Tissue Engineering of the Urethra—Clinical Applications

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Tissue engineering (TE) is a promising approach for repair/substitution of damaged tissues and organs. Urethral strictures are common and serious health conditions that impair quality of life and may lead to serious organ damage. The TE approach is promising and effective, but many issues remain that need to be addressed for broader adoption of TE in urethral repair. Better design of trials, better cooperation of research groups and centralization could lead to reduction of costs and slowly proceed to commercialization and routine use of TE products for urethral reconstruction.

Keywords: tissue engineering ; urethra ; urethral stricture ; urethral reconstruction

1. Introduction

Tissue engineering (TE) is an interdisciplinary field, which combines elements from biology, material science, medicine and engineering to produce new approaches and therapies for tissue and organ regeneration. It refers to the use of building blocks comprised of cells and scaffolds, either derived from extracellular matrix (ECM) or synthetic materials for tissue repair [1]. Scaffolds are defined as materials that have been engineered to cause desirable cellular interaction. They serve as a support for cells but also provide a biochemical and physical environment similar to native tissue [2].

The urethra is the duct connecting the urinary bladder to the body exterior to produce urine. In males, it is a part of the genital tract as well. Due to the significant differences between male and female urethra, the male urethra consists of functionally and anatomically defined parts (prostatic, membranous, spongious urethra containing the bulbar urethra and the penile urethra) [3][4]. Urethra may be affected by many pathological processes and thus negatively affect the quality of life or even lead to organ impairment. For example, congenital birth defects of urethra, such as hypospadias (1 in every 300 births) [5], and acquired urethral abnormalities, such as urethral strictures (1 in every 1000 men > 65 year of age) are the most common [6]. Urethral strictures are most common among adults and most often occur as a result of scarring, which replaces the vascular tissue of the corpus spongiosum, leading to ischemic spongiofibrosis. Replacement of damaged urethra by scar tissue leads to a reduction of its lumen, with the gradual formation of lower urinary tract obstruction [7].

Treatment of strictures usually involves a surgical procedure such as urethral dilation (UD) or direct vision internal urethrotomy (DVIU). No statistically significant difference in surgical outcome between DVIU and UD was described by Steenkamp et al. [8], but both procedures become less effective with increasing stricture length. Patency rates vary considerably between 8% and 77% after DVIU [9][10].

The most important predictive factor for stricture recurrence is length of stricture. Steenkamp et al. indicated that with each 1 cm increase in the stricture length, the risk of recurrence is increased by 1.22 (95% CI: 1.05–1.43) [8]. In addition, in the systematic review of a case series, a weighted average patency rate was 71.2% vs. 23.2% for strictures less or more than 1 cm, respectively ($p < 0.0001$) [11].

Because of these drawbacks, EAU guidelines recommend not using DVIU/UD as solitary treatment for long (>2 cm) segment strictures. On the other hand, better long-term success rates are associated with an open reconstructive treatment, urethroplasty. These procedures are usually multi-staged interventions, often with the use of buccal or skin autologous grafts or flaps [12].

A systematic review by Mangera et al. [13] showed an average patency rate of 90.5% with the use of all types of grafts for staged penile urethroplasties with an average follow-up of 22.2 months. However, buccal mucosa harvesting is painful and not complication-free (bleeding, postoperative infection, pain, swelling, salivary duct disorders, restricted mouth

opening, scar formation, contracture, loss of sensation due to nerve injury, impairment of mouth opening, smiling, whistling, diet and speech) [14][15][16].

Progress and development in TE has the potential to overcome these limitations. Despite huge progress in pre-clinical settings, clinical application of TE products in urethral repair remains challenging.

2. Tissue Engineering of the Urethra

2.1. Small Intestinal Submucosa Grafts (SIS)

SIS was applied in urethral reconstruction with the length varying from 0.5 up to 10 cm in nine studies. Studies vary greatly in terms of number of patients, site of strictures, follow up period and technique used (**Table 1**).

Table 1. Overview of clinical studies.

