# W7: Tissue Engineering Therapy of the Lower Urinary Tract — The Potential and Hurdles

Workshop Chair: Karl-Dietrich Sievert, Germany
06 October 2015 11:00 - 12:30

| Start | End | Topic | Speakers |
|---|---|---|---|
| 11:00 | 11:15 | Introduction and explanation of stem cell applications in urology | Karl-Dietrich Sievert |
| 11:15 | 11:30 | Acellular matrix in urethral and bladder reconstruction | Leopoldo Alves Ribeiro-Filho |
| 11:30 | 11:45 | Fecal Incontinence: From Bench to Bedside | Massarat Zutshi |
| 11:45 | 12:00 | Stem Cell and Tissue Engineering Research: Where are we? | Margot Damaser |
| 12:00 | 12:10 | Discussion | All |
| 12:10 | 12:25 | Questions | All |
| 12:25 | 12:30 | Summary and close | Karl-Dietrich Sievert |

**Aims of course/workshop**

The aim of this course will be to report on the state of the art knowledge with respect to tissue engineering, stem cells in treating voiding dysfunction, and faecal incontinence, and to evaluate the latest prospective therapy options. Our aim is to enhance the knowledge base with regard to the potential of stem cell therapy for those interested in progressive translational medicine.

**Learning Objectives**

1. Obtain enhanced knowledge of current cell-based therapies being tested in animal models and clinical trials for both urinary and fecal incontinence, voiding dysfunction.

2. Understand complications encountered in testing and approving this progressive translational medicine

3. Gain insight to treatment approaches in the development pipeline.

# TISSUE ENGINEERING THERAPY OF THE LOWER URINARY TRACT— The Potential and Hurdles

Educational Value:

This is a workshop based on latest known technology to address the potential and challenges of tissue engineering and regenerative medicine as applied to treating urinary and fecal incontinence.

Description:

Regenerative Medicine is a promising treatment for the development of biomaterials that can restore, maintain, or improve tissue function. The creation of human tissue-engineered products, generated from autologous somatic cells or adult stem cells with or without seeding of biocompatible matrices has the potential to resolve the lack of tissues and organs for transplantation and to offer new options for reconstructive surgery. It is a rapidly evolving field in basic research, and while progress has been made, the translation to the clinic has yet to be realized. No cell-based therapy approach is currently in clinical practice in Urology, although a number of clinical trials have been completed or are ongoing in the field. These approaches hold much promise, but practical results and treatments available to patients have been slow to materialize, despite the publication of what might appear to be miracle cures, partly due to the difficulty of regulating this new paradigm for therapy which is neither a simple drug nor a device. These approaches will be discussed by experts in the field.

**Take Home Messages:** *Stem cells have generated a considerable amount of scientific and medical interest. Stem cell research, by increasing our understanding of normal cell development, allows us to understand, and possibly correct, the errors that lead to such medical conditions.*

**Chair:**

Karl-Dietrich Sievert, kd_sievert@hotmail.com, Urologist, Austria

*Karl-Dietrich Sievert is the Chair of Urology and Andrology at the SALK University Clinic/Paracelsus Medicine Private University (PMU) in Salzburg, Austria. Professor Sievert's clinical and research interests include oncology (investigation of advanced detection tools and reduced invasiveness and improved functional outcome with anatomical and clinical findings), neuro-urology (diagnosis and treatment of urological nerve disorders), incontinence (pathophysiology), reconstructive surgery (medical devices, tissue engineering and stem cell treatments) and the progressive and innovative treatment of spinal cord injured patients, such as early SNM implantation, which he won a 2010 Klee Innovation Prize award. He has initiated novel clinical trials to investigate the outcome of incremental or combined antimuscarinics dosages to increase effectiveness without side effects. In recent years he became one of the few urologic experts in the stem cell and tissue-engineering field who has focused on the real-time processes of bringing research initiatives from the laboratory to clinic.*


- Tissue engineering in urothelium regeneration. Vaegler M, Maurer S, Toomey P, Amend B, Sievert KD. Adv Drug Deliv Rev. 2014 Dec 1. pii: S0169-409X(14)00287-7. doi: 10.1016/j.addr.2014.11.021. Epub ahead of print
- Tissue engineering and regenerative medicine: Bench to bedside in Urology Margot S. Damaser, PhD and Karl-Dietrich Sievert, MD, PhD Adv Drug Deliv Rev. 2014, Epub ahead of print
- Stem cell therapy for voiding and erectile dysfunction. Vaegler M, Lenis AT, Daum L, Amend B, Stenzl A, Toomey P, Renninger M, Damaser MS, Sievert KD. Nat Rev Urol. 2012 Aug;9(8):435-447. doi: 10.1038/nrurol.2012.111. Epub 2012 Jun 19. Review.

