The current state of tissue engineering in the management of hypospadias

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Abstract | Hypospadias is a congenital malformation resulting from the disruption of normal urethral formation with varying global prevalence. Hypospadias repair, especially that of proximal hypospadias (in which reconstruction of a long urethra is necessary), remains a surgical challenge despite more than two decades of surgical technique development and refinement. The lack of tissue substitutes with mechanical and biological properties similar to those of native urethra is a challenge for which the field of tissue engineering might offer promising solutions. However, the use of tissue-engineered constructs in preclinical studies is still hindered by complications such as strictures or fistulae, which have slowed progression to clinical application. Furthermore, the generation of uniform tubular constructs remains a challenge. Exciting advances in the application of nanotechnology and 3D bioprinting to urethral tissue engineering might present solutions to these issues.

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https://doi.org/10.1038/s41585-020-0281-4

Hypospadias is a complex congenital malformation characterized by the interruption of normal urethral development, resulting in a urethral meatus located proximal to the orthotopic meatal location at the tip of the glans penis in men[1] (FIG. 1). Hypospadias often coexists with deficiency of the ventral prepuce and chordee, which is an abnormal penile curvature. Presentation of hypospadias is variable, ranging from mild forms with the meatus located at the corona (distal) to severe forms with the meatus in the perineum (proximal) (FIG. 1). Current management, especially for proximal hypospadias, is far from optimal[2,3]. Repair is challenging owing to the variable anatomy and quality of the urethral plate. Those patients with fibrotic and deficient urethral plates often require staged repair with urethral plate augmentation with preputial or buccal tissue[2]. Complications such as urethral strictures, fistulae and dehiscence often lead to multiple surgical procedures for each individual. Thus, tissue engineering, which combines the principles of engineering and biological sciences to provide constructs that replace or enhance the regeneration of damaged or deficient native tissue, has a role in hypospadias repair[4].

In this Review we discuss the considerable effect of hypospadias on affected individuals and the need for improved management options. We describe advances in urethral tissue engineering and barriers to the clinical application of tissue-engineered constructs in hypospadias repair.

Prevalence

Hypospadias affects 1 in 200–300 newborn male babies in the USA[5,6]. Numerous studies have been performed globally and have shown a mean hypospadias prevalence of 19.9 in 10,000 in Europe, 34.2 in 10,000 in the USA, 5.2 in 10,000 in South America, 0.6–69.0 in 10,000 in Asia, 5.9 in 10,000 in Africa, and 17.1–34.8 in 10,000 in Australia[7]. However, data on the true prevalence and trends of hypospadias are difficult to interpret given the variability in hypospadias awareness and reporting in different geographical regions[7]. A study of 23 EUROCAT registries noted a large amount of variability between registries, with reported prevalence ranging from 5.10 in 10,000 births in south Portugal to 36.83 in 10,000 births in Germany[8]. Variations in reported prevalence might be caused by factors including geographical differences in genetic and environmental factors, increased practitioner awareness of hypospadias over time and disparities in hypospadias reporting between registries[7].

Genetic and environmental factors

Hypospadias can be hereditary and can result from disruptions to various signalling pathways at the genetic and/or molecular levels[9–12]. A cohort study of >1.2 million boys in Denmark estimated the recurrence risk ratio of hypospadias for first-degree relatives to be 11.6 (95% CI 9.75–13.7), with possible transmission via maternal or paternal lines[9]. Mutations, copy number variations and single-nucleotide polymorphisms in *AR*, *SRD5A2*,

Key points

- Hypospadias is a common congenital abnormality with genetic, molecular and environmental causes that can result in functional or cosmetic issues for affected individuals.
- Distal hypospadias repair is relatively successful, but complication rates of proximal hypospadias repair are high even for experienced surgeons despite surgical technique refinement.
- Tissue engineering could address the lack of tissue substitutes with properties similar to those of native urethra for use in urethral reconstruction.
- For hypospadias in particular, improved understanding of the mechanical properties and biological support being provided by the corpus spongiosum is needed.
- Amongst the emerging tissue engineering technologies, nanotechnology could enable alteration of the microenvironment to improve wound healing and regeneration, and 3D bioprinting could be used to offer patient-tailored urethral constructs.
- Scientific barriers, such as identifying the ideal tissue-engineered urethral construct, and practical barriers, including institutional support and funding for translational research, hamper clinical application of tissue-engineered constructs in hypospadias repair.

HSD3B2, *WT1* and many other genes involved in early urethral and genital tubercle development are associated with hypospadias[10–13]. As the technology for whole-genome sequencing improves, more genetic variants associated with the hypospadias phenotype will probably be identified. The clinical implications of this emerging information will need to be thoroughly scrutinized and might help families identify predispositions to other associated conditions. For example, an infant with proximal hypospadias was noted to have a *WT1* gene alteration, which prompted early screening for Wilms tumour and resulted in early detection and treatment[14].

Environmental causes of hypospadias are more challenging to establish than genetic factors given the presence of multiple confounding factors. Studies have suggested associations between hypospadias and maternal exposure to endocrine-disrupting chemicals (EDCs) and medications such as diethylstilboestrol, which was historically given to women in the 1970s to prevent preterm labour, and valproate, which is an anticonvulsant used to treat bipolar disorder and seizures[15–20]. EDCs include synthetic chemicals found in industrial solvents, pesticides, plastics and medications, but can also include natural chemicals found in food products. Exposure can occur through occupation, use of products containing EDCs or pollution[21]. Paternal EDC exposure at the time of fertilization might be a potential risk factor for hypospadias, but has been inconsistently demonstrated in studies[16,22,23]. Hypospadias is also known to be associated with intrauterine growth retardation, which could be caused by maternal placental insufficiency[24,25].

Urethral development

Hypospadias results from interference in urethral formation. Thus, a basic understanding of normal human urethral development is necessary to comprehend this condition. Theories about how the human urethra develops have evolved over time from the 'ectodermal intrusion' theory to the current 'double zipper' hypothesis[3,26]. In 2015, Li et al.[1] analysed eight developing human male fetal specimens using optical projection tomography and observed progression of the urethral meatus from the scrotal folds to the glans penis along with proximal to distal fusion of the urethral groove with increasing gestational age[1]. This observation led investigators to propose the 'double zipper' hypothesis in which urethral development occurs via the processes of urethral groove formation via canalization of the urethral plate followed by fusion of the urethral groove. Failure of urethral plate formation or canalization or failure of urethral groove growth or fusion results in hypospadias[1].

Further study increased understanding of glanular urethral development, which is distinct from that of penile urethral formation. Examination of 48 human fetal penile specimens showed termination of the urethral plate cannulation at the proximal glans with continued extension of the solid urethral plate to the tip[27]. Urethral plate cannulation then occurs in an asymmetric, ventral-to-dorsal manner without formation of urethral grooves. The glanular urethra is then formed from mesenchymal confluence with subsequent reabsorption of endoderm cells ventral to the confluence[27]. The distinct differences in penile and glanular urethral formation might give insight into the differences in outcomes of proximal and distal hypospadias surgical repair. These observations have increased our knowledge of the distinct differences between penile and glanular urethral formation.

Hypospadias classification

Timing of urethral development disruption dictates the location of the urethral meatus, with earlier time points correlating with more proximal locations. Hypospadias is, therefore, classified by the ultimate position of the urethral meatus (FIG. 1). Many classification systems have been reported, but the two most commonly used classifications were proposed by Duckett[28] in 1996 and Hadidi[29] in 2004. Duckett categorized hypospadias into anterior (distal penile to glanular), middle (proximal penile and mid-shaft) and posterior (perineal to penoscrotal) hypospadias[28]. Hadidi divided hypospadias into three categories: glanular, distal (mid-shaft to coronal) and proximal (perineal to proximal penile)[29]. These classifications help divide hypospadias into different categories by meatal location, offering frameworks for reporting in data registries. However, meatal location alone is arguably insufficient to adequately represent the true severity of the phenotype, and other factors, including degree of ventral curvature and quality of the urethra plate, should also be considered. At present, further standardization is needed for hypospadias classification[30].

Clinical goals of management

Hypospadias is not life threatening but can result in functional issues with urination, intercourse and sperm deposition in severely affected individuals[31]. Thus, the main goals of management include reconstruction of the urethra with placement of the meatus as close to the tip of the glans as possible for proper aim of the urinary stream and semen deposition, correction of the penile curvature to minimize sexual dysfunction and achievement of acceptable cosmetic results to reduce the psychosocial effect on the affected individual. Thus, an understanding of urinary symptoms,

sexual function and fertility, and psychosocial outcomes in patients with hypospadias is important.

Urinary symptoms after hypospadias repair are improved; comparison of patient-reported outcome measures between men with uncorrected hypospadias, successful repair or failed repair showed that spraying or deviated streams were higher in those with failed repair (39%) or uncorrected hypospadias (37%) than in those with successful repair (13%) ($P = 0.003$)[32]. An internet survey of men, including 7.1% who had untreated hypospadias, showed that those with untreated hypospadias reported worse voiding and sexual function, more difficulty with intercourse and more ventral penile curvature than men who did not have hypospadias[33]. Evaluation of patients who underwent hypospadias correction revealed normal or mild voiding disturbances[34].

Sexual function and fertility are worse in those with proximal hypospadias. A survey was completed by 119 men who underwent hypospadias repair as children and was analysed according to primary urethral meatal locations (glanular, distal or proximal). The rate of mild erectile dysfunction was highest in those with proximal hypospadias (8.9% glanular, 50% distal and 72.2% proximal) who also reported decreased sexual quality of life compared with those who had distal or glanular hypospadias[35]. A study administered a questionnaire designed to evaluate fertility and psychosocial status to 167 adult men with a history of hypospadias and age-matched controls and found no overall differences in fertility, number of sexual partners or sexual interest between the two groups[36]. However, those with proximal hypospadias, who comprised 13% of the patients, did have lower sexual life satisfaction and lower reported fertility than those with distal hypospadias and those without hypospadias. Reported fertility was 22.7% in those with proximal hypospadias compared with 52% in those without hypospadias ($P = 0.002$) and 53.9% in those with distal hypospadias ($P = 0.001$)[36]. Hypospadias in certain patients has been hypothesized to be associated with undescended testes and impaired semen quality[37]. Prospective evaluation of semen parameters and erectile function in 73 adult patients with history of hypospadias showed poorer erectile function and semen quality in men with proximal hypospadias than in men with distal hypospadias and those without hypospadias (who had comparable semen parameters)[38]. Comparison of semen quality between men with isolated hypospadias and those with hypospadias and additional genital disorders showed that the latter have reduced semen quality[37].

