



## Review – Reconstructive Urology

# Tissue Engineering for the Lower Urinary Tract: A Review of a State of the Art Approach

Karl-Dietrich Sievert\*, Bastian Amend, Arnulf Stenzl

The Department of Urology, University of Tuebingen, Tuebingen, Germany

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### Abstract

**Objectives:** Tissue engineering (TE) has become synonymous with physiological and functional reconstructive approaches in medicine. Although the goals of TE are ambitious and have not yet been attained, significant milestones have been achieved and future possibilities are great. To examine these possibilities with a special emphasis on the lower urinary tract, we provide a review of the development of TE techniques and a high-level overview of related regulatory and legal issues.

**Methods:** Current trends in the field of TE, including the use of stem cells, scaffold optimization, and acellular tissue and growth factors, were reviewed and critically assessed through a comprehensive literature review using the PubMed database. Because of the rapid development of new TE approaches, recent abstracts from international urology conventions were included. A review of 2007 European Medicines Agency and Commission for Advanced Therapies legal regulations was also performed.

**Results:** Although several clinical TE approaches have been developed, most lack objective validation. A variety of TE techniques are currently under development or investigation, but thus far, no one approach is clearly superior on the basis of significant long-term studies. A medical product based on TE and stem cells can be successfully developed only with careful consideration of legal and ethical regulations.

**Conclusions:** TE holds the promise for a tremendous impact on reconstructive urology. However, research must be focused and intensified for the full potential clinical benefits to be made widely available. Because the product development is affected by legal regulations, consensus must be achieved.

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\* Corresponding author. Department of Urology, University of Tuebingen, Hoppe-Seyler-Str 3, D-72076 Tuebingen. Tel. +49 7071 298 4081; Fax: +49 7071 295092. E-mail address: [Karl.Sievert@med.uni-tuebingen.de](mailto:Karl.Sievert@med.uni-tuebingen.de) (K.-D. Sievert).

## 1. Introduction: from the enthusiastic start of tissue engineering to the mandated regulatory boundaries

During the last decade, reconstructive urology improved surgical outcomes through the use of specialized surgical equipment and the primary use of autologous or allogenic tissue. Tissue engineering (TE), with its subspecialty of stem cells, aims to treat and regenerate urological structures and organs so that full physiological function is restored [1].

The success of cell cultures depends on cell isolation, separation, and selection, as well as optimized conditions for the proliferation of single cell types [2]. In recent years, important developments such as fluorescence-activated cell sorting (FACS), immunomagnetic bead sorting, and magnetic-activated cell sorting (MACS<sup>®</sup>) enabled the selection of specific cell types [3]. Cell culturing is tailored to each individual cell type, and if stem cells are used, their differentiation to the targeted cell type is required. Biomaterials should be biocompatible, eliciting no host response, and biodegradable within a certain time after the seeded cells have built their own extracellular scaffold to replace the biomatrix. Matrices can be artificially produced or made as an acellular scaffold from tissue. Additional growth factors support the early phase of regeneration, which might also be enhanced by genetic engineering [4–8].

Probably the most exciting and controversial area of TE is stem cells. Initially, embryonic stem cells were used due to their totipotency. However, because of their source, their use has raised moral and ethical concerns. Today, pluripotent stem cells, or differentiable adult stem cells, can be separated from many different tissues, including bone marrow [5,9,10], striated muscle [11,12], fat [13], skin [14,15], synovial membrane [16], and, more recently, testicles [17,18] and amniotic fluid [19]. They can then be differentiated into a variety of cells. In addition to resolving all these specific basic research aspects of TE, legal discussions must support the movement of TE from theory to application in clinical trials and in standard “manufacturing” procedures.

Thus, the optimism of the “gold rush” in the final years of the 20th century, when tissue-engineered artificial bladder, kidney, and penis were announced to be within a few years’ reach, has subsided and given way to a more sobering and pragmatic view. This review evaluates the current state of the art in the field of TE and considers the near future, incorporates legal circumstances, and outlines realistic expectations.