**Speaker 1:**

Leopoldo Alves Ribeiro-Filho, leopoldofilho@yahoo.com, Urologist, Brazil

*Professor Ribeiro-Filho is a Brazilian urologist practicing at the University of Sao Paulo, Brazil and is also the Basic Science Editor of the International Brazilian Journal of Urology. After completing his residency in Urology in 1999, he joined a fellowship program in uro-oncology and tissue engineering at the University of California, San Francisco, mentored by Drs. Rajvir Dahiya, Peter Carroll and Emil Tanagho. He returned to Brazil in 2002, where he was awarded with a R01 NIH Grant to study biomarkers in bladder cancer. Since 2004, in addition to developing animal models for tissue engineering research, Dr. Ribeiro-Filho has been conducting the first and largest program in Latin America for urethral reconstruction and bladder augmentation in humans utilizing cadaveric organ-specific acellular matrix.*


- Acellular matrix in urethral reconstruction. Ribeiro-Filho LA, Sievert KD. Adv Drug Deliv Rev. 2014 Dec 2. pii: S0169-409X(14)00285-3. doi: 10.1016/j.addr.2014.11.019. Epub ahead of print Review.
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**Speaker 2:**

Massarat Zutshi, zutshim@ccf.org, Colorectal Surgeon, United States

*Professor Zutshi is a colorectal surgeon and staff in the Colorectal Surgery Department at Cleveland Clinic. Much of her practice is dedicated to pelvic floor dysfunction, lower bowel dysfunction, and anorectal disorders. She has a joint appointment in Biomedical Engineering in the Lerner Research Institute of the Cleveland Clinic and has become a leader in the nascent field researching regenerative therapies, including stem cells, and the application to lower bowel dysfunction and the injured anal sphincter.*


- Current status: new technologies for the treatment of patients with fecal incontinence. Salcedo L, Mayorga M, Damaser M, Balog B, Butler R, Penn M, Zutshi M. Stem Cell Res. 2013 Jan;10(1):95-102. doi: 10.1016/j.scr.2012.10.002. Epub 2012 Oct 16.
- Functional outcome after anal sphincter injury and treatment with mesenchymal stem cells. Salcedo L, Penn M, Damaser M, Balog B, Zutshi M. Stem Cells Transl Med. 2014 Jun;3(6):760-7. doi: 10.5966/sctm.2013-0157. Epub 2014 May 5.
- Current status: new technologies for the treatment of patients with fecal incontinence. Kaiser AM, Orangio GR, Zutshi M, Alva S, Hull TL, Marcello PW, Margolin DA, Rafferty JF, Buie WD, Wexner SD. Surg Endosc. 2014 Aug;28(8):227

**Speaker 3:**

Margot Damaser, damasem@ccf.org, Biomedical Engineer, United States

*Professor Damaser has 20 years experience utilizing animal models to investigate the mechanisms of development of pelvic floor disorders, including urinary and fecal incontinence and pelvic organ prolapse as they occur on their own, in concert with each other, and with spinal cord injury, diabetes, and aging. She is particularly interested in methods to regenerate & repair extracellular matrix and neuromuscular systems. To this end she investigates the mechanism of action of stem cell and cell-based therapy in these animal models. She also conducts preclinical testing of potential therapies, develops diagnostic and assessment techniques for use in animals, and investigates technologies for improved clinical therapies & diagnostics.*