Previous studies have mainly focused on surgical and functional outcomes of hypospadias repair, but contemporary studies are also starting to consider the psychosocial outcomes in those with history of hypospadias. Satisfaction with cosmetic appearance is highest in those with successful repair (93%) compared with those who have failed repair (77%) or uncorrected hypospadias (67%) ($P < 0.001$)[32]. A large, registry-based, cohort study of >4,000 individuals with a history of hypospadias showed that they were more likely to receive a disability pension than age-matched men without hypospadias, but no difference was observed with regard to marital status, education level, income and diagnosis of psychiatric diseases[39]. A questionnaire-based study of 167 men with a history of hypospadias showed that hypospadias did not affect marital status, employment, presence of children in the family or experience of bullying. However, those with a history of proximal hypospadias reported a tendency to avoid close relationships[40].

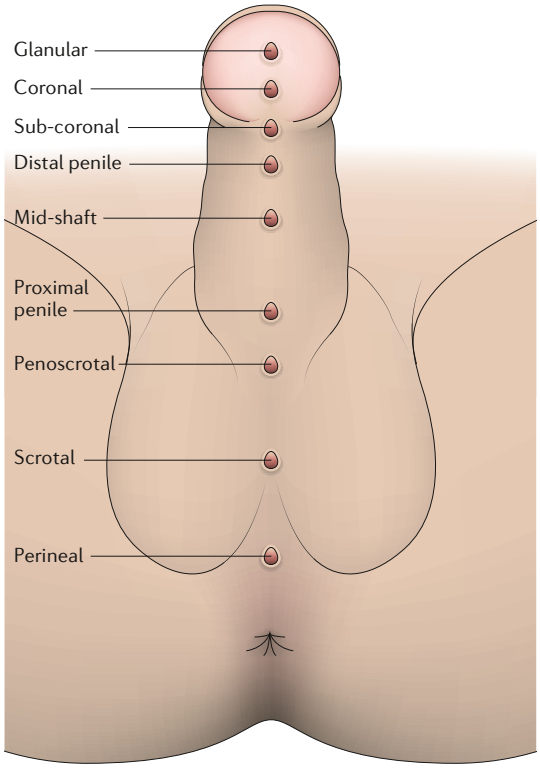

Fig. 1 | **The meatal locations in varying degrees of hypospadias.** The potential meatal locations of hypospadias in varying presentations, ranging from glanular hypospadias to perineal hypospadias.

Many studies to date have focused on physician-reported outcomes and have been limited by the absence of long-term follow-up data. However, increasing emphasis has been placed on the importance of patient-reported outcomes and the creation of measures to objectively evaluate cosmetic and functional results following hypospadias management[2,32]. Future studies incorporating these factors and long-term, regular follow-up monitoring might provide a more accurate representation of the success of current hypospadias management.

Current surgical management

Hypospadias treatment is surgical and involves repair of the urethral defect and correction of the ventral curvature. Surgical techniques for hypospadias repair have changed and continue to evolve[41]. Given the plethora of techniques used, no consensus exists on the best approach. The choice of approach varies with patient anatomy and surgeon preference[42].

Glanular and distal hypospadias

Hypospadias management depends on the severity of the condition (FIG. 2). In patients with glanular or distal hypospadias and minimal functional issues, observation

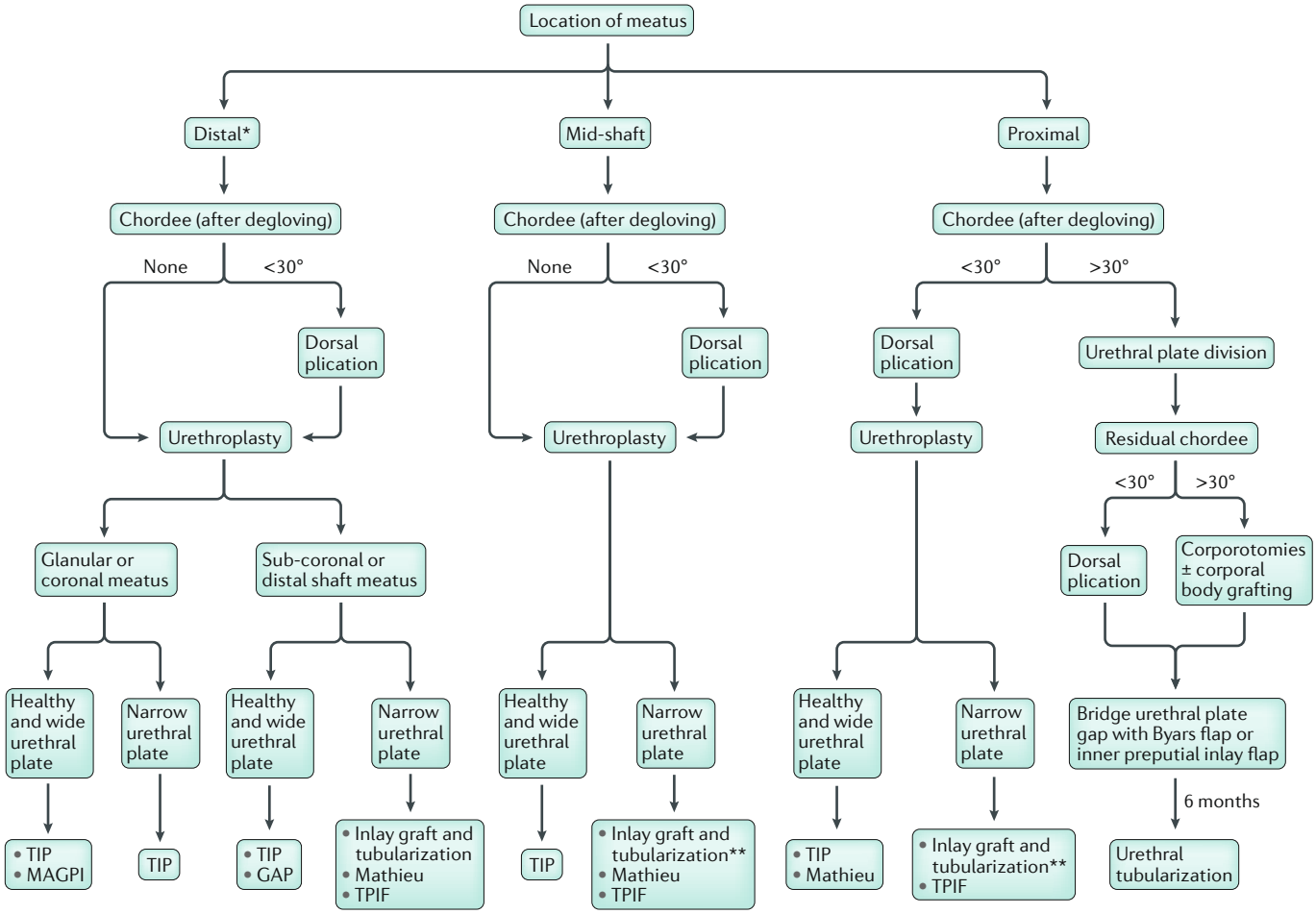

Fig. 2 | **Surgical management algorithm for hypospadias.** An algorithm showing the potential approach to hypospadias management. The surgical approach is guided by meatal location, degree of chordee and the quality of urethral plate. In a child with a distal hypospadias who has a straight urinary stream, is asymptomatic and has no penile curvature, consider nonoperative management (asterisk). Tubularization can be performed as a second-stage repair 6 months after chordee repair (double asterisk). Two-stage repair is recommended in patients with considerable chordee and/or an unhealthy urethral plate. GAP, glans approximation plasty; MAGPI, meatal advancement and glanuloplasty; TIP, tubularized incised plate urethroplasty; TPIF, transverse preputial island flap.

without surgical intervention might be chosen[43]. If the parents desire repair, the American Academy of Pediatrics recommends intervention between 6 to 12 months of age[44]. Various techniques have been described. The tubularized incised plate (TIP) repair, which is a commonly used technique, involves penile degloving and creation of a relaxing incision in the midline urethral plate[45]. The urethral plate is then tubularized and covered with a dartos flap[45]. The Mathieu technique uses a penile shaft skin flap measured to the distance from the meatus to the tip of the glans to generate the neourethra. The flap is flipped over the meatus and sutured to the lateral edges of the urethral plate[46]. The meatal advancement and glanuloplasty (MAGPI) technique involves making a longitudinal incision in the glanular groove and closing the incision in a Heineke–Mikulicz manner[46]. The glans approximation plasty (GAP) procedure, which is only feasible in those with wide urethral plates, involves tubularization of the urethral plate without a relaxing incision[46]. In patients in whom the urethral plate is of inadequate quality, a transverse preputial island flap (TPIF) technique, which involves harvesting a strip of inner preputial skin with a preserved blood supply to create the ventral wall of the neourethra, can be used[45].

To date, no consensus exists on the ideal method of repair, but distal hypospadias repair has relatively low complication and failure rates. In a study using a multicentre database of hypospadias surgery, the reoperation rate was 9% in distal hypospadias repair. Increased age at time of repair was associated with an increased risk of requiring cystoscopy and urethral dilation or incision. Each additional year of age at the time of initial repair conferred an increased risk of 15% for cystoscopy and 21% for urethral dilation or incision[47]. A meta-analysis comparing primary Mathieu and TIP repairs in distal hypospadias showed an increased incidence of urethral fistulas with the Mathieu approach compared with TIP (5.3% versus 3.8%, $P = 0.028$) and a higher incidence of meatal stenosis in the TIP group than in the Mathieu group (3.1% versus 0.7%, $P < 0.001$). However, this difference was no longer seen after excluding studies that

did not specify the follow-up duration[48]. A meta-analysis evaluated outcomes of TIP repairs and found fistula and reoperation rates of 5.7% and 4.5%, respectively, following primary distal repair[49]. Evaluation of urinary flow parameters after distal hypospadias repair revealed flow improvement with time and especially by 10 years postoperatively[50].

Mid-shaft hypospadias often have a greater degree of chordee than glanular or distal hypospadias[45]. If the chordee is <30° and the urethral plate is healthy and wide enough, the TIP or Mathieu technique can be used[45]. If the urethral plate is insufficient, the TPIF technique can be used[45]. A case series of 46 patients with mid-shaft hypospadias repaired using this approach showed a fistula rate of 2.2%[51]. Alternatively, the urethral plate can be augmented with an inner preputial inlay graft[45]. In this technique, the urethral plate is incised longitudinally from the hypospadiac meatus to the tip of the penis. The plate is elevated off the underlying corporal bodies and an appropriately sized inner preputial graft, which is removed from its native blood supply, is placed in this defect. The neourethra can be created via a single-stage repair or a two-stage repair with a 6-month hiatus to allow neovascularization of the graft[45]. A prospective study in 230 individuals who underwent repair using inlay grafts showed no incidences of meatal stenosis or urethral diverticulum; fistula formation occurred in 3.9% of individuals[52].