Material	Technique	Location	Follow-Up in Months (Mean)	Number of Patients	Results	Ref
collagen-based inert matrix - bladder submucosal graft	dorsal onlay	hypospadias - meatus penoscrotal 3 patients - meatus scrotal 1 patient	22	4	1 patient with subglandular fistula repaired using standard techniques all 4 patients as success	[17]
SIS	dorsal onlay	complete urethral stricture	16	1	100% success	[18]
bladder submucosa collagen based inert matrix	ventral onlay	N/A	37	28	24 patients (86%) success 4 patients slight caliber decrease	[19]
Unseeded SIS	endoscopic urethroplasty	bulbar urethral strictures	24	9	2 patients success (25%) 6 patients as failure 1 lost during follow up	[20]
acellular dermis (AlloDerm) + buccal mucosa	dorsal onlay + buccal mucosa ventral cover	4 cm segment of ventral penile urethra	6	1	100% success	[21]
SIS	dorsal onlay technique	bulbar urethras	18	9	8 patients success (89%)	[22]
SIS	dorsal onlay substitution urethroplasty	2 bulbar stricture 3 penile-bulbar stricture	14	5	1 patient success (20%)	[23]
SIS	onlay urethroplasty	10 patients bulbar urethra 31 patients bulbopenile area 9 patients distal penile urethra	31.2	50	40 patients success (80%)	[24]
SIS	14 patients dorsal inlay, 1 patient ventral onlay 5 patients dorsal onlay plus ventral onlay.	Anterior urethral stricture	21	20	17 cases success (85%)	[25]
in vitro cultured urothelial cells on acellular dermis	onlay	scrotal or perineal hypospadias	52	6	6 cases as success (100%)	[26]
autologous tissue-engineered buccal mucosa	dorsal onlay technique	urethral stricture secondary to to lichen sclerosus	33.6	5	0	[27]

Material	Technique	Location	Follow-Up in Months (Mean)	Number of Patients	Results	Ref
acellular bladder matrix (BAMG) and buccal mucosa	ventral onlay	11 patients bulbar stricture 7 pendulous stricture 12 combined	25	30	2 patients lost during follow-up buccal mucosa 15 (100%) BAMG 10 patients success (66%)	[28]
SIS	SIS endoscopically placed	bulbar urethral stricture	14.25	10	8 patients as success (80%)	[29]
seeded tubularised polyglycolic acid: poly(lactide-co-glycolide acid) scaffolds	urethral tubularised posterior urethroplasty	3 patients posterior urethral disruption 2 patients with previous failed posterior urethral repairs	71	5	100% success	[30]
seeded acellular dermis	ventral onlay	scrotal or perineal hypospadias	87	6	100% success	[31]
SIS	dorsal/ventral or dorsal plus ventral onlay	bulbar strictures (non-obliterative)	71	25	19 (76%) success	[32]
SIS	Augmentation urethroplasty Onlay and inlay technique	8 patients bulbar urethra 9 patients bulbopenile area 10 patients distal penile urethra 1 patient after failed hypospadias repair	24.8	28	24 patients success (85%)	[33]
TE autologous oral mucosa graft MukoCell®	ventral onlay, dorsal onlay, dorsal inlay and combined	penile in 3 (7.9%) cases, bulbar in 29 (76.3%), peno-bulbar in 6 (15.8%)	55	38	32 patients (84.2%) as success	[34]
TE autologous oral mucosa graft MukoCell®	ventral onlay	any etiology, location, length and severity	24	99	success rate 70.8% (46 of 65) and 76.9% (30 of 39)	[35]
acellular TE bovine pericardial patch	dorsal onlay technique	long segment anterior urethral strictures (involving penile and/or bulbar urethra	8	9	8 (88.9%) success	[36]

We found nine studies that used dorsal onlay technique for urethral repair. Patent urethral lumen with no evidence of stricture was used as a criterion for successful procedure in all studies. The first substitution urethroplasty using SIS was performed in 2003. However, this procedure was performed only on one patient with a history of long stricture of penile and bulbar urethra with the follow-up of 16 months. The patient had a satisfactory urodynamic–urine flow rate and the subjective outcome was reported as satisfactory [18]. A few years later, the same surgical technique was chosen in nine patients with 89% success rate [22]. One patient had stricture recurrence due to urinary infection, six patients reported having post-micturition dribbling.