- Stem cells as drug delivery methods: Application of stem cell secretome for regeneration. Tran C, Damaser MS. Adv Drug Deliv Rev. 2014 Oct 15. pii: S0169-409X(14)00215-4. doi: 10.1016/j.addr.2014.10.007. Adv Drug Deliv Rev. 2014, Epub ahead of print Review.
- Mesenchymal stem cells and their secretome preserve nerve and urethral function in a dual muscle and nerve injury stress urinary incontinence model. Deng K, Lin DL, Hanzlicek B, Balog BM, Penn MS, Kiedrowski MJ, Hu Z, Ye Z, Zhu H, Damaser MS. Am J Physiol – Renal Phsyiol. 2015. Epub ahead of print.
- Dissaranan, C., M.A. Cruz, M.J. Kiedrowski, B.M. Balog, B.C. Gill, M.S. Penn, H.B. Goldman, and M.S. Damaser (2014) Mesenchymal Stem Cell Secretome Promotes Elastogenesis and Facilitates Recovery from Simulated Childbirth Injury. *Cell Transplantation.* 23(11): 1395-406.
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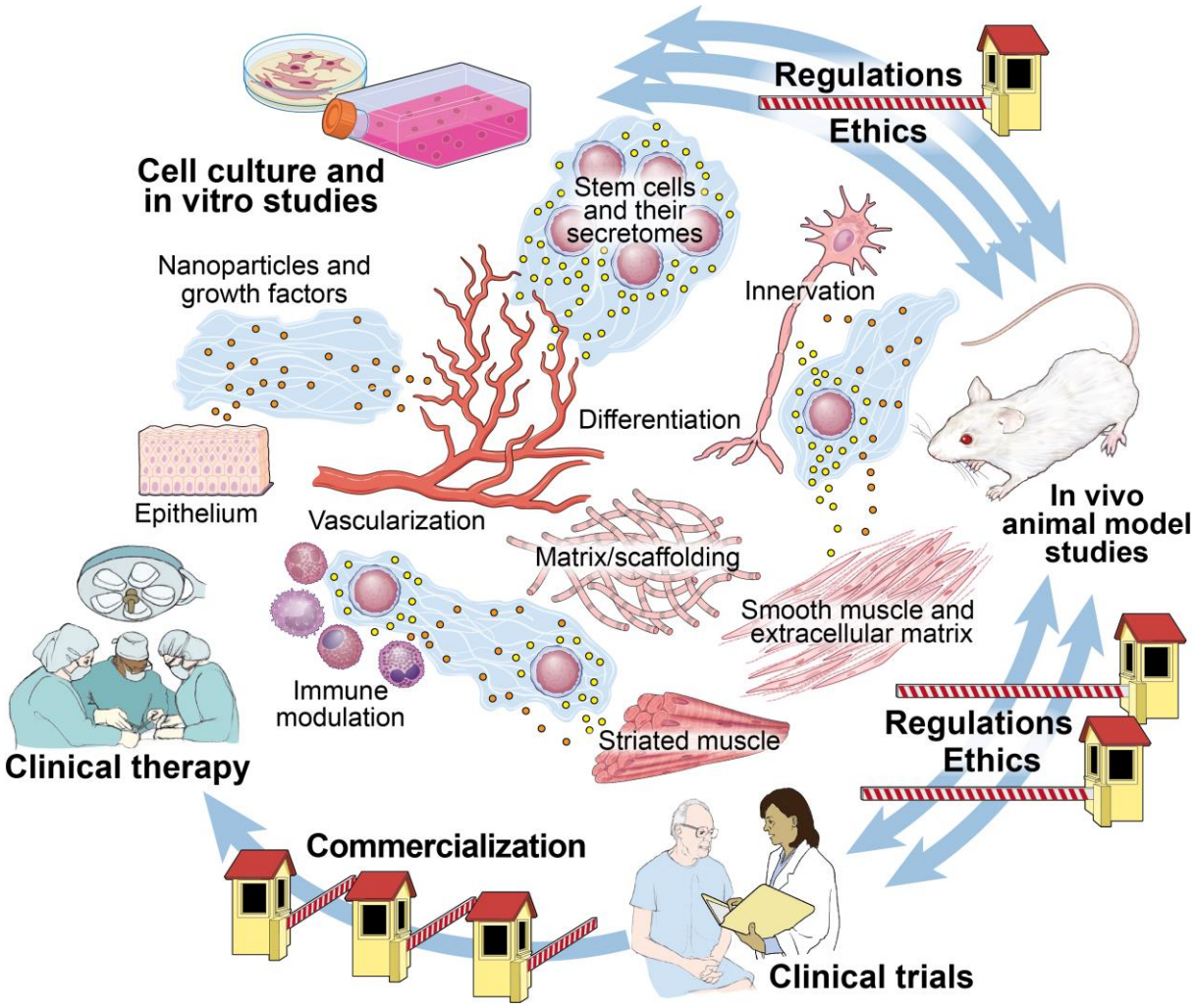


**Figure 1: Bench to bed of tissue engineering: Clinical opportunities and hurdles.**

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# Introduction and Explanation of Stem Cell Applications in Urology

Professor Karl-Dietrich Sievert

Chair of Urology and Andrology

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Urology, the surgical specialty addressing the diagnosis and treatment of the urinary tract in women and the reproductive and urinary tracts in men, has a strong history of successfully adopting innovative techniques in advance of other clinical specialties [1]. For example, Urology initiated clinical use of the first circulating tumor marker, systemic hormone-based therapy of cancer, and robotic-assisted minimally invasive surgery [2]. Urologists continue to be innovators, having recently adopted innovations in minimally invasive surgery and by conducting clinical and laboratory-based research to develop novel devices, therapies, and diagnostic techniques.