Proximal hypospadias

Proximal hypospadias repair is far more complex than glanular or distal hypospadias repairs (which can be completed in one operation) and often requires staged repair[43]. Proximal hypospadias repair is challenging owing to the often small and dysplastic urethral plates and severe chordee. If the chordee is <30° after penile degloving, the curvature can be corrected with dorsal plication. If the urethral plate is healthy, a single-stage approach with TIP can be attempted. If the urethral plate is insufficient, it can be augmented with an inner preputial inlay graft followed by single or two-stage reconstruction[45]. If the curvature is >30° after penile degloving, urethral plate division might be necessary with subsequent deep transverse incisions of the tunica albuginea or corporal body grafting if the severe chordee persists[2,45]. After chordee correction, the urethral defect needs to be bridged. Multiple techniques have been described, including the Byars flap in which the preputial skin flaps are rotated ventrally along their vascular pedicles to fill the gap. Alternatively, a dorsal inner preputial skin flap can be harvested and quilted to the underlying tunica albuginea to bridge the gap. The second stage of the repair, during which the urethral plate is tubularized, is usually undertaken 6 months after the first stage[45,46].

Complication rates are high in proximal hypospadias repair with a multivariate analysis showing that the only predictive factor for re-intervention was the presence of proximal hypospadias (OR 3.27, $P = 0.012$)[53,54]. The complication rate was 56% in a cohort who underwent single-stage or two-stage proximal hypospadias repair, with complications developing in 62% of those who underwent single-stage repair and in 49% of those who underwent staged repair[55]. In another study, 68% of the cohort who underwent two-stage proximal hypospadias repair with transposed preputial skin flaps required additional unplanned operations[56]. Similarly, the complication rate was 68% in 38 patients who underwent two-stage proximal hypospadias repairs using inner preputial flaps[57]. These results from surgeons experienced in hypospadias repair illustrate the considerable difficulty in proximal hypospadias management, which poses the challenge of needing to reconstruct long urethras with minimal healthy urethral tissues in patients undergoing primary repair and often deficient healthy urethral tissues in those undergoing re-repair[2]. Advances in urethral tissue engineering would have a large effect on proximal hypospadias repair by potentially creating comparable urethral substitutes for use in patients in whom the urethral plates are deficient or lacking.

Urethral tissue engineering

Repairing proximal hypospadias is challenging. As urethral plates with dysplastic tissue are deficient, substitutes for urethral tissue (such as buccal mucosal, bladder mucosal and postauricular grafts) are used to create the neourethra[46,58], which strains the autologous tissue donor sites, especially in patients requiring repeated interventions. Tissue engineering, which combines bioengineering principles, material sciences and aspects of nanotechnology, could lead to the creation of tissue with properties similar to those of normal urethra[59]. Scaffolds are integral to this process and provide the structural support necessary for cellular localization, maturation and ultimately tissue development[60]. The ideal scaffold is biocompatible and biodegradable, possesses mechanical properties similar to those of the native organ and can be successfully surgically manipulated[4]. Various scaffolds exist and can be classified according to whether they are made of natural or synthetic material[61]. Scaffolds made from natural polymers in part include those derived from collagen, chitosan, alginates, gelatin, elastin or silk. Natural scaffolds can also be made from decellularized tissue, such as small intestinal submucosa (SIS) or bladder acellular matrices (BAMs), which preserve tissue microarchitecture and growth factors[61]. Synthetic scaffolds are made of synthetic polymers including polyglycolic acid (PGA), polylactic acid (PLA) and poly(lactic-co-glycolic acid) (PLGA), among others[60,61]. Hybrid scaffolds are generated by using a combination of synthetic and natural materials[62]. Scaffolds can be acellular or cell-seeded with stem cells or differentiated cells. Cell-seeded scaffolds provide additional support by using autologous cells that aid in regeneration[61].

Acellular scaffolds

Acellular scaffolds promote local cellular migration and growth by providing the structural and growth factor support necessary for native cellular regeneration[60,61].

Naturally derived scaffolds. Various naturally derived scaffolds for facilitating urethral tissue regeneration have been investigated[59,63]. Collagen-based scaffolds, which can be moulded to the configuration of a human

urethra, have been commonly used[64]. Double-layered high-density collagen tubes were used to replace 2-cm native urethral defects in 20 male white rabbits that had undergone subtotal urethral excision[65]. Voiding cystourethrography was performed at 1, 3, 6 and 9 months and the regenerated urethras were evaluated by histology and immunohistochemistry. Fistula and stricture rates were both 20%. Urothelial and vascular regeneration were visualized at 1 month after the procedure and smooth muscle bundle formation was visualized at 6 months. Cellular organization improved over time and began to resemble that of the native rabbit urethra 6–9 months after the procedure[65].

Silk fibroin scaffolds are naturally derived scaffolds that are gaining popularity for bladder and urethral regeneration[66]. This scaffold purportedly has better elasticity than other biomaterials, including collagen and PLA, and is relatively nonimmunogenic compared with collagen and PLA[66,67]. In one study, acellular silk fibroin scaffolds used in ventral onlay urethroplasties were compared with SIS grafts or urethrotomy alone in adult male rabbits. Epithelial and smooth muscle regeneration were equivalent in both silk fibroin and SIS groups, but silk fibroin scaffolds elicited lower levels of inflammatory response[68]. Silk fibroin has also been used to develop composite scaffolds. A composite silk, keratin, gelatin and calcium peroxide (CPO) film that facilitates high continuous oxygen delivery had similar regenerative outcomes to SIS in reconstructions of rabbit urethral defects[69]. Furthermore, scaffolds without CPO had higher incidences of fistula, which was seen in all animals within this group. Complete, patent urethras were noted in the groups treated with films containing CPO or with SIS alone. Surprisingly, the CPO scaffolds had strong antimicrobial activities against *Escherichia coli* and *Staphylococcus aureus*. The authors postulated that this activity could be secondary to the degradation products of CPO and could potentially reduce the negative effects of infection on wound healing, but further investigation is warranted[69]. Lv et al.[69] used a complex composite scaffold instead of a commercially available scaffold, such as SIS[69]. Making SIS + CPO scaffolds is potentially possible, but the mechanical properties of the material, rate of oxygen diffusion and nature of scaffold degradation might not be ideal for continuous oxygen delivery. The investigators noted that methods for detecting the rate of oxygen delivery are still being refined. Improvements in techniques and understanding of how to facilitate oxygen delivery means that this biomaterial or the techniques involved potentially could provide additional benefits beyond what is currently commercially available.

The amniotic membrane is another biomaterial that has been used in urethral regeneration with or without the epithelium layer[70]. The membrane is rich in essential growth factors including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), which enhances wound healing and decreases inflammation, and has low immunogenicity[71,72]. In one study, human amniotic membrane xenografts were used to repair 10-mm urethral defects in 20 rabbits. Fistula occurred in one animal (5%) and strictures occurred in two (10%). Complete re-epithelialization of the xenograft was observed but no definitive muscle layer was seen[73]. The combination of amniotic membranes and buccal mucosa for urethroplasty to repair 10 × 5-mm surgically created urethral defects in male rabbits improved tissue healing and decreased inflammation compared with controls[74]. Amniotic membrane is an inexpensive source of biomaterial for urethral regeneration, but further research is necessary to develop standardized protocols for its processing, characterization and storage. Tissue properties vary between different regions of the amniotic membrane, making it a heterogeneous material to work with[72]. Future research might improve our understanding of these variations and potentially lead to the use of other biomaterials or additional growth factors to minimize this heterogenicity. Combinations of the membrane with other biomaterials including nanofibres might reduce its fragility and increase its suitability for widespread use[72,75].

Naturally derived decellularized scaffolds. Naturally derived matrices obtained via tissue decellularization are scaffolds that preserve tissue microarchitecture[61]. Commonly used decellularized scaffolds in tissue engineering include SIS and BAM[61]. In one study, single-layer SIS grafts were used for urethral replacement in male rabbits as onlays (nine animals) or for tubular replacement (nine animals)[76]. Animals either had a 15-mm defect created in the ventral penile urethra (18 animals) or had a 15-mm segment of their penile urethra excised (18 animals). In nine animals, the ventral defect was closed with SIS onlay grafts, and the results were compared with nine control animals that had spontaneous regeneration without repair. In the nine animals that had complete excision of the urethra, the defect was bridged with a 15-mm segment of tubularized SIS; regeneration in this group was compared with that in nine controls in which the excised urethral defect was not bridged. Histological examination revealed inferior healing with the SIS onlay grafts, which lacked formation of smooth muscle fascicles, compared with spontaneous regeneration; instead, abundant collagenized fibrous tissue was observed. Poor tissue healing, with development of fistulas or strictures, was observed in rabbits with a tubular defect, whether or not it was bridged with SIS. Repair with tubularized SIS grafts resulted in fibrosis with luminal obstruction. The authors concluded that SIS onlay alone was inferior to spontaneous regeneration, and SIS was unsuitable for use as a tubularized urethral substitute in this model[76]. Subsequently, use of single-layer SIS, four-layer SIS and buccal mucosa for the repair of 10 × 5-mm urethral defects was compared in 36 male rabbits, with 12 animals in each group. The degree of fibrosis, epithelial regeneration and neovascularization were similar across all groups but graft shrinkage was less in the group receiving the four-layer SIS repair[77].

BAMs are also commonly used in urethral regeneration. Urethroplasty was performed to repair a 1.5-cm urethral defect in rabbits using BAM either treated with peracetic acid or not[78]. Peracetic acid increased the porosity of the scaffold, which minimized exogenous

cellular components and increased cellular migration, infiltration and proliferation. Regeneration of the urothelium and smooth muscle was faster in the experimental group in which the increased porosity of the scaffold facilitated nutrient transfer and cellular viability[78]. A BAM hydrogel and silk fibroin composite scaffold was created that was pre-vascularized using a 2-week incubation period in the omentum of male rabbits and used to repair autologous urethral defects[79]. Repairs using the BAM composite scaffolds had increased vascularization and wider calibre urethras than repairs using composite scaffolds of collagen hydrogel and silk fibroin or silk fibroin-only scaffolds (which were similarly incubated in the omentum before use)[79]. Similar success was achieved using autologous urethral tissue-embedded acellular porcine bladder submucosa matrices (BSMs)[80]. Normal urethral lumen, complete epithelialization and muscle regeneration were observed in rabbits that underwent urethroplasty with autologous urethral tissue-embedded acellular BSM. Those rabbits that underwent urethroplasty with an acellular BSM graft had abundant fibrous tissue deposition and lacked circular smooth muscle bundles[80].

Preputial acellular matrices (PAMs) and decellularized urethral tissue have also been used as scaffolds for urethral regeneration. PAM generated from decellularized prepuces from circumcised boys were used to repair 5 × 5-mm ventral surgically created urethral defects in male rabbits[81]. The results were compared with those in rabbits in which defects were closed in layers (six animals), closed in layers with application of fibrin sealant (six animals), closed with PAM graft alone (six animals) or closed with a PAM graft and fibrin sealant (six animals). Animals with defects repaired using a PAM had a wide-calibre urethra with complete transitional cell layers over the graft at all time points[81]. Decellularization and recellularization of urethral tissue have been successfully performed and might offer new avenues for future urethral tissue regeneration[82,83]. One study evaluated the decellularization of eight human urethras with the surrounding corpus spongiosum. The scaffolds were transplanted into the omentum of 12 male rats to evaluate their suitability for in vivo recellularization[82]. Initial results were promising, with angiogenesis and epithelialization of the scaffold. In vivo recellularization was found to be more successful than in vitro cellularization[82]. In another study, 17 porcine urethras (9 female, 8 male) were decellularized and then seeded with human muscle progenitor cells, human skeletal myoblasts and adipose-derived stromal vascular fractions[83]. The overall tissue microstructure was maintained in the decellularization process and the urethra bioscaffold provided a suitable environment for cellular adhesion and proliferation.