Significantly better results in 50 patients were reported by Fiala et al. (24). Porcine SIS collagen-based matrix was used for bulbar, bulbopenile and the distal penile urethral strictures. Ventral onlay urethroplasty was performed, with a follow-up of 24–36 months; success rate was reported in 80% (40 patients), with no evidence of stricture recurrence. These occurred in the first 6 months postoperatively.

2.2. Bladder-Derived Matrices

Bladder submucosa collagen-based inert matrix as free graft substitute for urethral stricture repair was used for 28 patients. Ventral onlay technique was used in all cases and the mean follow-up was 37 months. A total of 24 patients (86%) were rated as success. A slight caliber decrease at the anastomotic sites on urethrography was reported in four patients. In one case, subcoronal fistula was developed and closed spontaneously 1 year after the procedure [19]. Finally, a randomized comparative study was performed in order to compare acellular bladder matrix (BAMG) and buccal mucosa in 30 patients with urethral strictures. Results showed that BAMG had a 53% success rate, compared to the application of

buccal mucosa graft, which had a 100% success rate. Authors divided these two groups into subgroups of patients with healthy and unhealthy urethral bed. In the subgroup of patients with healthy urethral bed (not undergone prior intervention for urethral stricture) there was a success rate of BAMG 89%. In the subgroup of patients with unhealthy urethral bed there was a success rate of BAMG only 33%. In case of buccal mucosa application, the success rate in both subgroups was 100%. Authors concluded that the use of BAMG is a viable option for urethral repair in patients with a healthy urethral bed and no spongiofibrosis [28].

2.3. Acellular Dermis Graft

Acellular dermis (AlloDerm, LifeCell Technologies, Maharashtra, India) was used only in one patient with severe comorbidities. However, this material was combined with buccal mucosa as staged therapy. During a 6-month follow-up time interval, the patient had no evidence of residual infection. Neourethra was functional and the patient was able to void normally [21].

2.4. Tissue Engineering Approach

Autologous urothelial cells were seeded on the acellular dermis in order to treat scrotal or perineal hypospadias and pronounced chordee [31]. The study involved six patients, urethroscopy and biopsy of the neourethras were performed at 3–4 and 6–8 years postoperatively. All patients could void without straining and urethroscopy showed a well-formed and wide penile urethra without sacculation or diverticula.

The same material was used in five patients who underwent urethroplasty [27]. Excision of entire graft due to scarring was necessary in one patient, partial excision in one patient, and there was stricture recurrence in three patients.

MukoCell® was applied in two studies. One study described using standard techniques such as ventral onlay, dorsal onlay, dorsal inlay and combined with MukoCell® in 38 patients. This study reported 32 patients (84.2%) as success and 6 patients (15.8%) as failure due to the need to undergo further urethral reconstruction [34]. The second study was a multicenter, prospective, monitored non-interventional observational trial [35] which included 99 patients with recurrent urethral strictures. To manufacture the graft, biopsy of oral mucosa was harvested and submucosa was separated and used to establish primary culture of the epithelial cells. Success rates ranged between 85.7% and 0% depending on high or low surgical experience.

3. Summary

While many preclinical studies have been performed, TE is still not used as an alternative treatment in routine clinical practice, except for a select patient group with a history of failed repairs [37][38][39].

Potential advantages of regenerative techniques are now overlooked by several reasons. In summary, these reasons are: (i) quality of clinical studies, (ii) cost and complexity of TE constructs, (iii) regulatory issues and legislation aspects.

For common use of TE in clinical practice, large RCTs must prove superiority or at least non inferiority over conventional treatment. RCT allows valid inferences considering cause and effect of clinical interventions [40]. Without proper RCTs, no direct comparisons with current clinical practice (buccal urethroplasty) can be made. A recent systematic review of urethral TE showed that complex two-stage urethroplasty has complication-free rates and functionality of approximately 62%, 67%, and 36% [41], which is similar to the outcome of TE urethras. This suggests that urethral TE may be a valid alternative for further investigation [42].