Current urological treatments are varied and include active surveillance, pharmacological therapies, device and prosthetic implants, and ambulatory as well as major surgery, including reconstruction of the genitourinary system after trauma or disease. In addition to cancer, urologists often treat urological complications of aging or systemic disease or injuries, involving repair or restoration of components of genitourinary organs and their innervation and vascularization. These urologic conditions, as well as the innovative nature of this surgical specialty, position Urology for clinical application of regenerative medicine and tissue engineering techniques in the very near future, an opinion recently embraced in an important viewpoint article in *Nature Reviews Urology* [1]. Therefore, urologic applications serve well as examples of the capabilities of regenerative medicine and tissue engineering for a broad variety of clinical applications.

Regenerative medicine addresses the process of replacing, engineering, or regenerating human cells, tissues, or organs to restore or establish normal function, by treatment with either autologous or allogenic stem or stromal cells [3]. Tissue engineering extends regenerative medicine to research growth of organs and tissues *ex vivo* in the laboratory followed by safe implantation *in vivo* for applications when the body cannot heal itself, even with regenerative medicine techniques. The utilization of different types of stem or stromal cells with diverse capabilities and capacities could offer effective therapeutic options to patients with a variety of benign urologic disorders, including lower urinary tract dysfunction, urinary incontinence, neurogenic bladder, and erectile dysfunction, as well as urethral and bladder trauma. With aging of the population and the dominance of diseases and disorders of the elderly in Urology, novel therapies, once adopted clinically, have great potential to influence care in other clinical specialties as well [4].

No cell-based therapy is currently in clinical practice in Urology, although a number of clinical trials have been completed or ongoing in the field [5]. Therefore, the goal of this special issue of *Advanced Drug Delivery Reviews* is to draw together the expertise of researchers in bioengineering, biomaterials, stem cells, gene therapy, and cell and matrix biology, as well as clinicians, who provide a Urological roadmap for future progress. In aggregate, these papers create a body of work that comprehensively and systematically highlights innovations in the field and sets the standard for future research and clinical care. As a result, the

collection of outstanding papers in this special double issue of *Advanced Drug Delivery Reviews* encompass the current state of the art of research on urological applications of regenerative medicine and tissue engineering and outline the directions of future work. To date, there have not been any Urologic journal issues that have discussed this topic with such a wide overview.

Since a primary mechanism of action of stem cell therapy is via paracrine, autocrine, and endocrine secretions [6], regenerative medicine and tissue engineering could be considered as modern methods of drug and gene therapy, which have great potential as applied to Urologic conditions. Tran reviewed this literature and suggest mechanisms for application of stem cells as modern drug delivery therapies for urological conditions [7]. In addition, gene therapy can be used to manipulate the secretions of stem cells to provide improved therapeutic outcomes, as reviewed by Li *et al.* [8], suggesting that stem cells can be manipulated prior to transplantation to provide personalized cells for personalized medicine.

Urologic reconstruction and other surgeries often involve implantation of prostheses, such as grafts or mesh, which could potentially be utilized as modern cell or drug delivery systems to improve outcomes as discussed by Hakim *et al.*, using the example of treating stress urinary incontinence [9]. The strength of this special double issue is the comprehensiveness with which this important area of research is addressed. Van Ba and colleagues provide an overview of this field with their paper entitled *Bladder tissue engineering: a literature review* [10]. Ribeiro-Filho systematically summarizes the potential utility of *Acellular matrix in urethral reconstruction* [11], while Lin and colleagues comprehensively review *Biomatrices for bladder reconstruction* [12].

Important and relevant areas of research in this field are given particular focus by Vaegler *et al.* in their paper, *Tissue engineering in urothelium regeneration* [13] and by Osman *et al.* with *Tissue-engineered buccal mucosa for urethroplasty: Progress and future directions* [14]. The interaction between scaffolds, cells, and drug delivery are ably reviewed in the papers by Mauney and colleagues [15] and Sharma and colleagues [16], again suggesting the opportunities for personalized therapies based on an individual patients' need.

With ongoing emphasis on improving surgical outcomes, there is great clinical demand to develop advanced reconstructive techniques with improved postsurgical wound healing. In the chapter entitled *Wound healing in Urology* [17], Ninan and colleagues summarize the current status of wound healing and ongoing experimental research with cell and other therapies to improve outcomes. Hakim and colleagues take an alternate approach and suggest that one successful urologic surgery could be further improved with incorporation of cell-based therapy in their chapter entitled, *Sling surgery for urinary incontinence and the application of cell-based therapy* [9]. As in all medical fields, accurate and relevant animal models are essential for success of development and testing of novel therapies. The paper by Herrera-Imbrodaa and colleagues [18] provides a background from which judgments can be made regarding appropriate choices of animal models for different investigative paradigms.

