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Review

Urothelial or oral mucosa cells for tissue-engineered urethroplasty: A critical revision of the clinical outcome

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Abstract *Objective:* To report the clinical outcome of urethral reconstruction by cultured urothelial or oral mucosa cells for tissue-engineered urethroplasty.
Methods: We systematically searched for studies reporting the use of tissue-engineered techniques for hypospadias and urethral stricture repair in humans in PubMed and Embase (OvidSP) through January, 1990 to June, 2018. We excluded studies based on titles that clearly were not related to the subject, studies in which tissue-engineered biomaterial were used only in laboratory or experimental animals, and in the absence of autologous cultured epithelial cells. Studies were also excluded if they were not published in English, had no disease background and adequate follow-up. Finally, we search all relevant abstract presented at two of the main urological meetings in the last 10 years: European Association of Urology (EAU) and American Urological Association (AUA).
Results: A total of six articles, reporting the clinical use of tissue-engineered techniques in humans, were fully reviewed in our review. The epithelial cells were harvested from the urethra (10 patients), the bladder (11 patients) and the mouth (104 patients). The tissue-engineered grafts were used in children for primary hypospadias repair in 16 cases, and in adults for posterior and anterior urethral strictures repair in 109 cases. Tissue-engineered grafts were showed working better in children for primary hypospadias repair than in adults for urethral strictures repair.
Conclusion: One hundred and twenty-five patients received tissue-engineered urethroplasty using cultured epithelial cells for primary hypospadias or urethral strictures repair. The studies

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demonstrate a high degree of heterogeneity respect to epithelial cells (from urethra, bladder, and mouth), type of scaffold, etiology, site of urethral stricture, number of patients, follow-up and outcomes.

1. Introduction

Tissue engineering (TE) combines the field of cell biology with material science, in order to generate tissues and organs that might be used for regeneration, replacement or reconstruction of human's bodies. Although the goal of TE is to give an answer to unmet clinical needs, so far, poor quality results, unclear in animal models, unrealistic hopes and lack of funds covered the topic. However, in the past 10 years there has been an exponential growth in those therapies, and, despite relatively small number of clinical successes, great optimism and excitement remain about the potential effects or implications.

Such development involved urology since the end of twenty century, when Romagnoli et al. [1], by the first, reported the use of cultured urethral mucosa cells for primary hypospadias repair.

Afterward, it was driven by the plight of patients requiring healthy tissues or organ when conventional reconstruction is unsuitable (*i.e.* bladder reconstruction in congenital diseases) or grafts are not available (*i.e.* urethral reconstruction with oral mucosa graft) [2,3]. Although the gold standard for urethral reconstruction is represented by the use of oral mucosa graft, it is not always possible: Patients refusing mouth graft harvesting, patients with congenital small mouth (*i.e.* Chinese population), patients with small mouth opening for previous trauma or surgery in the mandibular arch, patients requiring bilateral grafts harvesting (it represents a significant predictor of patient dissatisfaction), patients requiring a rectangular graft harvesting for two-stage urethroplasty and patients with recurrent urethral stricture who already undergone previous grafts harvesting form both cheeks [4—6].

At dawn of urethral reconstruction, organ or tissue decellularization was believed to represent a potentially rich source for TE, and some groups investigated its role for serving as bodily conduit or reservoir [7—10]. These early attempts of clinical translation served to highlight critical barriers to progress, such as vascularization, biomechanical proprieties and neuronal regulation. Urological community understood those unmet clinical needs and new clinical experiences emerged in the last years.

The aim of this paper is to make a "clinical" revision of outcomes of the TE urethroplasty in humans.

2. Materials and methods

We systematically searched for studies reporting the use of TE techniques for hypospadias and urethral stricture repair in humans in PubMed and Embase (OvidSP) through January, 1990 to June, 2018. We excluded studies based on titles that clearly were not related to the subject, studies in which TE biomaterial were used only in laboratory or experimental animals, and in the absence of autologous cultured epithelial cells. Studies were also excluded if they were not published in English text, had no disease background and adequate follow-up. Finally, we search all relevant abstract presented during two of main urological meetings in the last 10 years: European Association of Urology (EAU) and American Urological Association (AUA).