Naturally derived acellular scaffolds offer the structural architecture, growth factors and extracellular matrix proteins that help facilitate regeneration. However, these scaffolds are limited by protein variability between batches and their poor efficacy in the repair of long urethral defects[61,84]. Dorin et al.[84] showed that the use of acellular matrices to repair tubularized defects longer than 0.5 cm results in considerable collagen deposition and fibrosis[84]. Despite this limitation, these acellular scaffolds still have potential applications in the repair of smaller defects.

Synthetic scaffolds. Synthetic scaffolds derived from PLA, PLGA, poly-L-lactide-co-ε-caprolactone (PLCL), polytetrafluoroethylene (PTFE) and others have also been used in tissue regeneration with varying results[59,63]. A study of urethral replacement with PTFE in ten dogs showed development of fibrous tubes around the graft without evidence of regeneration of normal urethral tissue[85]. In another study in dogs, 4 cm of urethra was replaced with a graft of polyglactin fibre mesh coated with polyhydroxybutyric acid and evidence of patent neourethra was seen by 1 year[86]. Composite scaffolds such as a combination of poly(L-lactide) (PLLA) and poly(ethylene glycol) (PEG) have also been created. Scaffolds of PLLA–PEG seeded with human amniotic mesenchymal cells were used to repair 20-mm urethral defects in male rabbits and resulted in no strictures or fistula formation, whereas strictures or fistula were seen in 72.2% of control animals repaired with primary closure[87]. Studies of PLCL have shown promising results in vitro, suggesting it has the ability to effectively support the growth of human urothelial cells[88,89]. Human urothelial cells were seeded on human amniotic membranes and synthetic PLCL, and cellular viability, proliferation and differentiation were compared. PLCL provided better cellular support[89]. A comparison of the characteristics of different types of PLCL matrix (smooth, textured and compression-moulded) and of their ability to support cellular adhesion and growth showed that smooth and textured surfaces offered the best support[88].

The mechanical properties of these materials can be more easily controlled and dependably reproduced than those of naturally derived biomaterials. The risk of inflammatory response, material inelasticity and an asynchronous rate of scaffold degradation relative to tissue regeneration make using these materials challenging[66,88]. Nevertheless, emerging studies suggest that certain polymers such as PLCL might have potential in urothelial tissue engineering.

Role of growth factors

Acellular scaffolds can be used to enrich the regenerative environment with growth factors, as well as provide 3D support for cellular growth. Growth factors have been used to enhance regeneration via fusion proteins such as a collagen-binding VEGF (CBD-VEGF) protein[90]. In this study, a 5-cm segment of the anterior urethra in beagle dogs was replaced with a tubularized collagen scaffold with (five animals) or without (five animals) CBD-VEGF. CBD-VEGF was dissolved in phosphate-buffered saline and the solution was dripped on to the collagen scaffolds of the experimental group before implantation. The neourethras were evaluated at 6 months with retrograde urethrography, urodynamic studies and immunohistochemistry. Improved smooth muscle generation and neovascularization were seen in the neourethra of the animals receiving the CBD-VEGF-treated scaffold compared with the neourethra in those receiving the collagen-only scaffold. However, strictures were seen

in all animals in both groups, but these were worse in those receiving the collagen-only scaffold. The diameters of the neourethra at the centre, at the anastomosis and in the middle in the two groups were evaluated. The mean neourethral diameter in the animals receiving the CBD-VEGF-treated scaffold was significantly larger than in those receiving collagen-only scaffold (1.86 ± 0.19 mm versus 0.92 ± 0.13 mm, $P < 0.05$). However, both mean neourethral diameters were significantly smaller than that of normal urethras (5.94 ± 0.65 mm, $P < 0.01$)[90]. A similar study was performed using a fusion protein of collagen-binding domain and basic fibroblast growth factor (CBD-bFGF) to reconstruct 5-cm urethral defects in male beagle dogs[91]. The urethras were reconstructed with collagen tubes with (five animals) and without (five animals) CBD-bFGF. Fewer fistulae were seen in the animals receiving the CBD-bFGF-modified tube than in those receiving the unmodified tube (20% versus 60%), and more favourable regeneration was also seen in those receiving the CBD-bFGF-modified tube[91].

Similarly, collagen biomatrices incubated with growth factors (including VEGF, fibroblast growth factor 2 and heparin-binding epidermal growth factor) were used to repair 1-cm urethral defects in a New Zealand white rabbit model (32 animals total, 16 each in the experimental and control groups)[92]. Incubation with the growth factors improved extracellular matrix deposition, neovascularization and urothelium regeneration. However, these improvements in regeneration did not translate to positive functional outcomes. Relative urethral narrowing was seen at 2 weeks in the four animals that received the growth factor-supplemented biomatrix compared with those that received the unsupplemented biomatrix. Diverticula formed in the remaining 11 of 12 rabbits with the growth factor-supplemented biomatrix; in 1 of the 11 rabbits a fistula had formed at 24 weeks. The animals that received the unsupplemented biomatrix had no strictures, fistulae or change in urethral calibre[92]. The results of these studies suggest the polarizing effects when using VEGF, for example. Tissue neovascularization is accomplished but accompanied by abnormal tissue formation including fistula and diverticula formation.

Further work is necessary to understand the proper timing of growth factor release in the regeneration process, the optimal growth factor combinations and subsequent concentrations necessary, and the most effective methods of delivery to facilitate optimal cellular migration and development for urethral formation. Further studies are needed to explore these avenues. Developing the optimal growth factor and scaffold combinations would also require further evaluation[92,93].

Cell-seeded scaffolds

Cell-seeded scaffolds have emerged as promising alternatives to acellular scaffolds. Cellularization promotes vascularization and the development of urothelial barriers to urine, mitigating local inflammation and fibrosis caused by urine leaks[62,94,95]. A large meta-analysis did not show superiority of cell-seeded scaffolds over acellular scaffolds, but in preclinical studies they have been associated with a lower rate of adverse effects[63].

Cell sources. Cell sources used for seeding in urethral tissue engineering can be classified into three main categories: terminally differentiated cells, stem cells and progenitor cells[61]. Terminally differentiated cells, which include smooth muscle cells, epithelial cells and fibroblasts, can enhance the development of the epithelial or muscle layers in the regenerated urethras[96]. Smooth muscle cells and urothelial cells can be obtained from bladder biopsies[96,97]. Epithelial cells can be obtained from foreskin tissue or oral mucosal biopsies[96]. Fibroblasts can be obtained from skin biopsies[96]. Obtaining these cells might require procedures such as bladder, skin or oral biopsies, but some studies have demonstrated successful collection of urothelial cells in voided urine specimens or bladder washes[96,97]. Stem cells have the potential to differentiate into various cell types necessary for urethral regeneration, unlike differentiated cells, and can also enhance angiogenesis[98]. Sources of stem cells include embryonic stem cells, induced pluripotent stem cells and adult stem cells (which include urine-derived stem cells (USCs), bone marrow-derived mesenchymal stem cells (BMSCs), adipose-derived stem cells (ADSCs), human umbilical cord-derived stem cells and urethral stem cells)[96]. Embryonic stem cells have the highest differentiation potential, but ethical concerns regarding the cell sources have limited their use, especially with the advent of induced pluripotent stem cells[99,100]. Induced pluripotent stem cells are adult differentiated cells that are induced to resemble pluripotent stem cells. However, some concerns remain regarding risk of teratoma formation with the use of these cell types[100]. Adult stem cells can be obtained from various sources, including bone marrow and adipose tissue, but are multipotent and only have the ability to differentiate into some cell types[100]. Progenitor cells, such as endothelial progenitor cells (EPCs), are primitive cells that are programmed to differentiate into a specific cell type but are not terminally differentiated[96,100,101]. These cells can be obtained through differentiation of induced pluripotent stem cells or from umbilical cord blood and adult peripheral blood[96]. EPCs are being studied for their role in vascularization of engineered tissue[101].

Terminally differentiated cells. Scaffolds seeded with terminally differentiated cells have shown enhanced urethral regeneration in many studies. In a study in male dogs, urethral defects of >0.5 cm were treated using long, cell-seeded, tubularized urethral constructs. Autologous bladder epithelial and smooth muscle cells were seeded onto 6-cm collagen tubular matrices, which were used for urethroplasty in 15 animals. The results were compared with those in six control animals in which defects were repaired with unseeded constructs. Cell-seeded scaffolds promoted both epithelial and smooth muscle regeneration, whereas only epithelial regeneration was seen with the unseeded control constructs[95]. Similar successes were demonstrated with two-layer engineered urethras generated from epithelial sheets derived from oral mucosa and muscle sheets made of collagen matrices seeded with muscle-derived cells[102] and tubularized acellular bladder matrices seeded with autologous epithelial and smooth muscle cells[103]. In a study in female dogs,

silk fibroin matrices seeded with autologous oral keratinocytes and fibroblasts were used for repair of 5-cm urethral defects in five animals. Retrograde urethrography demonstrated that urethras repaired with acellular silk fibroin matrices developed strictures to varying degrees in all five control animals but showed wide-calibre urethras in all the animals in the experimental group[104]. Denuded amniotic scaffolds seeded with rabbit urethral epithelial cells were used in the repair of 5 × 10-mm urethral defects in male New Zealand white rabbits. Overall, six animals were treated with seeded denuded amniotic scaffold and six control animals were treated with intact, non-seeded human amniotic membrane. Of the six animals in the non-seeded group, one developed a fistula and another developed a serious infection. No complications were seen in the animals receiving the cell-seeded scaffold. Successful neovascularization and smooth muscle formation was seen in the experimental group, suggesting more complete urethral regeneration than in the unseeded group[105]. Differentiated cells are readily available cell sources that can enhance urethral regeneration and can be obtained through typically well-tolerated biopsies.