Urologists treat localized disease, defects, or disorders that occur as a result of local injury or disease or as complications of systemic diseases such as heart disease, spinal cord injury, or diabetes. Such conditions need local delivery of treatments, but they may also need a more systemic approach and support. Andersson summarizes the current status of investigation of treatments aimed at restoring bladder function in his paper entitled *Potential of stem cell treatment in detrusor dysfunction* [19], while Klein and colleagues focus on

restoration of urethral function in *Mesenchymal stromal cells for sphincter regeneration* [20]. Alwaal and colleagues review current testing of *Stem cell treatment of erectile dysfunction* [21] and Sadri-Ardekani *et al.* review current knowledge regarding *Regenerative medicine for the treatment of reproductive system disorders: current and potential options [22].* The urological complications of spinal cord injury, which are of great importance to patients and increase costs to the healthcare system, are considered by Cruz and colleagues in the paper entitled *Biomarkers in spinal cord injury and ensuing bladder dysfunction* [23].

Many urologic conditions involve neuromuscular systems, including the somatic system, as well as the sympathetic and parasympathetic autonomic systems, repair of which could be specifically addressed by tissue engineering and regenerative medicine techniques. Current treatment options are limited, urologic diseases are mostly localized, a large population is affected, and the demand for improved treatments is rising. To address this aspect of application of regenerative medicine to Urology, Faroni and colleagues review the literature on methods of nerve regeneration, experimental strategies and future perspectives [24] while Handschin and colleagues provide a state of the art assessment of *External physical and biochemical stimulation to enhance skeletal muscle bioengineering [25].*

Regenerative medicine and tissue engineering have ushered in new methodologies to overcome current clinical shortcomings. These approaches hold much promise, but practical results and treatments available to patients have been slow to materialize, despite the publication of what might appear to be miracle cures, partly due to the difficulty of regulating this new paradigm for therapy which is neither a simple drug nor a device. Much work still needs to be done to move innovation from the laboratory bench to the patients' bedside, including proof of concept in multiple animal models prior to initiating a clinical study. Existing ethical guidelines and regulations need to be carefully followed to progress from the laboratory testing to clinical testing and from clinical trials to clinical practice. These important aspects of innovation development are reviewed by Beriain in his paper entitled *The ethics of stem cells revisited* [26], Ram-Liebig and colleagues in their paper entitled *Regulatory challenges for autologous tissue-engineered products on their way from bench to bedside in Europe* [27], and Knoepfler in his paper entitled *From Bench to FDA to Bedside: US Regulatory Trends for New Stem Cell Therapies* [28].

Adv Drug Deliv Rev. 2015 Mar;82-83:v-vii. doi: 10.1016/j.addr.2015.01.006. Epub 2015 Jan 24.

## References and suggested reading

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Notes:

# Acellular matrix in urethral and bladder reconstruction

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## Urethral reconstruction: a major challenge

Urethral reconstruction has always been a challenge even for skilled urologists. Conventional urethroplasty, skin flaps and buccal mucosa grafting have been used to treat urethral strictures, but success rates of these classical procedures vary dramatically (70-90%) and a considerable number of patients need to undergo multiple procedures. After recurrent surgical failures, in some dramatic cases, when classical operative techniques have been exhausted, abdominal urinary diversion may be indicated [1].

Urologists have tested many other different autologous tissues for urethral reconstruction: artery [2], vein [3], ureter [4], appendix [5], tunica vaginalis [6], bladder mucosa [7], and even lyophilized human dura mater [8]. Unfortunately, results of these numerous creative attempts were disappointing. Furthermore, the large number of reports published on different biological tissues indicates that finding the ideal urethral substitute has always been a main goal and a difficult task for reconstructive urology.

## Bladder augmentation: are intestinal segments a good option?

Gastrointestinal segments are commonly used for augmentation cystoplasty in patients with contracted bladders. However, several complications may occur such as infection, mucous production, stone formation and cancer. Animal and human studies demonstrated that Bladder Acellular Matrix Graft (BAMG) provides an excellent scaffold for the ingrowth of host bladder wall components, inducing tissue regeneration.

## Acellular matrix: principles and rational

The ideal material for urethral/bladder replacement must act as a frame for the progressive in-growth of all host wall components and finally become an integrated part of the urethral/bladder wall with the same mechanical and functional properties as the host. Furthermore, this biomaterial must not elicit immune response, fibrosis or tissue contraction. In other words, the ideal urethral substitute should provide the creation of a suitable microenvironment for tissue regeneration.