A total of six articles, reporting the clinical use of TE techniques in humans, were fully reviewed. The studies demonstrate a high degree of heterogeneity respect to epithelial cells (from urethra, bladder, and mouth), type of scaffold, aetiology, site of urethral stricture, number of patients and follow-up. The epithelial cells were harvested from the urethra in 10 patients [1,11], from the bladder in 11 patients [12,13], and from the mouth in 104 patients [14,15]. The TE grafts were used in children for primary hypospadias repair in 16 cases [1,11,13], and in adults for posterior and anterior urethral strictures repair in 109 cases [12,14,15].

3. Results

3.1. Urethral or oral mucosa stem cells for urethral regeneration?

In our review, we found some studies suggesting the use of urethral mucosa and while other studies used the oral mucosa as source of stem cells for cultures. Corradini et al. [16] investigated the differences between urethral and oral stem cells for urethral regeneration. In their study, 19 biopsies from urethra and 21 from oral mucosa were obtained from patients, during reconstructive surgery, in order to determine whether urethra or oral mucosa can be equally useful for urethra engineering, making a comparison of clonogenic ability, proliferative potential and stem cell markers [16]. Urethral and oral mucosas were removed from the same donor, and their cells were used for developing primary cultures and cell characterization. Furthermore authors investigated *in vitro* long-term regenerative properties of both tissues by life span, clonal analysis and markers of different clonal types [16]. The results revealed the same high proliferative potential for urethra and oral mucosa cultures with maintenance of specific markers; karyotype and growth factor dependence confirmed the normal phenotype of cultured cells [16]. Clonal analysis of the proliferative compartment highlighted a very different proportion of stem and transient amplifying cells, characterized by

dissimilar cell size profile and marker expression. Corradini et al. [16] concluded that both tissues can be cultured and preserve their stem cells *in vitro*, suggesting that they can be equally useful for TE of the urethral tract, even if few limited differences appeared between oral mucosa and urethra.

3.2. Urethral reconstruction using urethral mucosa cells

In 1990 Romagnoli et al. [1] were the first authors reporting the urethral reconstruction by using TE autologous graft of cultured urethral epithelium, in two children with primary hypospadias. A small biopsy specimen of urethral mucosa was taken from external urethral meatus, and treated with trypsin to produce a suspension of single cells, seeded in dishes and cultured [1]. The cultured epithelium was extended into the penile shaft as first-stage urethroplasty and 10 days later was tabularized up to glans [1]. The authors reported that the two patients developed urethral fistula that was closed using the standard technique and at 6 and 18 month's follow-ups the patients showed normal urinary and erectile function.

In 1993, the same authors described the manufacturing of cultured urethral mucosa cells mounted on polytetrafluoroethylene tube and used for one-stage anastomotic urethroplasty in eight boys with hypospadias [11]. Patients were discharged from the hospital 10 days postoperatively after removal of catheter; the polytetrafluoroethylene tube was removed 20 days postoperatively [11]. All patients underwent periodically endoscopic examination up to 2 years postoperatively. One patient developed urethral fistula, which was closed by standard surgical technique; all the patients developed meatal stenosis requiring dilation. The authors did not report the long-term outcomes of these eight patients [11].

3.3. Urethral reconstruction using muscle and epithelial cells from the bladder

In 2011 Raya-Rivera et al. [12] reported the urethral reconstruction by using TE bladder muscle and epithelial autologous cells, in five boys with complex poster urethral strictures. A suprapubic incision was made, a bladder biopsy was taken and primary cultures of smooth bladder muscle and urothelial cells were collected [12]. Epithelial cells were seeded onto the luminal surface and muscle cells onto the outer surface of tubular collagen scaffold. A polyglycolic acid biodegradable mesh was tabularized and sized according to the stricture length. It was sutured to the distal and proximal urethral ends in anastomotic fashion [12]. At median follow-up of 71 months, the authors reported a success in all five patients [12]. Fossum et al. [13] reported the urethral reconstruction in six patients with scrotal or perineal hypospadias using cultured autologous urothelial cell transplants. The urothelial cells were harvested by bladder washing; the same authors described the original technique in 2003 [17]. With a median follow-up of 7.25 years, all patients were classified as success with good cosmetic appearance and functional outcome [13].