Stem and progenitor cells. The use of stem cells and progenitor cells in urethral tissue engineering has been shown to modulate the local regenerative environment and promote angiogenesis, which helps reduce the risk of fibrosis and graft failure[98]. SIS were seeded with autologous USCs (which are capable of differentiating into urothelial and smooth muscle cells) obtained using bladder washes[106]. The seeded scaffolds were used to repair surgically generated urethral defects in 12 male New Zealand white rabbits, and the results were compared with those in 12 control animals treated with acellular SIS. Retrograde urethrography and histological analyses were performed at 2, 3, 4 and 12 weeks. Faster urothelial regeneration and higher vessel density were seen in the cell-seeded group than in the acellular group, and no evidence of fibrosis was observed in the cell-seeded group compared with the acellular group[106]. Similar success was observed using human BMSCs combined with $CD34^+$ haematopoietic stem/progenitor cells (HSPCs) in modulating inflammation and wound healing in a rat urethroplasty model[107]. Elastomeric synthetic scaffolds made from poly(1,8-octanediol-co-citric acid) were co-seeded with BMSCs and $CD34^+$ HSPCs and used to repair 6-mm long urethral defects. A reduction in pro-inflammatory cytokine levels and increased neovascularization were observed in the seeded group compared with the unseeded group[107]. Synthetic polymers have also been successfully seeded with ADSCs[108]. ADSCs were seeded on PGA mesh and cultured statically for a week. This construct was then incubated in a bioreactor for 5 weeks, which resulted in the formation of a muscular urethral tube with organized collagen fibres and myoblasts[108]. In a study in male New Zealand white rabbits, 5-mm urethral defects were repaired using ADSC-seeded (12 animals) and unseeded (12 animals) PLA membranes. Normal urethral architecture developed in the animals receiving the seeded membrane but not in those receiving the unseeded membrane, which lacked the smooth muscle layers[109]. In a study in male dogs, 3-cm circumferential urethral defects were repaired using human amniotic membrane scaffolds seeded with allogenic BMSCs plus EPCs, EPCs alone or BMSCs alone, or using unseeded scaffolds (five animals in each group); five animals received a sham operation. Complete epithelial regeneration and vasculature development were observed in the animals receiving scaffolds seeded with BMSCs plus EPCs or with EPCs, in contrast to those receiving scaffolds seeded with BMSCs only or unseeded scaffolds and those receiving a sham operation. Animals repaired using scaffolds seeded with BMSCs only or unseeded scaffolds developed monolayers of epithelial cells at the anastomosis and scar tissue with collagen deposition[110]. Stem cells and progenitor cells seem to promote regeneration and vascularization and might be key in facilitating angiogenesis and prevention of fibrosis from ischaemia in urethral regeneration.

Clinical trials

Since the early 1990s, fewer than 30 clinical trials detailing applications of biomaterials in urethral repair have been conducted[62,63] (Supplementary Table 1), and, of these, only 5 were focused on hypospadias repair[111–115]. In 1990, a graft of autologous urethral epithelial cells mounted on petroleum gauze was first used in hypospadias repair in two patients, both of whom developed small fistulae requiring reintervention[115]. This technique was further developed and tubular grafts of autologous squamous urethral epithelium supported by tubular PTFE (Gore-Tex) were created and used in eight patients with proximal hypospadias. Of these patients, one developed a fistula requiring intervention, and all developed mild stenosis[114]. In 1999, BAM grafts were used for urethral repair in four patients with hypospadias, one of whom developed a fistula[111]. In one study in 2012, six patients with proximal hypospadias were treated using acellular dermis seeded with autologous urothelial cells harvested by bladder washings[112]. These children were followed for a median of 7.25 years and five of the six demonstrated good urinary flow with bell-shaped uroflow curves. One required internal urethrotomy for a partially obstructed flow[112]. A pilot study in 2013 investigated SIS as onlay grafts for hypospadias repair in 12 patients. Of these patients, six had distal hypospadias, four had mid-shaft hypospadias and two had proximal hypospadias. Graft infection occurred in three patients who eventually developed graft failure and presented with complete disruption or with a large fistula with distal stenosis. Small fistulae occurred in three patients. No further intervention was required in 6 of the 12 patients[113]. Amniotic membranes have also been used in proximal hypospadias repair, but the outcomes of these procedures have not been reported[71]. In a study of the use of tissue-engineered urethras, urethral repair was conducted in five children using polyglycolic acid:PLGA scaffolds seeded with autologous smooth muscle and urothelial cells[116]. Normal urethral calibres were observed by 3 months after implantation. Fistula and urinary infections were not seen during 6 years of follow-up. Notably, these children had urethral disruptions secondary to trauma,

and the success in these children may not be generalizable to hypospadias repair[116]. To date, clinical trials on the use of tissue engineered constructs in hypospadias repair are sparse, and interpretation of results is limited by small cohort sizes. Results were promising in some patients, but fistula remains a persistent issue that can potentially be addressed with new advances in urethral tissue engineering.

Importance of the corpus spongiosum

The urethra has been the main focus of many current tissue-engineering studies, but the role of abnormalities of the corpus spongiosum in hypospadias and its importance in hypospadias repair is gaining increased attention. The corpus spongiosum is the spongy tissue that surrounds the urethra and provides mechanical and vascular support[117]. Histological studies have shown that all components of a normal corpus spongiosum are present in hypospadias, but less organized smooth muscle fibres are seen next to the vascular channels[118]. Collagen subtype I is predominant in the corpus spongiosum underlying the urethral plates of patients with hypospadias, suggesting the relative inelastic nature of this tissue[119]. Furthermore, elastosonographic evaluation of normal and hypospadiac penises has shown that patients with hypospadias have a stiffer corpus spongiosum[120]. The quality of the corpus spongiosum has been identified as an indicator of outcomes of surgical hypospadias repair[121]. A prospective study examined the outcomes in 60 adolescents >16 years old and 60 children <5 years old who underwent TIP urethroplasty by a single surgeon for hypospadias repair and found increased complication rates with a poorly developed spongiosum ($P < 0.001$)[121]. These findings led to some studies using acellular corpus spongiosum as scaffolds in urethral regeneration to see if they would provide better structural support for urethral regeneration. Comparison of the mechanical properties of seeded acellular spongiosum matrices (ACSMs) with those of seeded BAM, SIS and PGA showed that ACSMs were more elastic and withstood more stress before breaking; these parameters suggest improved structural support, which might help reduce risk of urethral diverticulum or fistula[122]. Porcine ACSMs seeded with autologous corporal smooth muscle cells and lingual keratinocytes were subsequently used to repair urethral defects in male New Zealand white rabbits[123]. Wide-calibre urethras were seen in the six animals in the experimental group in contrast to the urethras seen in animals repaired with unseeded ACSMs or ACSMs seeded with keratinocytes only (six animals each). Animals in the experimental group exhibited a stratified epithelial layer and smooth muscle bundles in the neourethra after 6 months[123]. Researchers are evaluating the corpus spongiosum to improve understanding of its architecture for application in tissue engineering[117].

Barriers to clinical translation

Tissue-engineered urethral constructs have been slow to transition into the clinical setting. Scientific and logistical barriers are probably reasons for this protracted bench to bedside progression.

Scientific barriers

Multiple scientific barriers remain in the use of tissue-engineered urethral constructs for hypospadias repair. The ideal urethral construct that is biocompatible, offers good structural support, and facilitates angiogenesis and cellular regeneration remains to be elucidated. Also, no surgical hypospadias animal model has been developed, which might preclude translation of preclinical results to the clinical setting.

Finding the ideal construct. Urethral tissue engineering has made slow progress over the past three decades. Preclinical trials have demonstrated variable success, with some showing high fistula and stricture rates[63,84]. These results might be secondary to the combinatory effects of poor angiogenesis (especially in tubular constructs) and heightened inflammatory responses caused by urine leaks[61,124]. These limitations are especially detrimental for finding the ideal urethral construct for proximal hypospadias repair for which long tubular constructs in the setting of abnormal corpus spongiosum is necessary. Urethral tissue-engineered constructs would benefit patients with hypospadias, urethral strictures, urethral disruptions or lichen sclerosis, but the pathophysiological differences between these disease processes mean that the qualities needed for the ideal urethral construct differ for each. Urethral strictures, which can be secondary to trauma or infections, are characterized by fibrosis of a segment of the corpus spongiosum causing constriction of the urethra[125]. The urethra and corpus spongiosum abutting the diseased segment are typically healthy, which is not necessarily the case in hypospadias[118]. In patients with lichen sclerosis who experience urethral strictures and meatal stenosis secondary to a heightened inflammatory response, incorporation of anti-inflammatory factors in the scaffold could be crucial[125]. Mediating the inflammatory response is necessary for hypospadias repair, but those without lichen sclerosis might not require such robust mediators[126]. Most patients with urethral disruption show strictures with localized disease and otherwise healthy wound beds and corpus spongiosum.

Tissue-engineered constructs for hypospadias repair share many features with those used in the aforementioned disease processes. However, proximal hypospadias generally requires repair using longer constructs that are prevascularized or are able to facilitate rapid angiogenesis than isolated short segment strictures. These constructs can often be used in re-do surgical procedures with less than ideal wound beds for vascularization. Furthermore, these constructs must also quickly regenerate a urethral barrier to minimize leaks that would heighten the local inflammatory response. As hypospadias repair is often performed in children, prolonged urinary diversion with catheters is not ideal. Emerging data on the importance of the role of the corpus spongiosum show that further research is also needed to understand the nutrient and structural support provided by the corpus spongiosum and the findings need to be incorporated into new urethral constructs for hypospadias repair.

Animal models. All preclinical studies are performed in animals with surgically created urethral defects and, therefore, might not reflect the anatomical and tissue quality challenges posed by hypospadias repair. These animals have no underlying pathology and have abundant urethral tissue, well-vascularized wound beds and a healthy corpus spongiosum, which is not the case in patients with hypospadias.

Practical barriers. Practical barriers hinder translation of preclinical knowledge to the clinical setting. Generation and use of a seeded construct in the clinical setting requires robust laboratory and clinical support and coordination. Researchers must be able to reliably obtain autologous cells through invasive or noninvasive means. The generated scaffold needs to offer suitable culture conditions to facilitate cellular growth and proliferation while maintaining the qualities of an ideal scaffold, which would be biocompatible and biodegradable, would possess the mechanical properties similar to those of the native organ and could be successfully surgically manipulated. Once the construct is implanted, the local microenvironment needs to be conducive to wound healing with sufficient neovascularization and modulated inflammatory responses. Logistically, the clinical and research team must work closely to coordinate the timing of cell acquisition or derivation, generation of the cell-seeded urethral construct and eventual urethroplasty. This process is time consuming and costly, which could hinder the progress in centres that do not have adequate resources. Once a product has been proven to be effective, it must undergo rigorous regulatory evaluation including safety testing and confirmation of aseptic manufacturing processes before approval and widespread use[127,128]. The costs of these trials could raise the price of these products and affect their future use.

Future directions

Urethral tissue engineering has been studied for many years with increasingly promising results. However, for repair of hypospadias specifically, creation of a long, tubular construct with the ability to facilitate robust angiogenesis and fast regeneration is required. New technologies could improve generation of this type of construct. Amongst the various advances are the use of nanotechnology and 3D bioprinting[4]. Nanotechnology enables researchers to directly influence the cellular microenvironment and influence processes such as vascularization and wound healing[129]. In particular, nanofibres composed of synthetic or natural polymers are effective in altering the microenvironment as modules of drug delivery or sensors for cellular migration[129,130]. Extracellular matrix-mimicking nanofibres have been used to evaluate the spatial and temporal processes of cell emergence onto damaged or organized matrices, which can potentially be used to characterize the process of wound healing in hypospadias repair and identify risk factors for fistula formation[129]. They are also effective in local drug delivery. Nanofibre-coated stents capable of eluting EW-7197, an oral transforming growth factor-β type 1 receptor kinase inhibitor that has antifibrotic properties, were created and evaluated.