Badylak, in 1989, successfully tested the use of autogenous small intestinal submucosa matrix (SIS) as a vascular graft in the infrarenal aorta in dogs [9]. Significant regenerative results were obtained in large abdominal wall defects[10], larynx [11] , bladder [12] and urethra [13]. In the decellularization process, cells and antigenic epitopes are removed from native tissue, resulting in a 3-D structure that is basically constituted by collagen fibers, elastin and glicosaminoglicans(GAGs) and, thus, immunologically well-tolerated [14].

## Types of Matrices

The term acellular matrix may be applied to different types of scaffolds generally used in regenerative medicine. Biological matrices may be animal- or human-derived, with all cells removed from the original organ during preparation. Different decellularization methods

(physical, chemical, and enzymatic) may be used. Obviously, different protocols have distinct effects on the extent of cell removal and extracellular matrix (ECM) composition and structure [15]. In other words, the same type of tissue treated by different decellularization protocols may present variations in GAGs and growth factors composition, resulting in distinct regenerative profiles.

Synthetic matrices are totally manufactured polymer-based scaffolds. Some authors have tested non-degradable materials, such as silicone and polytetrafluoroethylene (PTFE) for urethral reconstruction with poor results. They reported erosion, calcification, and fistula formation. There was no adequate tissue regeneration. On the contrary, scaffolds made of biodegradable polymers such as polyglycolic acid (PGA), polylactic acid (PLA), and polylactic co-glycolic acid (PLGA) have produced some good results when used for urethral reconstruction specially on a cell-seeded human model, since as just one patient out of five needed transurethral incision [16].

## Cell-free *versus* cell-seeded matrices

One important aspect of the scaffold-based urethroplasties that often generates some debate among researchers is the use of cell-free *versus* cell-seeded matrices. The cell-free matrices have some very attractive advantages such as ease of production, storage, transportation and may be considered an "off the shelf" material. Since additional surgical procedures for graft harvesting may not be needed, operative time and morbidity may be reduced. Homologous (cadaveric) or heterologous tissue maybe used for matrix preparation, since all cells will be removed. Cells, membranes and DNA induce a strong immune response. Since acellular matrix is cell-free, the treated scaffold does not elicit immune reaction. On the other hand, heterologous or even homologous cells, if seeded, will induce tissue rejection. In humans, only autologous cell have been used for urethral tissue engineering.

Cell-seeded matrices represent a very attractive strategy especially for tubular implants. Biological scaffolds have been seeded with several different types of cells such as mesothelial (collected from omentum) [17], autologous bladder epithelial and smooth muscle cells [18], bone marrow mesenchymal stem cell [19]. Coculture protocols may have some advantages. However, cell-seeded scaffolds, although promising in initial clinical trials, consist in extremely complex procedures in terms of logistics. If urothelial cells are selected to be used (preferred by most of the authors) [16], for instance, for each patient treated, a cystocopy is required for harvesting autologous urothelial tissue from the bladder. Clean cell culture facilities are mandatory, especially for human treatment. Once the construct has been adequately cellularized, prompt surgical implantation must be scheduled, since delays may lead to graft failure. The total cost of a cell-seeded graft can be up to six times greater than that of cell-free matrix. [20]. Thus, the future clinical use of cell-seeded matrices may be restricted to a selected group of patients.

## Acellular Matrix for urethral reconstruction

Actually, decellularization protocols routinely use hyperosmolar solutions and enzymes that kill the vast majority of infectious agents. At the University of São Paulo, Brazil, we had performed more than 200 urethral reconstructions with acellular matrices in humans over the last 10 years. These matrices were produced at our lab by enzymatic conversion of human cadaveric urethras and bladders with a protocol based on the use of DNAses. In 2014, our group reported long-terms outcomes of the 44 initial patients treated with ventral onlay urethral acellular matrix grafts (UAMG) [21]. These patients presented long and complex strictures (3 to 18 cm) and history of multiple previous urethral procedures (3 to 30 procedures/ patient). During follow-up (24 to 113 months, median 42 months) peak flow rates ranged from 5.4 to 25.2 ml/s (median 17.6 ml/s)in those patiens who were able to void. Six patients (14%) needed an endoscopic urethrotomy to treat partial restenosis (0.5 to 1.0 cm

long) 2 to 8 months after the UAMG reconstruction and have been presenting a stable flow rate over the last 24 months (8.6 to 17.2ml/s). Nine patients (including the two who had graft infection) had been requiring urethral dilations (20%). Complete restenosis of the graft occurred in 5 patients(11%), who were submitted to a new UAMG procedure. Two out of these five patients who underwent a UAMG salvage procedure were able to urinate, but they need urethral dilations.