3.4. Urethral reconstruction using oral mucosa cells

In 2008, Bhargava et al. [14] were the first authors reporting the urethral reconstruction by using TE autologous graft of cultured oral mucosa cells, in five patients with urethral strictures associated with genital lichen sclerosus. Oral mucosa biopsies were obtained from each patient and keratinocytes and fibroblasts were isolated and cultured, seeded onto sterilized donor de-epidermised dermis, and maintained at air-liquid interface for 7—10 days to obtain full-thickness grafts [14]. These TE grafts were used for one-stage (two cases) or two-stage (three cases) anterior urethroplasty [14]. At mean follow-up of 33.6 months, one patient required complete excision of the grafted area and one patient partial graft excision for fibrosis and hyper proliferation of tissue [14]. Furthermore, three patients required some forms of postoperatively instrumentation (urethrotomy or dilation) [14]. In 2011, these authors suggested a pretreatment of the de-epidermised acellular derma scaffold with the use of glutaraldehyde and β-aminopropionitrile, to reduce the contraction of TE oral grafts [18].

In 2014 Lazzeri et al. [19] reported the preclinical and clinical examination of TE graft of autologous oral mucosa (MukoCell®) for urethral reconstruction. The main aim of their work was about the TE graft safety. Oral mucosa cells were generated from a small oral mucosa biopsy and cultured on the surface of biocompatible scaffold [19]. Evaluation of tumorigenic study in nude mice did not reveal macroscopic and microscopic malignancies attributable to MukoCell® in different examined tissues and organs. Migration of transplanted cells into distant organs was excluded and the grafts were degraded 40 days after implantation in the majority of animals [19]. Preliminary results about 70 patients demonstrated no peri- or postoperative adverse events related to TE grafts [19].

In 2015 the same group of authors reported the legal framework, the manufacturing procedure, pharmacology, pharmacokinetic, toxicology and clinical development of this TE oral mucosa graft [20]. Twenty-one patients were included in this study. Stricture site was bulbar in 18 (85.7%) cases, and peno-bulbar in three (14.3%) cases. The mean stricture length was 5.5 cm (range 2—8 cm) with a median follow-up of 18 months (range 13—22 months) [20]. Out of 21 patients, 17 (80.9%) were classified as success, and four (19.1%) as failures. No expected or unexpected adverse reaction related to the implant (MukoCell®) was reported [20].

In 2017 the same group of authors, published a multicenter, prospective, monitored observational trial on the results of TE autologous oral mucosa graft for urethral reconstruction in 99 patients with urethral stricture of any etiology, severity location and length (real-world data) [15]. The primary and secondary outcomes were success rate and safety at 12 and 24 months postoperatively [15]. Twelve and 24 months' success rates for evaluable patients in all centers were 70.8% and 76.9% respectively. The success rates ranged 0%—85.7% in case of low or high surgical experience in urethral surgery [15].

In 2018, Barbagli et al. [21] reported the surgical techniques and long-term results used for the implant of TE oral mucosa graft in 38 patients [21]. Out of 38 patients, 32 (84.2%) were classified as success, and six (15.8%) as

failures. The ventral onlay technique showed 85.7% success rate, the dorsal onlay 83.3%, the dorsal inlay 80% and the combination of them 100%. The bulbar urethroplasty technique showed 93.1% success rate, the penile 66.7% and the peno-bulbar 50%. Table 1 summarizes studies reporting the clinical outcomes of different materials used for a TE urethroplasty.

4. Discussion

Cossu et al. [22] showed that the use of TE urethral reconstruction might represent a safe and potentially effective opportunity for patients. However, the current survey showed the limits and controversies in the topic of TE urethral reconstruction as well. We presented studies suggesting the use of urethral or bladder mucosa for manufacturing the TE graft and other studies suggesting the use of oral mucosa. Corradini et al. [16] characterized tissue cultures of the urethra and oral mucosa and proved that they were equally useful for TE of the urethral tract. One of the main challenges of such studies is to address technical details. Corradini et al. [16] focused on clonogenic ability and proliferative potential and found by clonal analysis of stem and transient amplifying (TA) cells that both clones of urethral and oral stem cells differ in size and have different cell size from respective transiently proliferating cells. These data represent one of the highest quality in the field of TE urethral reconstruction. Stem cells were identified by nuclear expression of p63 alpha transcription factor and B cell-specific moloney murine leukemia virus integration site 1 confirming their proliferative potential and related epithelial signature. Safety of culture process was proven by the regulation of p63 expression in clonal conversion, the capability to undergo replicative senescence, and maintenance of normal karyotype. Moreover, the adhesion dependence reassured with regards to the absence of growth, in case of migration in the bloodstream [16]. From a clinical point of view, oral mucosa is easily accessible in any patient and biopsy under simple local anesthesia is easy, non-invasive and painless for patient. Urethral mucosa is more difficult to access and may be painful.