## 2. Methods

This review is based on a search of the PubMed database of the National Center of Biotechnology Information and recently published presentations at international urology conventions for literature addressing current developments in TE and stem cells. The identified literature was critically reviewed.

The literature analysis is accompanied by a comparative discussion of the current primary trends in TE basic research followed by clinical trials, with consideration of legal and ethical issues and the integration of good medical practices (GMP) of the European Medicines Agency (EMA) and the Committee for Advanced Therapy (CAT).

## 3. Results

Between January 2004 and June 2007, 347 publications addressing either TE or stem cells for the reconstruction of the urinary tract were published, one third of which were reviews (Table 1). In 2006 and 2007, respectively, there were marked increases in the number of TE and stem cell abstracts accepted for presentation at the three key urology conventions: the European Urology Association accepted 14 and 21 abstracts, the American Urology Association 9 and 32, and the DGU (Deutschen Gesellschaft für Urologie), 7 and 11.

The main goals underlying the most current TE research remains the determination of the optimum scaffold that can be seeded by cells, the best source of stem cells, and the optimal way to differentiate stem cells in urological reconstruction and regeneration. Several hundred companies are approaching the field of TE or already selling TE medical products. Research and practice needs to bear in mind the increasing regulations of the EMA, CAT, and the United States Food and Drug Administration, depending on the confederation or country.

### 3.1. Organ-specific reconstruction

#### 3.1.1. Urothelium

TE urothelium can play a key role in reconstructive urology involving urethral reconstruction (eg, repair of urethral structures, fistula, or hypospadias) and bladder reconstruction (eg, bladder exstrophy). Because no equal substitute for urothelium exists, buccal mucosa was evaluated and found to be the most adequate substitute in urethral reconstruction [20]. TE has the potential to minimize surgery time and improve the quality of repair by identifying a new source of urothelium for reconstruction through the seeding of stem cells on small intestine submucosa (SIS) (as an acellular tissue), the omentum [21], or artificial scaffolds [22,23]. Beyond these successes, a functional multilayer urothelial sheath

**Table 1 – PubMed analysis: January 2004–February 2007**

Keywords	Articles	Reconstructive urology	Reviews	Basic research
Tissue engineering				
Urology	17	16	16	0
Urothelium	26	26	3	23
Urinary tract	113	95	23	72
Bladder	89	81	22	59
Urethra	17	17	8	9
Sphincter	5	4	3	1
Erectile dysfunction	6	5	4	1
Penis	16	8	7	1
Stem cell				
Urology	6	5	5	0
Urothelium	21	15	0	15
Lower urinary tract	34	6	0	6
Bladder	83	30	6	24
Urethra	13	9	5	4
Sphincter	7	7	3	4
Erectile dysfunction	15	12	3	9
Penis	26	11	3	8
Total		347	111	236

was recently cultivated in vitro from bladder wash-separated urothelium cells [24]. It was further demonstrated from a bladder wash that progenitor cells could be isolated, grown, and expanded to urothelium and smooth muscle cells [25].

### 3.1.2. Bladder

The generation of a complete bladder wall requires not only a multilayer urethral scaffold, but also vascularization and innervation of the united smooth muscle structure to be regenerated. The addition of growth factors (nerve growth factor in combination with vascular endothelial growth factor, for example) might enhance the regeneration of acellular matrix [26]. Further development of scaffolds and acellular matrices has made the use of stem cells even more successful. Although SIS is available for reconstructive surgery, it has not yet been verified how many layers are required for successful regeneration, whereas it has been demonstrated that commercially available SIS can suppress the growth of urothelium in vitro [27]. Seeding of scaffolds with stem cells might help to generate a bladder wall [28], as confirmed by Ram-Liebig et al [29], which might result in an even better bladder wall regeneration when omnipotent stem cells are used [19,23,30,31]. However, it has not yet been determined which kind of scaffold might best support regeneration without a prolonged phase of regeneration or even extensive fibrous tissue [32]. One of the most recent and exciting publications reported the use of tissue-engineered autologous bladder tissue for reconstruction in humans [33]. Although this study demonstrates the potential

of scaffolds seeded with tissue-engineered cells, extensive work is needed to verify which setting best supports the clinical goal.