## Acellular Matrix for bladder reconstruction

Our first patient to undergo a bladder augmentation cystoplasty procedure with acellular matrix was a 52 year-old lady with an overactive contracted bladder (capacity of 120 ml) post-hysterectomy (leiomyomata treatment) in August 2005 [22]. The patient presented short voiding intervals (30 minutes), nocturia (10-12 times) and bilateral ureteral reflux. Tuberculosis and cancer were ruled out. A human bladder, harvested from a cadaveric donor, was enzymatically converted into a BAMG in our lab. Through an extraperitoneal approach, three transverse dissection lines were performed on the anterior bladder wall, preserving the mucosa while splitting the muscular wall for about 50% of the bladder circumference. BAMG strips were individually placed covering the intact mucosa lining on each one of three dissection lines and sutured to the edges of detrusor wall using 5.0 Dexon. Surgical time: 120 minutes. The patient stayed with the indwelling catheter and received antibiotics for 1 month. No immunosuppressors were prescribed.

There were no immediate post-operative complications. The patient was able to urinate after catheter removal. No self-catheterization was necessary. After 120 months, her voiding intervals increased (4- 5 hours) and nocturia reduced (1x). Post-operative cystogram showed a normal-looking bladder with no ureteral reflux. Bladder capacity increased to 420 ml and she had normal urinary flow (Qmax = 21 ml/s) and a residue of 50 ml. Cystoscopy revealed that the BAMG was totally integrated to the bladder wall.

## Conclusions

- Clinically, cell-free acellular matrices are safe, easy to work with, “off-the-shelf”, and have demonstrated long term success rates between 70 – 80% in most series. Unseeded biological acellular matrices represent the most common type of scaffold used in humans to the present time.
- successful cell-seeded matrices for urethral reconstruction have been reported more recently, probably reflecting the progress in cell biology and innovations in the development and characterization of natural and synthetic biomaterials for use as scaffold components. In terms of cell-seeded scaffolds used for human urethroplasties, there are only three reports with limited number of patients.
- Prospective trials comparing seeded and cell-free acellular matrix grafts with each other and also with traditional techniques are needed in order to determine the most accurate clinical indications for each approach. Probably, in a near future, both cell-free and cell-seeded matrices will become an important part of the clinical armamentarium in urethral reconstruction with different and specific indications for each one.
- Acellular matrix augmentation cystoplasty is a simple technique that may represent one more option in the treatment of contracted bladders.

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# Fecal Incontinence: from Bench to Bedside

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## Take Home Points:

- Currently research in cell-based therapies is focused in animal models and for anal sphincter dysfunction.
- Previously stem cell therapy was thought to be successful due to ability of cells to differentiate and replace damaged or diseased tissue; however, the current thinking suggests that stem cells also exert functional benefit by secreting bioactive factors that trigger local and systemic responses to injury
- Tissue engineering utilizes constructs that use stems cells seeded onto scaffolds that may replace entire complexes.
- Although most animal research in the field have focused on autologous cell sources, the use of allogeneic stem cells which is already being used in other fields of medicine needs to be explored.
- Understanding the mechanisms of stem cells in injured tissue will pave the options for their use in tissues that have been injured in the remote past.

## What are stem cells?

Stem cells are undifferentiated cells that can differentiate into specialized cells and can divide to produce more stem cells. There are two broad types of stem cells: embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and adult stem cells, which are found in various tissues. In adults stem cells act as a repair system for the body, replenishing adult tissues. In a developing embryo, stem cells can differentiate into all the specialized cells—ectoderm, endoderm and mesoderm but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.

## Where do stem cells come from?

Stem cells most commonly are used from the bone marrow but may be derived from adipose tissue, muscle tissue, Wharton's Jelly and the dental bud.

## How are stem cells thought to work therapeutically?

Stem cells can act via paracrine and autocrine mechanism. They need to be guided to the site of injury and initiate repair based on the signals received from the injured cells. Incomplete repair or fibrosis is due to loss of these signals over time. This has been well documented in the cardiac tissues. To be able to regenerate tissues where injury has occurred in the remote past some kind of a low grade injury to mimic the tissue environment as it occurs after an acute injury is needed. Some forms of conditioning injuries are electric current and hypoxia. Alternatively these signals can be introduced via cytokines that are expressed during acute injury at the site of repair to trick the body into initiating repair.

## Current research on stem cells to treat anal sphincter dysfunction

Current research has focused on the acute injury and mechanisms related to cell mediated repair. One human trial evaluated muscle derived autologous stem cells after electrical stimulation.