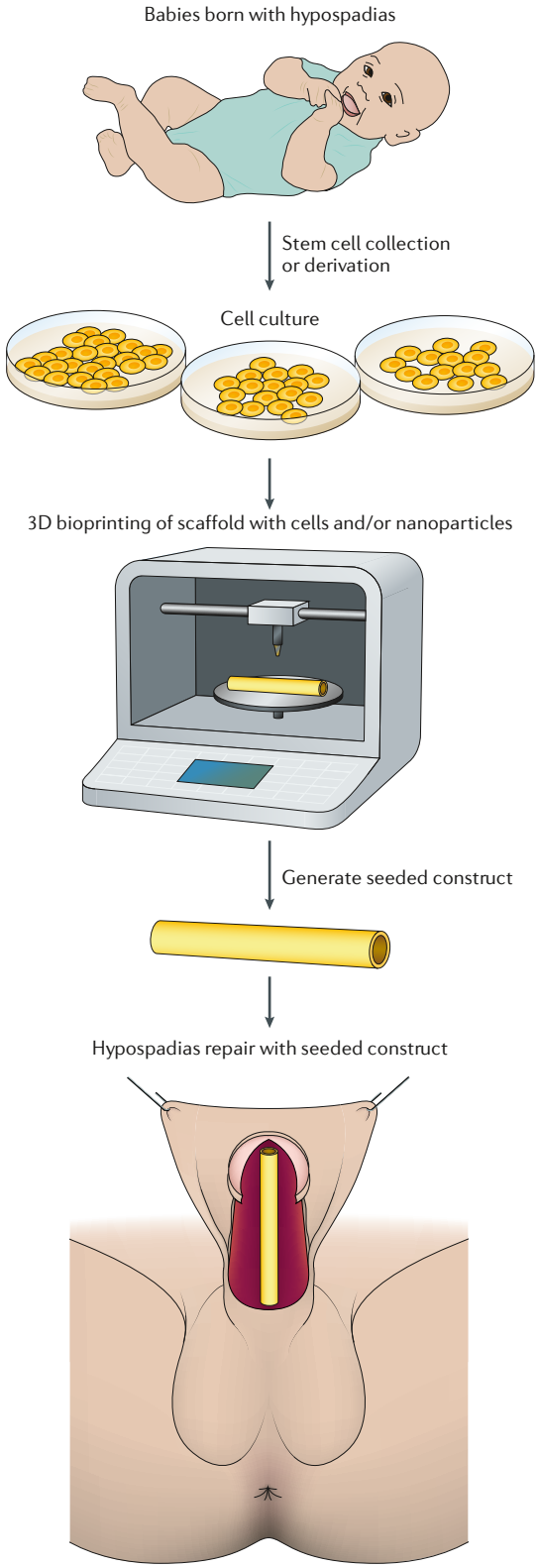

Fig. 3 | **Future applications of 3D bioprinting and nanotechnology in hypospadias repair.** The use of 3D bioprinting and nanoparticles along with cellularization to create urethral constructs for hypospadias repair. Autologous stem cells can be collected, cultured and incorporated into 3D bioprinted urethral constructs tailored to the patient's needs and supplemented with nanoparticles for use in repair.

These stents were placed into the proximal and distal urethras of three male dogs and granulation tissue formation was compared with that in three dogs with control nanofibre-coated stents. An increased mean luminal diameter (8.16 ± 1.46 mm versus 6.51 ± 1.27 mm at 4 weeks) and reduced collagen deposition were observed in the animals receiving the EW-7197 stents[130]. Nanotechnology in the form of peptide amphiphiles could also be used to modulate the local inflammatory response and angiogenesis. Peptide amphiphiles contain a hydrophobic alkyl segment attached to a peptide domain that contains a β-sheet-forming segment[131]. This structure enables the peptide amphiphiles to self-assemble in aqueous environments into nanofibres with high aspect ratio[131]. The peptide domain could be used to deliver signals to the local environment and improved bladder regeneration in a rat model by reducing inflammatory markers and increasing vascularization[131]. Use of this technology to modulate the local environment could help researchers overcome challenges such as poor vascularization and robust inflammatory responses that occur with currently available acellular or seeded scaffolds.

3D bioprinting also has applications in tissue engineering. The use of the now commercially available 3D bioprinter might enable urethral constructs to be printed with specifications tailored to each patient's needs (FIG. 3). Furthermore, the precision and accuracy of the printer might help make cell seeding more uniform than conventional methods. A 3D bioprinter was used to generate a composite polycaprolactone–PLCL spiral scaffold seeded with urothelial cells and smooth muscle cells delivered into the inner and outer layers of the scaffold via cell-laden hydrogels[132]. Cells remained viable after 1 week of printing and the construct's mechanical properties resembled those of a rabbit urethra[132]. This study demonstrated the feasibility of this approach. In the future, 3D bioprinting could help streamline the creation of seeded tubular urethral constructs with the added benefit of patient-tailored designs, increasing the efficiency of generation of these tissue-engineered urethras for clinical applications.

Conclusions

Hypospadias is a complex congenital malformation that remains challenging to manage despite decades of surgical technique modifications and refinements. The lack of tissue with properties similar to those of the urethral plate and the limited supply of usable autologous tissues has led to the development of biomaterials suitable for urethral replacement. These biomaterials include collagen, SIS, silk, amniotic membrane, acellular matrix and synthetic based scaffolds, but they have had limited success. Cellularization has been employed to further enhance the regeneration process. Although these tissue-engineered constructs have shown promising results, stricture and fistula formation still occur and might be a result of poor tissue vascularization, protracted inflammatory responses and fibrosis. Applications of technological advances may help reduce these barriers. The role of and support provided by the corpus spongiosum and the use of hypospadiac animal models still need to be fully elucidated to drive translational relevance. Clinical trials are still lacking, which could be because of the combined effect of scientific barriers, logistical challenges and funding. The development of promising constructs should lead to an increase in funding support to facilitate more clinical trials. This transition from preclinical to clinical settings is necessary to determine whether regeneration at the tissue level truly translates to desirable functional outcomes.

Published online: 05 February 2020

1. Li, Y. et al. Canalization of the urethral plate precedes fusion of the urethral folds during male penile urethral development: the double zipper hypothesis. *J. Urol.* **193**, 1353–1359 (2015).
2. Gong, E. M. & Cheng, E. Y. Current challenges with proximal hypospadias: we have a long way to go. *J. Pediatr. Urol.* **13**, 457–467 (2017).
3. Baskin, L. et al. Development of the human penis and clitoris. *Differentiation* **103**, 74–85 (2018).
4. Shafiee, A. & Atala, A. Tissue engineering: toward a new era of medicine. *Annu. Rev. Med.* **68**, 29–40 (2017).
5. Cunha, G. R., Sinclair, A., Risbridger, G., Hutson, J. & Baskin, L. S. Current understanding of hypospadias: relevance of animal models. *Nat. Rev. Urol.* **12**, 271–280 (2015).
6. Paulozzi, L. J., Erickson, J. D. & Jackson, R. J. Hypospadias trends in two US surveillance systems. *Pediatrics* **100**, 831–834 (1997).
7. Springer, A., van den Heijkant, M. & Baumann, S. Worldwide prevalence of hypospadias. *J. Pediatr. Urol.* **12**, 152.e1–152.e7 (2016).
8. Bergman, J. E. et al. Epidemiology of hypospadias in Europe: a registry-based study. *World J. Urol.* **33**, 2159–2167 (2015).
9. Schnack, T. H. et al. Familial aggregation of hypospadias: a cohort study. *Am. J. Epidemiol.* **167**, 251–256 (2008).
10. Carmichael, S. L. et al. Hypospadias and genes related to genital tubercle and early urethral development. *J. Urol.* **190**, 1884–1892 (2013).
11. Kon, M. et al. Molecular basis of non-syndromic hypospadias: systematic mutation screening and genome-wide copy-number analysis of 62 patients. *Hum. Reprod.* **30**, 499–506 (2015).
12. Bouty, A., Ayers, K. L., Pask, A., Heloury, Y. & Sinclair, A. H. The genetic and environmental factors underlying hypospadias. *Sex. Dev.* **9**, 239–259 (2015).
13. Shih, E. M. & Graham, J. M. Jr. Review of genetic and environmental factors leading to hypospadias. *Eur. J. Med. Genet.* **57**, 453–463 (2014).
14. Dabrowski, E. et al. Proximal hypospadias and a novel WT1 variant: when should genetic testing be considered? *Pediatrics* **141**, S491–S495 (2018).
15. Giordano, F. et al. Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. *Birth Defects Res. A Clin. Mol. Teratol.* **88**, 241–250 (2010).
16. Kalfa, N. et al. Is hypospadias associated with prenatal exposure to endocrine disruptors? A French collaborative controlled study of a cohort of 300 consecutive children without genetic defect. *Eur. Urol.* **68**, 1023–1030 (2015).
17. Klip, H. et al. Hypospadias in sons of women exposed to diethylstilbestrol in utero: a cohort study. *Lancet* **359**, 1102–1107 (2002).
18. Kalfa, N., Paris, F., Soyer-Gobillard, M. O., Daures, J. P. & Sultan, C. Prevalence of hypospadias in grandsons of women exposed to diethylstilbestrol during pregnancy: a multigenerational national cohort study. *Fertil. Steril.* **95**, 2574–2577 (2011).
19. Rodriguez-Pinilla, E. et al. Risk of hypospadias in newborn infants exposed to valproic acid during the first trimester of pregnancy: a case-control study in Spain. *Drug. Saf.* **31**, 537–543 (2008).
20. Jentink, J. et al. Valproic acid monotherapy in pregnancy and major congenital malformations. *N. Engl. J. Med.* **362**, 2185–2193 (2010).
21. Diamanti-Kandarakis, E. et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr. Rev.* **30**, 293–342 (2009).
22. Morales-Suarez-Varela, M. M. et al. Parental occupational exposure to endocrine disrupting chemicals and male genital malformations: a study in the Danish National Birth Cohort study. *Env. Health* **10**, 3 (2011).
23. Estors Sastre, B. et al. Occupational exposure to endocrine-disrupting chemicals and other parental risk factors in hypospadias and cryptorchidism development: a case-control study. *J. Pediatr. Urol.* **15**, 520.e1–520.e8 (2019).
24. Yinon, Y. et al. Hypospadias in males with intrauterine growth restriction due to placental insufficiency: the placental role in the embryogenesis of male external genitalia. *Am. J. Med. Genet. A* **152A**, 75–83 (2010).
25. Hussain, N. et al. Hypospadias and early gestation growth restriction in infants. *Pediatrics* **109**, 473–478 (2002).
26. Glenister, T. W. The origin and fate of the urethral plate in man. *J. Anat.* **88**, 413–425 (1954).
27. Liu, X. et al. Human glans and preputial development. *Differentiation* **103**, 86–99 (2018).
28. Duckett, J. W. in *Adult and Pediatric Urology* 3rd edn (eds Gillenwater, J. Y., Grayhack, J. T., Howards, S. S. & Duckett, J. W.) 2549–2590 (Mosby Year Book, 1996).
29. Hadidi, A. T. in *Hypospadias Surgery* (eds Hadidi, A. T. & Azmy, A. F.) 79–82 (Springer, 2004).
30. Snodgrass, W., Macedo, A., Hoebeke, P. & Mouriquand, P. D. Hypospadias dilemmas: a round table. *J. Pediatr. Urol.* **7**, 145–157 (2011).