One of the important drawbacks of TE for investigation and development is that the trial of a TE construct is not simply a test of a new medicinal product. The development of TE constructs includes trials of a complete process in vitro, in vivo or ex-vivo (construction, extensive testing for cytotoxicity, biodegradability, biomechanics, implantation, follow-up, regenerative effect of the TE product in the patient, analysis, and the final functional outcome) [43]. Moreover, standardization of a surgical intervention is difficult; new TE products may have unique properties for the surgeon who conducts an implantation of the TE product. The surgical technique may be refined and changed over time due to the surgical learning curve. Consequently, in multicenter studies, the differences in skills and experiences of the operating teams may introduce further variation, provided there is not a robust number of patients [44]. This can be clearly seen in a multicenter study [35], where the outcome of the implanted TE constructs clearly depended on surgical experience.

The major disadvantage of the TE approach is the high cost of production and lack of off-the-shelf availability products. These limitations can be (and must be) overcome by continuous scientific developments with industry involvement. As

was mentioned before, high-quality phase 1 and 2 studies are needed with long-term follow up, which should be followed by careful commercialization. Hand in hand, it is necessary to create multidisciplinary high-volume centers with appropriate experience in TE that follow GMP protocols. Establishing dedicated working groups of clinical and biotechnology experts should be the cornerstone of the transition of TE products into clinical practice. The clinical and research teams must work closely to coordinate the timing of cell harvesting, cell-seeding, TE construct maturation (bioreactors) and eventual urethroplasty. These interdisciplinary teams will be able to conduct trials (preclinical and clinical) in an effective way, based on simultaneous, continuous cooperation. This process is time consuming and expensive, but the cost-effectiveness of TE will ultimately improve after broader adoption of this technique. It is necessary to involve industry and financing to facilitate large-scale production of scaffolds and biomaterials based on standardized protocols. Determining the cost-effectiveness of TE products may be very complex because it is not easy to determine what to include in the calculation of costs of treatment. TE construction contains biologically active molecules and/or cells and its behavior in the body is less predictable than that of a medical device with “stable” and predictable properties [45].

When considering cost-effectiveness in urology, it is important to illustrate involvement of industry and urologic surgeons into robotic surgery. Even though robotic surgery is clearly not cost effective with comparable surgical outcomes (in comparison to open and laparoscopic surgery), it is widely popular and robotic centers are growing in numbers [46].

Another reason for slow adoption of TE products into clinical practice can be the extensive culture time required for TE constructs. On the other hand, reconstructive urethral surgeries are usually performed on an elective basis, so this should not be a crucial problem.

Regenerative medicine and TE involve cell therapies, gene therapy and biomedical engineering techniques. That is why a TE product is difficult to define due to the extent and complexity it encompasses. These products are now regulated in specific legislations [47].

Any medicinal product that can be used in the EU market requires registration, assessment and approval by the Committee for Advanced Therapies at the European Medicines Agency. The process requires substantial financial, laboratory and human resources, and such products must undergo meticulous regulatory evaluation such as safety testing and confirmation of GMP before approval and widespread use [48]. By definition, a TE product is categorized as “an advanced therapy medicinal product (ATMP) that contains or consists of engineered cells or tissues and is presented as having properties for, or is used in or administered to human beings with a view to regenerating, repairing or replacing a human tissue” [49]. Consequently, TE products must undergo strict and complex assessment before entering markets to be widely and commonly used in clinical practice. This can also influence scientists and clinicians considering development and testing of TE urethral replacements, especially when appropriate material such as buccal mucosa or skin is readily available.

Urethral TE has made slow progress in clinical practice so far. The TE approach is promising and effective in the management of urethral strictures, but in simple cases and short strictures where local tissues or buccal mucosa are available, this should remain the gold standard. Though significant progress to achieving a safe and reliable TE construct has been made, many issues remain that need to be addressed: better design of trials, namely RCTs, better cooperation of research groups and centralization of AMTEP that could lead to reduction of costs and slowly proceed to commercialization of “off the shelf” products. The development and subsequent approval of a TE product require further significant financial and human resources. So far, research of TE of the urethra has not yet been translated into a clinically available material. 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 TE urethras for clinical applications.

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