## Conclusions

We need to tread carefully so that we do not do trials that are unsuccessful because the researchers were hasty in their use of stem cells without studying the mechanisms of such repairs. More animal research is needed to start therapy in an area that does not have acute injury.

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# Stem Cell and Tissue Engineering Research: Where are we?

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## Take Home Points:

- Currently stem cell-based therapies are being tested in animal models and clinical trials for incontinence and voiding dysfunction
- Conventional stem cell therapy has focused on the ability of cells to differentiate and replace damaged or diseased tissue; however, mounting evidence suggests that stem cells also exert functional benefit by secreting bioactive factors that trigger local and systemic responses to injury
- For tissue engineering applications, stems cells can be seeded onto scaffolds to facilitate the incorporation of the graft by native tissue.
- Although most clinical trials in the field have focused on autologous cell sources, the use of allogeneic stem cells offers the potential for 'off-the-shelf' treatment with disease-free cells; this approach is already being used in other fields of medicine
- Further research is needed to better understand the mechanisms of stem cell therapeutic action in order to optimize treatment algorithms

## What are stem cells?

Stem cells by definition can self-perpetuate indefinitely and can differentiate a variety of types of cells, depending on the local cellular environment. Embryonic stem cells are derived from an early stage embryo. They are pluripotent or totipotent and can differentiate into all adult cell types. In contrast, adult stem cells are multipotent and classically are thought to be able to differentiate only into a limited number of types of cells. Adult stem cells make up a distinct minority of cells in a given tissue and are generally slow cycling, multipotent and are referred to by their tissue of origin. The use of human embryonic stem cells has been limited in the United States and in most European countries because of their tumorgenic potential and due to ethical concerns with harvesting of embryonic cells, resulting in government regulations limiting their use (1-3). This talk will focus on characterization and use of adult stem cells since they are the ones most utilized to develop therapies for incontinence and voiding dysfunction.

## Where do stem cells come from?

Although bone marrow is the classic site for extraction of adult stem cells, they can be obtained from almost any tissue or even body fluids, including but not limited to muscle, fat,

hair, and urine (4). Stem cells are not identical in each tissue and therefore the source of therapeutic stem cells may impact efficacy. Adult stem cells from all these sites have been utilized as therapeutic agents in preclinical animal studies or clinical trials and their use to treat incontinence and voiding dysfunction will be discussed in this talk.

## How are stem cells thought to work therapeutically?

Stem cell therapy in urology has, for the most part, presumed that stem cells have their therapeutic effect by differentiating into muscle tissue, for example to increase muscle mass and strength of urethral sphincter muscle (5;6). Similarly, tissue engineering for reconstruction has been developed via ex vivo differentiation of stem cells. Stem cells can also act via paracrine and autocrine mechanisms via their secretions (4;7). In some studies, therapeutic effects resulting from stem cell therapy appear disproportionate to the number of cells that engraft to injured organs, supporting this mechanism of action. The profound possibilities of this mechanism of stem cell therapeutic effect are best illustrated by a study in which stem cells were injected into the hamstring muscle of hamsters with heart failure. Although cells were unable to migrate out of the muscle, they generated significant cardiac improvement, suggesting a systemic effect of stem cell secretions: a systemic paracrine mechanism of action (8).

## Current research on stem cells to treat stress urinary incontinence

Clinical trials have shown that autologous muscle derived stem cells are safe and have promise as a therapy for stress urinary incontinence symptoms (6). However, clinical studies cannot determine the mechanism of action of stem cells, partly because, in humans, the cells cannot be tracked in vivo. Therefore, although therapy has entered the clinical trial stage of testing, determination of mechanism of action in animal models remains necessary. This talk will summarize the results of research in animals and humans testing stem cells as potential therapy for stress urinary incontinence.

## Current research on stem cells to treat voiding dysfunction

Several studies have demonstrated that stem cells can restore contractility in rats with bladder outlet obstruction (9;10), likely via the paracrine effect of secretions of the stem cells. Several similar studies in animals have demonstrated the ability of stem cells to initiate bladder remodeling after prolonged obstruction—even in cases where only a few stem cells homed, survived, and differentiated into smooth muscle tissue—suggesting the importance of a paracrine mechanism of action (11;12).

## Conclusions

The studies conducted to date demonstrate that stem cells hold great therapeutic potential for treatment of urological disorders. However, much research is yet to be done, particularly into mechanism of action of the cells, ideal cell population to utilize for therapy, and optimal delivery method. Because of the hype and great potential for stem cells there is also the potential for misuse of the term as well as unethical research to advance a specific agenda. These have occurred in the Urological field and researchers ought to be vigilant to guard against them.

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