### 3.1.3. Urethra

Urethral reconstructive surgery is changing rapidly. New techniques and engineered materials are a part of our future. In 2003, Weiser et al [34] first reported single-layer SIS as an effective and safe commercially available material for the two-stage repair of different types of hypospadias. Despite the limited experience with SIS in urethral reconstruction, some studies have reported a high success rate of 80–85% [35,36]; however, others such as Hauser et al [37] reported failures in 4 of 5 patients. In our own experience, failure occurred ventrally when SIS was used as a single layer in 6 of 10 adult patients; however, the outcome was functionally successful in our limited experience with children [38].

Ribeiro-Filho et al [39] successfully used organ-specific acellular tissue in seven patients with a clinical follow-up of 5–30 mo, confirming the animal model of Sievert et al [40,41]. Further, acellular bladder matrix was used for urethral reconstruction in complex urethral strictures with a success rate of 80% with a mean long-term follow-up of 25 mo [42,43]. A new approach in humans, presented by Bhargava et al [44], uses tissue-engineered buccal mucosa; this approach might reduce the need for extended buccal mucosa harvesting. For further information regarding scaffolds, see section 3.2.2.

In addition to the use of (un)seeded acellular scaffold, Nagele et al [45] demonstrated that tissue-engineered urothelium might help to further improve

outcomes in urethral stricture repair. Still in the experimental stage, this same group improved their culture media, thus increasing the success rate of multilayer urothelium to 76% [46]. The effectiveness of this approach was verified with the use of a new marker, p63 [47].

#### 3.1.4. Urethral sphincter

In reconstructive urology, stress urinary incontinence probably draws the most public attention. The functional outcome is supposed to be improved with injection of muscle precursor cells [48]. The use of progenitor stem cells might create the opportunity to restore the loss of sphincteric function [49]. Myogenic stem cells are another possible source; in the rat model, these cells survived and remained integrated into the muscular structure for more than 120 d [50]. Application of myogenic stem cells functionality was investigated in the pig model and demonstrated a dependence on the amount of applied cells [51], whereas administration of muscle-derived cells alone increased the contractility of the urethral sphincter strips in the organ bath [52].

In 2004, Strasser et al [53] reported a cure rate of 83% in 42 patients following the application of myoblasts mixed with collagen into the rhabdosphincter. Between September 2002 and March of 2005, these authors [54] also treated 184 stress urinary incontinent patients with a cure rate of 79.4% by injecting fibroblasts mixed with 2.5 ml collagen submucosally and previously cultivated myoblasts into the rhabdosphincter. In a phase I/II clinical trial [55] in 32 women with stress urinary incontinence, a 50% symptom improvement was seen in 83% of patients following periurethral injection of human cord blood stem cells.

In a recently published comparison of autologous myoblasts and fibroblasts versus collagen as a treatment for stress urinary incontinence, 90.5% and 9.5% of patients, respectively, were cured (dry) 12 mo after the treatment [56]. As Novara and Artibani [57] commented "...some doubts might be raised about allocation concealment and ascertainment bias, as suggested by some statistically significant differences between baseline characteristics in the two arms. Further re-evaluation with a longer follow-up is needed and multi-institutional studies are highly recommended. If the data is confirmed, this approach is likely to cause a substantial change in the treatment of female stress urinary incontinence."