31. van der Horst, H. J. & de Wall, L. L. Hypospadias, all there is to know. *Eur. J. Pediatr.* **176**, 435–441 (2017).
32. Keays, M. A. et al. Patient reported outcomes in preoperative and postoperative patients with hypospadias. *J. Urol.* **195**, 1215–1220 (2016).
33. Schlomer, B., Breyer, B., Copp, H., Baskin, L. & DiSandro, M. Do adult men with untreated hypospadias have adverse outcomes? A pilot study using a social media advertised survey. *J. Pediatr. Urol.* **10**, 672–679 (2014).
34. Jaber, J., Kocherov, S., Chertin, L., Farkas, A. & Chertin, B. Voiding patterns of adult patients who underwent hypospadias repair in childhood. *J. Pediatr. Urol.* **13**, 78.e71–78.e75 (2017).
35. Chertin, B. et al. Objective and subjective sexual outcomes in adult patients after hypospadias repair performed in childhood. *J. Urol.* **190**, 1556–1560 (2013).
36. Ortqvist, L. et al. Sexuality and fertility in men with hypospadias; improved outcome. *Andrology* **5**, 286–293 (2017).
37. Asklund, C. et al. Semen quality, reproductive hormones and fertility of men operated for hypospadias. *Int. J. Androl.* **33**, 80–87 (2010).
38. Kumar, S. et al. Fertility potential in adult hypospadias. *J. Clin. Diagn. Res.* **10**, PC01–PC05 (2016).
39. Skarin Nordenvall, A. et al. Psychosocial outcomes in adult men born with hypospadias: a register-based study. *PLoS One* **12**, e0174923 (2017).
40. Ortqvist, L. et al. Psychosocial outcome in adult men born with hypospadias. *J. Pediatr. Urol.* **13**, 79.e1–79.e7 (2017).
41. Hadidi, A. T. History of hypospadias: lost in translation. *J. Pediatr. Surg.* **52**, 211–217 (2017).
42. Baskin, L. S. & Ebbers, M. B. Hypospadias: anatomy, etiology, and technique. *J. Pediatr. Surg.* **41**, 463–472 (2006).
43. Steven, L. et al. Current practice in paediatric hypospadias surgery; a specialist survey. *J. Pediatr. Urol.* **9**, 1126–1130 (2013).
44. American Academy of Pediatrics. Timing of elective surgery on the genitalia of male children with particular reference to the risks, benefits, and psychological effects of surgery and anesthesia. *Pediatrics* **97**, 590–594 (1996).
45. Morrison, C. & Cheng, E. Y. in *Operative Techniques in Plastic Surgery* (eds Chung, K C., et al.) 3041–3051 (Wolters Kluwer, 2019).
46. Subramaniam, R., Spinoit, A. F. & Hoebeke, P. Hypospadias repair: an overview of the actual techniques. *Semin. Plast. Surg.* **25**, 206–212 (2011).
47. Lee, O. T., Durbin-Johnson, B. & Kurzrock, E. A. Predictors of secondary surgery after hypospadias repair: a population based analysis of 5,000 patients. *J. Urol.* **190**, 251–255 (2013).
48. Wilkinson, D. J., Farrelly, P. & Kenny, S. E. Outcomes in distal hypospadias: a systematic review of the Mathieu and tubularized incised plate repairs. *J. Pediatr. Urol.* **8**, 307–312 (2012).
49. Pfistermuller, K. L., McArdle, A. J. & Cuckow, P. M. Meta-analysis of complication rates of the tubularized incised plate (TIP) repair. *J. Pediatr. Urol.* **11**, 54–59 (2015).
50. Hueber, P. A. et al. Long-term functional outcomes of distal hypospadias repair: a single center retrospective comparative study of TIPs, Mathieu and MAGPI. *J. Pediatr. Urol.* **11**, 68 e61–67 (2015).
51. Liang, W. et al. Surgical repair of mid-shaft hypospadias using a transverse preputial island flap and pedicled dartos flap around urethral orifice. *Aesthetic Plast. Surg.* **40**, 535–539 (2016).
52. Ahmed, M. & Alsaid, A. Is combined inner preputial inlay graft with tubularized incised plate in hypospadias repair worth doing? *J. Pediatr. Urol.* **11**, 229.e1–229.e4 (2015).
53. Spinoit, A. F. et al. Grade of hypospadias is the only factor predicting for re-intervention after primary hypospadias repair: a multivariate analysis from a cohort of 474 patients. *J. Pediatr. Urol.* **11**, 70.e1–70.e6 (2015).
54. Pippi Salle, J. L. et al. Proximal hypospadias: a persistent challenge. Single institution outcome analysis of three surgical techniques over a 10-year period. *J. Pediatr. Urol.* **12**, 28.e1–28.e7 (2016).
55. Long, C. J. et al. Intermediate-term followup of proximal hypospadias repair reveals high complication rate. *J. Urol.* **197**, 852–858 (2017).
56. Stanasel, I. et al. Complications following staged hypospadias repair using transposed preputial skin flaps. *J. Urol.* **194**, 512–516 (2015).
57. Tiryaki, S. et al. Unexpected outcome of a modification of bracka repair for proximal hypospadias: high incidence of diverticula with flaps. *J. Pediatr. Urol.* **12**, 395e1–395.e6 (2016).
58. Lanciotti, M. et al. Proximal hypospadias repair with bladder mucosal graft: our 10 years experience. *J. Pediatr. Urol.* **13**, 294.e1–294.e6 (2017).
59. de Kemp, V., de Graaf, P., Fledderus, J. O., Ruud Bosch, J. L. & de Kort, L. M. Tissue engineering for human urethral reconstruction: systematic review of recent literature. *PLoS One* **10**, e0118653 (2015).
60. Howard, D., Buttery, L. D., Shakesheff, K. M. & Roberts, S. J. Tissue engineering: strategies, stem cells and scaffolds. *J. Anat.* **213**, 66–72 (2008).
61. Orabi, H. et al. Tissue engineering of urinary bladder and urethra: advances from bench to patients. *ScientificWorldJournal* **2013**, 154564 (2013).
62. Atala, A. et al. The potential role of tissue-engineered urethral substitution: clinical and preclinical studies. *J. Tissue Eng. Regen. Med.* **11**, 3–19 (2017).
63. Versteegden, L. R. M. et al. Tissue engineering of the urethra: a systematic review and meta-analysis of preclinical and clinical studies. *Eur. Urol.* **72**, 594–606 (2017).
64. Versteegden, L. R. et al. Tubular collagen scaffolds with radial elasticity for hollow organ regeneration. *Acta Biomater.* **52**, 1–8 (2017).
65. Pinnagoda, K. et al. Engineered acellular collagen scaffold for endogenous cell guidance, a novel approach in urethral regeneration. *Acta Biomater.* **43**, 208–217 (2016).
66. Sack, B. S., Mauney, J. R. & Estrada, C. R. Jr. Silk fibroin scaffolds for urologic tissue engineering. *Curr. Urol. Rep.* **17**, 16 (2016).
67. Altman, G. H. et al. Silk-based biomaterials. *Biomaterials* **24**, 401–416 (2003).
68. Chung, Y. G. et al. Acellular bi-layer silk fibroin scaffolds support tissue regeneration in a rabbit model of onlay urethroplasty. *PLoS One* **9**, e91592 (2014).
69. Lv, X. et al. Structural and functional evaluation of oxygenating keratin/silk fibroin scaffold and initial assessment of their potential for urethral tissue engineering. *Biomaterials* **84**, 99–110 (2016).
70. Jerman, U. D., Veranic, P. & Kreft, M. E. Amniotic membrane scaffolds enable the development of tissue-engineered urothelium with molecular and ultrastructural properties comparable to that of native urothelium. *Tissue Eng. Part. C. Methods* **20**, 317–327 (2014).
71. Oottamasathien, S., Hotaling, J. M., Craig, J. R., Myers, J. B. & Brant, W. O. Amniotic therapeutic biomaterials in urology: current and future applications. *Transl Androl. Urol.* **6**, 943–950 (2017).
72. Ramuta, T. Z. & Kreft, M. E. Human amniotic membrane and amniotic membrane-derived cells: how far are we from their use in regenerative and reconstructive urology? *Cell Transpl.* **27**, 77–92 (2018).
73. Shakeri, S. et al. Application of amniotic membrane as xenograft for urethroplasty in rabbit. *Int. Urol. Nephrol.* **41**, 895–901 (2009).
74. Gunes, M. et al. A novel approach to penile augmentation urethroplasty using buccal mucosa and amniotic membrane: a pilot study in a rabbit model. *Urology* **87**, 210–215 (2016).
75. Adamowicz, J. et al. New amniotic membrane based biocomposite for future application in reconstructive urology. *PLoS One* **11**, e0146012 (2016).
76. El-Assmy, A., El-Hamid, M. A. & Hafez, A. T. Urethral replacement: a comparison between small intestinal submucosa grafts and spontaneous regeneration. *BJU Int.* **94**, 1132–1135 (2004).
77. Kawano, P. R. et al. Comparative study between porcine small intestinal submucosa and buccal mucosa in a partial urethra substitution in rabbits. *J. Endourol.* **26**, 427–432 (2012).
78. Huang, J. W. et al. Reconstruction of penile urethra with the 3-dimensional porous bladder acellular matrix in a rabbit model. *Urology* **84**, 1499–1505 (2014).
79. Cao, N. et al. Prevascularized bladder acellular matrix hydrogel/silk fibroin composite scaffolds promote the regeneration of urethra in a rabbit model. *Biomed. Mater.* **14**, 015002 (2018).
80. Chun, S. Y. et al. Urethroplasty using autologous urethral tissue-embedded acellular porcine bladder submucosa matrix grafts for the management of long-segment urethral stricture in a rabbit model. *J. Korean Med. Sci.* **30**, 301–307 (2015).
81. Kajbafzadeh, A. M. et al. The application of tissue-engineered preputial matrix and fibrin sealant for urethral reconstruction in rabbit model. *Int. Urol. Nephrol.* **46**, 1573–1580 (2014).
82. Kajbafzadeh, A. M. et al. Future prospects for human tissue engineered urethra transplantation: decellularization and recellularization-based urethra regeneration. *Ann. Biomed. Eng.* **45**, 1795–1806 (2017).
83. Simoes, I. N. et al. Acellular urethra bioscaffold: decellularization of whole urethras for tissue engineering applications. *Sci. Rep.* **7**, 41934 (2017).
84. Dorin, R. P., Pohl, H. G., De Filippo, R. E., Yoo, J. J. & Atala, A. Tubularized urethral replacement with unseeded matrices: what is the maximum distance for normal tissue regeneration? *World J. Urol.* **26**, 323–326 (2008).
85. Anwar, H., Dave, B. & Seebode, J. J. Replacement of partially resected canine urethra by polytetrafluoro-ethylene. *Urology* **24**, 583–586 (1984).
86. Olsen, L., Bowald, S., Busch, C., Carlsten, J. & Eriksson, I. Urethral reconstruction with a new synthetic absorbable device. An experimental study. *Scand. J. Urol. Nephrol.* **26**, 323–326 (1992).
87. Lv, X. et al. Electrospun poly(L-lactide)/poly(ethylene glycol) scaffolds seeded with human amniotic mesenchymal stem cells for urethral epithelium repair. *Int. J. Mol. Sci.* **17**, 1262 (2016).
88. Sartoneva, R. et al. Characterizing and optimizing poly-L-lactide-co-epsilon-caprolactone membranes for urothelial tissue engineering. *J. R. Soc. Interface* **9**, 3444–3454 (2012).
89. Sartoneva, R. et al. Comparison of a poly-L-lactide-co-epsilon-caprolactone and human amniotic membrane for urothelium tissue engineering applications. *J. R. Soc. Interface* **8**, 671–677 (2011).
90. Jia, W. et al. Urethral tissue regeneration using collagen scaffold modified with collagen binding VEGF in a beagle model. *Biomaterials* **69**, 45–55 (2015).
91. Tang, H. et al. Collagen scaffolds tethered with bFGF promote corpus spongiosum regeneration in a beagle model. *Biomed. Mater.* **13**, 031001 (2018).
92. Nuininga, J. E. et al. Urethral reconstruction of critical defects in rabbits using molecularly defined tubular type I collagen biomatrices: key issues in growth factor addition. *Tissue Eng. Part. A* **16**, 3319–3328 (2010).
93. Lee, K., Silva, E. A. & Mooney, D. J. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. *J. R. Soc. Interface* **8**, 153–170 (2011).
94. Fu, Q. & Cao, Y. L. Tissue engineering and stem cell application of urethroplasty: from bench to bedside. *Urology* **79**, 246–253 (2012).
95. Orabi, H., AbouShwareb, T., Zhang, Y., Yoo, J. J. & Atala, A. Cell-seeded tubularized scaffolds for reconstruction of long urethral defects: a preclinical study. *Eur. Urol.* **63**, 531–538 (2013).
96. Zou, Q. & Fu, Q. Tissue engineering for urinary tract reconstruction and repair: progress and prospect in China. *Asian J. Urol.* **5**, 57–68 (2018).
97. Nagele, U. et al. In vitro investigations of tissue-engineered multilayered urothelium established from bladder washings. *Eur. Urol.* **54**, 1414–1422 (2008).
98. Sharma, A. K. & Cheng, E. Y. Growth factor and small molecule influence on urological tissue regeneration utilizing cell seeded scaffolds. *Adv. Drug. Deliv. Rev.* **82–83**, 86–92 (2015).
99. Davis, N. F. et al. Biomaterials and regenerative medicine in urology. *Adv. Exp. Med. Biol.* **1107**, 189–198 (2018).
100. Panda, A. Stem cell in urology — are we at the cusp of a new era? *Transl Androl. Urol.* **7**, 653–658 (2018).
101. Peters, E. B. Endothelial progenitor cells for the vascularization of engineered tissues. *Tissue Eng. Part. B Rev.* **24**, 1–24 (2018).
102. Mikami, H. et al. Two-layer tissue engineered urethra using oral epithelial and muscle derived cells. *J. Urol.* **187**, 1882–1889 (2012).
103. De Filippo, R. E., Kornitzer, B. S., Yoo, J. J. & Atala, A. Penile urethra replacement with autologous cell-seeded tubularized collagen matrices. *J. Tissue Eng. Regen. Med.* **9**, 257–264 (2015).
104. Xie, M. et al. Tissue-engineered buccal mucosa using silk fibroin matrices for urethral reconstruction in a canine model. *J. Surg. Res.* **188**, 1–7 (2014).
105. Wang, F. et al. Urethral reconstruction with tissue-engineered human amniotic scaffold in rabbit urethral injury models. *Med. Sci. Monit.* **20**, 2430–2438 (2014).
106. Liu, Y. et al. Urethral reconstruction with autologous urine-derived stem cells seeded in three-dimensional porous small intestinal submucosa in a rabbit model. *Stem Cell Res. Ther.* **8**, 63 (2017).

