS grafting for hypospadias, infection could be prevented by incorporating antimicrobial agents, modified biofilms and bacterial-interference agents into SIS biomaterial. Also, the use of an antibiotic-coated catheter could help in preventing infection. Although the urethral catheter was left in for 10–14 days, it might be of value to leave it for longer, especially in patients with posterior and 'cripple' hypospadias. The scaffold can be injured when the catheter is removed and the unepithelialised SIS graft is still in contact with urine, leading to fibrosis and stricture, as in these cases healing and epithelialisation are slower.

To our knowledge this is the first study of using a SIS graft for hypospadias repair, and with the longest follow-up (36 months). The shortest follow-up was 6 months, which represents a limitation to our study, as a minimum of 12 months is recommended to report the outcome of urethroplasty, especially in hypospadias repair [1,2]. Other limitations include the heterogeneity of the hypospadias (distal, mid-shaft and proximal), the patients' age (infants and teenagers) and previous surgery (untreated or failed cases) that might generate confusion in the interpretation of the results. The heterogeneity was considered useful as a trial of the SIS graft urethroplasty in various situations of hypospadias type, age and previous surgery, to outline the possible uses of the SIS graft in hypospadias repair. Other limitations include the few patients, few cases of proximal and complicated hypospadias, a lack of histopathology from the regenerated and failed repairs, and that there was no uroflowmetry for all the repaired cases. Thus further studies with more patients having proximal hypospadias and repeat repair are needed to better evaluate the outcomes of SIS in hypospadias repair, and to determine its indications.

In conclusion, SIS promotes urethral regeneration in hypospadias surgery when used as an onlay graft. It could be used as an alternative substitute for urethral reconstruction when genital skin is insufficient or lacking, as in circumcised patients or for repeat repairs. The failure of SIS grafting does not complicate a future repair. Graft infection is a major cause of failure and every effort should be made to prevent it. Further studies are required to validate these preliminary results.

**Conflict of interest**

None.

**Source of funding**

None.

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