#### 3.1.5. Penis

In the reconstructive field, the search for a shelf-storable material for treatment of penile deviation

continues. Begemann et al [58] found no significant difference between dermis, fascia, or SIS for repair of Peyronie's disease. Other studies have reported a similar success rate but demonstrated that it is less favorable compared with vein graft [59]. The primary advantage of SIS using Surgisis<sup>®</sup> is availability and minimization of surgery. However, long-term positive outcome and proof that no DNA fragments have been transmitted are still unconfirmed.

#### 3.1.6. Erectile dysfunction

In the treatment of erectile dysfunction, the use of mesenchymal stem cells transduced with adenovirus containing endothelial nitric oxide synthase (eNOS), in particular, seems to be a future option because of the lack of eNOS-protein, NOS activity, and cyclic guanosine monophosphate levels in this condition [60]. Bakircioglu et al [61] reported successful treatment of erectile dysfunction by intracavernous injection of adeno-associated virus-brain-derived neurotrophic factor, which was recently patented and will soon be evaluated in a clinical trial. Kwon et al [62] reported the possibility of autologous tissue-engineered penile corpora cavernosa replacement with smooth muscle and endothelial cells in the rabbit model.

May et al [63] used artificial nerve guides, especially biodegradable ones containing growth-promoting factors or cells for the repair of cavernous nerve lesions to reestablish erectile function. A novel way to track follow nerve regeneration may be to label cells with superparamagnetic iron oxide, even during long-term follow-up [64].

### 3.2. Cell sources and seeding matrices

#### 3.2.1. New stem cell sources

New sources of stem cells are currently under investigation. Renninger et al [18] used spermatogonial stem cells for tissue regeneration and reported their differentiation into the three human germ layers, which might become a stem cell source for the adult male. Amniotic fluid mesenchymal stem cells might represent another potential new stem cell source for allogenic [65] or autologous use either in infants or, after storage, in adults.

#### 3.2.2. Scaffolds

Scaffolds may use not only artificial materials but also acellular tissue or a combination of the two; the latter approaches have been used in small trials for specific indications without any cell seeding [66]. In urological research, outcomes have ranged from the inhibition of cell proliferation in vitro [27] to successful use in clinical applications [67]. Compared with

the easily available porcine SIS, the “self produced” SIS seems to have higher potential in three-dimensional tissue regeneration [68].

A combination of collagen-based acellular matrix and polyglycolic acid polymers might even further improve tissue regeneration [69]. In addition, other materials, such as fibrinogen-polydioxanone, might support more rapid regeneration [70]. Major reviews of urinary bladder TE based on acellular tissue were published by Atala and Korossis et al [71,72].

3.3. *Legislative environment (medical device or product)*

Tissue engineering is an emerging technology, which, in many areas and for most indications, must still be considered experimental. In contrast to the technique of intracytoplasmic sperm injection, which surprised the medical world with its use in a legally unregulated environment [73], tissue engineering entered the market with legal regulation and patient safety guidelines already firmly in place. Thus, observance of strict standards and tight restrictions is necessary, which, in turn, places a considerable burden on scientists and limits their opportunities to perform necessary research.

Any experimental research must be assessed vigorously with regard to its compliance with the applicable GMP and good laboratory practice (GLP) requirements. The regulatory framework must not merely be considered after successful development of a novel approach in a laboratory setting. Rather, it must guide all considerations of laboratory research from the beginning, even when initiating work

associated with the development of a novel technique or setting. Also, the requirements must be met at all stages of research. This standard process will also apply to culture media and the like that are intended for use in GMP-compliant standard operating procedures for later clinical application, according to the country-specific drug laws.

The legal regulatory environment for TE is developing rapidly and therefore undergoing constant change. It is expected that by 2010 EMEA and CAT guidelines and regulations will replace European country-specific ones in addition to those developed by the two other major markets (United States and the world). The EMEA is intended to provide a legislative framework for human TE and tissue-engineered products that encompasses the marketing and safeguarding (consumer and user protection while enabling an effective common market).