It was interesting to note that, in our survey, TE grafts showed better when used in children for primary hypospadias repair [1,11,13] than in adults for urethral strictures repair [12,14,15]. Unfortunately, no more largest series of children treated using these techniques are available now, and no any evolution over time of these techniques and outcomes updating are described and this represents a strong limit of these studies. In our survey there was also a great difference between urethral and oral mucosa for TE graft arrangement. The epithelial cells were harvested from the urothelium in 22 patients [1,11–13], and from the oral mucosa in 104 patients [14,15].

Atala [23] reported that: "Although the field of TE medicine continues to expand and progress, additional challenges remain such as cost, patient selection, regulatory, and financial issues". Some authors suggested the use of TE oral mucosa graft in patients with urethral strictures associated with genital lichen sclerosus, reporting high failure rates [14,18]. Although the involvement of urethra in genital lichen sclerosus represents a pathological serious problem and any type of urethroplasty shows high failure rate [24,25], the results of this study may have been not promising enough for further investigations. Adequate patient selection represents the first step in introducing new TE technologies in surgery to better evaluate the real efficacy of material.

We would like to emphasize the important limits in some articles we reviewed in the present study. The majority of these studies reported only occasional and anecdotal report of their techniques and outcomes [1,11,12,14]. Some of the TE techniques are employed in a small series of patients, and also when the results are encouraging, the authors never extend the use of these techniques in larger

Table 1 The main data of articles reporting outcomes of tissue-engineered urethroplasty using cultured epithelial cells in humans.

Reference in the text	Journal year	Type of cells	Site of original cells	No. of patients	Urethral Pathology	Site of strictures	Success rate (%)	Mean follow-up
Romagnoli et al. [1]	New Engl J Med 1990	Urethral	External urinary meatus	2	Hypospadias		100	18 months
Romagnoli et al. [11]	J Urol 1993	Urethral	External urinary meatus	8	Hypospadias		100	18 months
Raya-Rivera et al. [12]	Lancet 2011	Bladder urothelium	Bladder	5	Traumatic strictures	Posterior urethra	90	71 months
Fossum et al. [13]	Acta Paediatrica 2012	Bladder urothelium	Bladder	6	Hypospadias		100	7.25 years
Bhargava et al. [14]	Eur Urol 2008	Oral mucosa	Mouth	5	Lichen sclerosus strictures	Anterior urethra	0	33.6 months
Ram-Liebig et al. [15]	EBioMedicine 2017	Oral mucosa	Mouth	99	Urethral strictures	Anterior urethra	85	24 months

series of patients [12]. Additional important challenges remain such as cost, regulatory, legal and financial issues as documented by some authors [3,20]. A further limitation of our paper is the re-publication of results from the same pool of cases.

Although TE products might have a high impact on patients' health, only a few of them will be approved. TE techniques are complex and require a high level of specialized laboratories. Although the price of these products may be considered in an acceptable range of few thousand euros, it could be even lower. As one of factors, determining the final price depends on clinical trial costs, and some considerations have to be done. In our opinion, due to the specify of these products and their health benefit, it would be advantageous to reconsider their regulatory requirements. The simplification of the latter would allow the acceleration of the access of these products into the market, a faster availability for the patients and a decrease in their costs and their price, making their reimbursement less challenging for public health insurances in different countries.

According to the controversial data we collect in our review, we hope that, in the future, TE urethral reconstruction studies should comply with the following characteristics: Preclinical and clinical examination with regard to its safety; manufacturing of the graft should be simple and easily reproducible in any country; adequate selection of patients; increasing number of patients; long-term follow-up.

5. Conclusion

The use of TE oral mucosa represent, today, a real, safe and efficient opportunity for our patients with urethral stricture diseases. However, our survey also showed that still many limits and controversies remain in the topic of TE urethral reconstruction as reported in the current literature. At present, cost, regulatory, legal and financial issues represent important factors that restrict and slow down the wide use of these technologies in many countries.

Author's contribution

Study design: Guido Barbagli, Massimo Lazzeri.
Data acquisition: Axel Heidenreich, Vahudin Zugor, Leonidas Karapanos.
Data analysis: Massimo Lazzeri.
Drafting of manuscript: Guido Barbagli.
Critical revision of the manuscript: Massimo Lazzeri.

Conflicts of interest

Guido Barbagli is an advisor for UroTiss Europe. The other authors declare no competing interests.

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