107. Liu, J. S. et al. Bone marrow stem/progenitor cells attenuate the inflammatory milieu following substitution urethroplasty. *Sci. Rep.* **6**, 35638 (2016).
108. Wang, Y., Fu, Q., Zhao, R. Y. & Deng, C. L. Muscular tubes of urethra engineered from adipose-derived stem cells and polyglycolic acid mesh in a bioreactor. *Biotechnol. Lett.* **36**, 1909–1916 (2014).
109. Wang, D. J. et al. Repair of urethral defects with polylactid acid fibrous membrane seeded with adipose-derived stem cells in a rabbit model. *Connect. Tissue Res.* **56**, 434–439 (2015).
110. Chen, C. et al. Transplantation of amniotic scaffold-seeded mesenchymal stem cells and/or endothelial progenitor cells from bone marrow to efficiently repair 3-cm circumferential urethral defect in model dogs. *Tissue Eng. Part. A* **24**, 47–56 (2018).
111. Atala, A., Guzman, L. & Retik, A. B. A novel inert collagen matrix for hypospadias repair. *J. Urol.* **162**, 1148–1151 (1999).
112. Fossum, M., Skikuniene, J., Orrego, A. & Nordenskjold, A. Prepubertal follow-up after hypospadias repair with autologous in vitro cultured urothelial cells. *Acta Paediatr.* **101**, 755–760 (2012).
113. Orabi, H., Safwat, A. S., Shahat, A. & Hammouda, H. M. The use of small intestinal submucosa graft for hypospadias repair: pilot study. *Arab. J. Urol.* **11**, 415–420 (2013).
114. Romagnoli, G., De Luca, M., Faranda, F., Franzi, A. T. & Cancedda, R. One-step treatment of proximal hypospadias by the autologous graft of cultured urethral epithelium. *J. Urol.* **150**, 1204–1207 (1993).
115. Romagnoli, G. et al. Treatment of posterior hypospadias by the autologous graft of cultured urethral epithelium. *N. Engl. J. Med.* **323**, 527–530 (1990).
116. Raya-Rivera, A. et al. Tissue-engineered autologous urethras for patients who need reconstruction: an observational study. *Lancet* **377**, 1175–1182 (2011).
117. Ottenhof, S. R. et al. Architecture of the corpus spongiosum: an anatomical study. *J. Urol.* **196**, 919–925 (2016).
118. Erol, A., Baskin, L. S., Li, Y. W. & Liu, W. H. Anatomical studies of the urethral plate: why preservation of the urethral plate is important in hypospadias repair. *BJU Int.* **85**, 728–734 (2000).
119. Hayashi, Y. et al. Characterization of the urethral plate and the underlying tissue defined by expression of collagen subtypes and microarchitecture in hypospadias. *Int. J. Urol.* **18**, 317–322 (2011).
120. Camoglio, F. S., Bruno, C., Zambaldo, S. & Zampieri, N. Hypospadias anatomy: elastosonographic evaluation of the normal and hypospadic penis. *J. Pediatr. Urol.* **12**, 199.e1–199.e5 (2016).
121. Bhat, A. et al. Comparison of variables affecting the surgical outcomes of tubularized incised plate urethroplasty in adult and pediatric hypospadias. *J. Pediatr. Urol.* **12**, 108.e1–108.e7 (2016).
122. Feng, C. et al. Evaluation of the biocompatibility and mechanical properties of naturally derived and synthetic scaffolds for urethral reconstruction. *J. Biomed. Mater. Res. A* **94**, 317–325 (2010).
123. Feng, C., Xu, Y. M., Fu, Q., Zhu, W. D. & Cui, L. Reconstruction of three-dimensional neourethra using lingual keratinocytes and corporal smooth muscle cells seeded acellular corporal spongiosum. *Tissue Eng. Part. A* **17**, 3011–3019 (2011).
124. Abbas, T. O., Mahdi, E., Hasan, A., AlAnsari, A. & Pennisi, C. P. Current status of tissue engineering in the management of severe hypospadias. *Front. Pediatr.* **5**, 283 (2017).
125. Mundy, A. R. & Andrich, D. E. Urethral strictures. *BJU Int.* **107**, 6–26 (2011).
126. Hofer, M. D. et al. Androgen supplementation in rats increases the inflammatory response and prolongs urethral healing. *Urology* **85**, 691–697 (2015).
127. Ram-Liebig, G. et al. Regulatory challenges for autologous tissue engineered products on their way from bench to bedside in Europe. *Adv. Drug. Deliv. Rev.* **82–83**, 181–191 (2015).
128. Lu, L. et al. Tissue engineered constructs: perspectives on clinical translation. *Ann. Biomed. Eng.* **43**, 796–804 (2015).
129. Sharma, P. et al. Aligned fibers direct collective cell migration to engineer closing and nonclosing wound gaps. *Mol. Biol. Cell* **28**, 2579–2588 (2017).
130. Han, K. et al. EW-7197 eluting nano-fiber covered self-expandable metallic stent to prevent granulation tissue formation in a canine urethral model. *PLoS One* **13**, e0192430 (2018).
131. Bury, M. I. et al. The promotion of functional urinary bladder regeneration using anti-inflammatory nanofibers. *Biomaterials* **35**, 9311–9321 (2014).
132. Zhang, K. et al. 3D bioprinting of urethra with PCL/PLCL blend and dual autologous cells in fibrin hydrogel: an in vitro evaluation of biomimetic mechanical property and cell growth environment. *Acta Biomater.* **50**, 154–164 (2017).

Acknowledgements
The authors thank S. Macomber who contributed to the production of the figures for this article.

Author contributions
Y.Y.C., M.I.B., E.M.Y., M.D.H. and A.K.S. researched data for the article, Y.Y.C., E.Y.C. and A.K.S. made substantial contributions to discussion of content, Y.Y.C. wrote the manuscript and all authors reviewed and edited the article before submission.

Competing interests
The authors declare no competing interests.

Peer review information
Nature Reviews Urology thanks W. Daamen, P. de Graaf and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Supplementary information
Supplementary information is available for this paper at https://doi.org/10.1038/s41585-020-0281-4.