To successfully bring an idea to the clinic, one must initiate the work with the goal of marketing the final product (Fig. 1). The researcher must keep in mind that a product typically requires a minimum 5-yr development time and an investment of more than €10 MM, as well as continual responsiveness to ongoing ethical concerns. An early risk analysis (Fig. 2) is critical in planning a product development course that is consistent with EMEA risk management guidelines for medicinal products in human use (EMEA/CHMP/96268/2005). The risk analysis must identify risk factors and risk minimization activities associated with the quality and safety of the product, and determine the extent and focus of the data required during nonclinical and clinical



Fig. 1 – EMEA and CAT guidelines development.

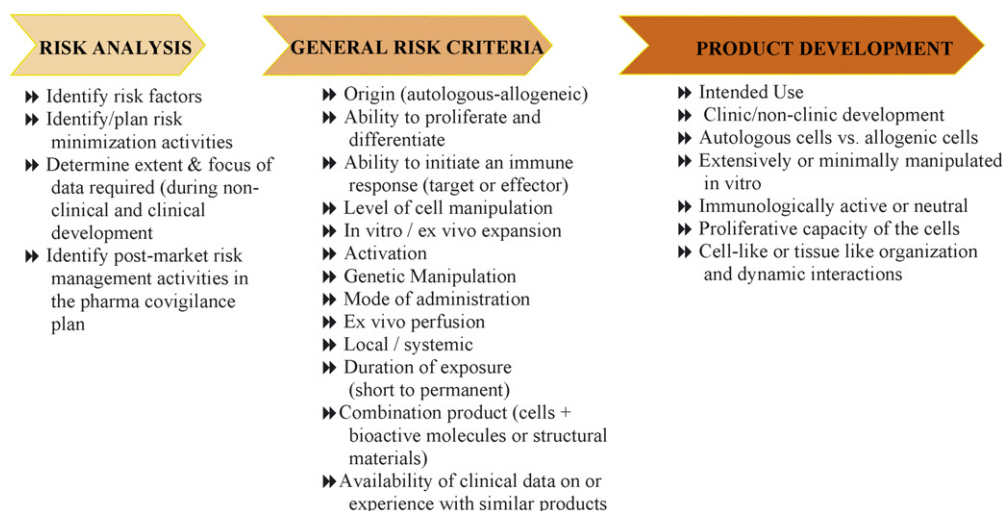


Fig. 2 – Early risk analysis with EMEA and CAT guidelines.

development. The analysis should also specify postmarket risk management activities in the pharmacovigilance plan.

In extremely limiting conditions (autologous products with small cell numbers), the analysis of structural/functional characteristics of a combination product may necessitate development of a model product composed of noncellular components combined with cellular component(s) with the same characteristics but proven availability.

#### 3.4. Requirements regarding components

##### 3.4.1. Cellular components

The identity of the cellular components, depending on the cell population and origin, should be characterized in terms of phenotypic and genotypic profiles, addressing the phenotype of the cells and relevant markers (eg, histocompatibility, genetics). The ratio of nonviable and viable cells should be determined and specified because cell viability is an important parameter for product integrity and directly correlates with biological activity.

##### 3.4.2. Culture and media

The optimized consistency/repeatability of the cell culture process must be demonstrated with respect to the intended clinical function of the cells. The influence of growth factors on cell populations or subpopulations must be shown. Synthetic media is recommended, but autologous serum is preferred and is superior to reagents of human origin (eg, albumin and immunoglobulins). For cells or tissues of animal origin (eg, supportive cells), specific EMEA regulations should be followed [74]. Bovine serum

should be irradiated and/or alternative synthetic media should be considered [75].

##### 3.4.3. Impurities

The impact of degradation products on cell component(s) should be addressed. Any material capable of introducing degradation products into, for example, biodegradable materials, should be thoroughly characterized. If genetically modified cells are used in the product, any additional proteins expressed from the vector (eg, antibiotic resistance factors, selection markers, etc) should be analyzed and their presence in the product should be justified.

#### 3.5. Potency

Potency should be defined as the time required for a predefined effect (eg, restoration of function or repair of anatomical structure) or calculated as the measured effect in a defined time period. An in vitro assay can be performed by assessing the expression of markers that have been demonstrated to be directly or indirectly (surrogate markers) related to the target biological activity, such as cell surface markers, activation markers, or specific genes. Also, a physiological response under defined conditions such as differentiation of specific cell types and/or secretion of tissue-specific proteins (eg, extracellular matrix components) can be used as a basic principle to test potency. A potency assay should be performed in vitro and in vivo with a validated number of cells.

Major cellular functions must be monitored continuously using surrogate markers and appropriate technology. In vivo assays for potency may

also be useful, especially when experimental animal models are available [76].

### 3.6. Production development

#### 3.6.1. Release criteria

All release testing should be performed using methods validated no later than the time of submission of an application. Specifications for release testing should include identity, purity, impurities, sterility, potency, cell viability, and total cell number. If the structure is an essential characteristic of the product, the structural characteristics of the active substance or finished product should be defined and justified. If the primary function of the cell-based medical product (CBMP) is excretion of specific proteins, specifications regarding these excreted proteins should be set.

#### 3.6.2. Stability testing

A shelf life for cells under specified storage conditions should be determined for all intermediates, for the components of the combined CBMP, for the active substance, and for the finished product. These values should be supported by experimental data with regard to the maintenance of cell integrity and product stability during the defined periods of validity.

#### 3.6.3. Kinetics, migration, and persistence

Cells may migrate within the host, which can present clinical concerns regarding adverse reactions deriving from displaced, possibly differentiating cells. This possibility should be evaluated in animals using appropriate methods for specific identification of the cells.

#### 3.6.4. Validation of the manufacturing process

The entire manufacturing process, including cell harvesting, cell manipulation, the maximum number of cell passages, combining product components, filling, packaging, and so forth, should be validated. A combined CBMP can correspond to a combination of active substances forming a final product, or, in some cases, the supportive structures can be considered excipients or devices, creating a mixed situation. In any case, the combination must be consistently produced.

It is recommended that the manufacturing process be validated regularly, depending on the product characteristics, for adventitious agents, identity, potency, viability, purity/impurities, and other product-specific parameters. If the intended use of the CBMP is, for example, to restore the function of deficient cells (tissue regeneration), functional tests

should be conducted to demonstrate that function is restored. If possible, studies should be conducted to determine the minimally or optimally effective amount of cell-based medicinal product needed to achieve the desired effect. In addition to cell numbers or concentrations, emphasis must be placed on the required specific characteristics (eg, differentiation stage and heterogeneity) of the applied cells or tissues.

### 3.7. Clinical efficacy

During the pivotal clinical studies, changes should not be introduced to the manufacturing process and final product. Clinical efficacy studies should adequately demonstrate efficacy in the target patient population, using clinically meaningful end points to establish an appropriate dose schedule that results in the optimal therapeutic effect. They should also evaluate the duration of the therapeutic effect of the administered product and enable a risk-benefit assessment, taking into account the existing therapeutic alternatives for the target population.

## 4. Conclusions

Reconstructive urology will experience significant changes in the coming years. Urological surgery will become less invasive and simultaneously ensure better outcomes. There will be a choice of different treatment options hopefully offering therapeutic approaches more tailored to different urological diseases. Minimization of hospitalization time and an increase in therapeutic success rates are primary objectives in the development of TE in reconstructive urology of the lower urinary tract.

Despite the fact that TE is a young field, it promises to influence urological treatment in the near future. It will change surgical techniques—even minimize some reconstructive techniques—and improve quality of life. Any experimental work has to be assessed vigorously with respect to its compliance with applicable GMP and GLP requirements. The regulatory framework must be considered at all stages of the research process to produce a novel treatment that is a CBMP compliant with the legal framework and all ethical guidelines.

### Conflicts of interest

The authors have nothing to disclose.

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