

## Stem cell applications for pathologies of the urinary bladder

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### Abstract

New stem cell based therapies are undergoing intense research and are widely investigated in clinical fields including the urinary system. The urinary bladder performs critical complex functions that rely on its highly coordinated anatomical composition and multiplex of regulatory mechanisms. Bladder pathologies resulting in severe dysfunction are common clinical encounter and often cause significant impairment of patient's quality of life. Current surgical and medical interventions to correct urinary dysfunction or to replace an absent or defective bladder are sub-optimal and are associated with notable complications. As a result, stem cell based therapies for the urinary bladder are hoped to offer new venues that could make up for limitations of existing therapies. In this article, we review research efforts that describe the use of different types of stem cells in bladder reconstruction, urinary incontinence and retention disorders. In particular, stress urinary incontinence has been a popular target for stem cell based therapies in reported clinical trials. Furthermore, we discuss the relevance of the cancer stem cell hypothesis to the development of bladder cancer. A key subject that should not be overlooked is the safety and quality of stem cell based therapies introduced to human subjects either in a research or a clinical context.

**Key words:** Stem cells; Urinary bladder; Urethra; Bladder reconstruction; Incontinence; Cancer stem cells; Safety

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**Core tip:** This article reviews the current status of the stem cell based therapies in the field of treating urinary tract pathologies. We provide a particular focus on bladder reconstruction models that were based on stem cellular therapies. Stress incontinence and voiding dysfunction represent common clinical problems that could benefit from advancing the stem cell field. A

brief highlight is given to bladder cancer stem cells and therapeutic value that could arise from controlling their behavior. We also note the pressing need for more robust regulations and quality guidelines to prevent the transfer of research findings prematurely into routine clinical practice.

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## INTRODUCTION

The bladder and urethra compose the lower urinary tract. Pathologies of this part of the urinary system are common and clinically significant. Due to the bladder role as a versatile and dynamic reservoir of urine, it has unique structural and regulatory mechanisms that need to be thoroughly considered when cell and tissues engineering based therapies are thought of. Its complex mucosal-muscular-neural elements work together efficiently to produce a synchronized effortless urinary filling and emptying process. For instance, the bladder mucosa hosts a particular type of epithelium, "the transitional epithelium or urothelium", with cellular, intercellular and architectural features that make it expansible, durable, and resilient to life long persistent irritation by urine<sup>[1]</sup>. Additionally, the detrusor muscle that surrounds the mucosa has a peculiar pattern of fibre arrangement to enable instant and complete emptying of the bladder once it is contracted. It can adjust to chronic outflow obstruction by hypertrophy and hyperplasia<sup>[2]</sup>. The neurological control of the bladder and its urethral sphincter is compounded by autonomic, sensory and motor neural interaction<sup>[3]</sup>. Multiple other hormonal and metabolic factors contribute to the integrity of the bladder function. Disturbance in one or more of these elements results in dysfunction in the vital process of urination which could significantly impair a patient's quality of life and may, in severe cases, cause disability or even death. Surgical removal of the bladder due to cancer or end stage benign disease necessitates the replacement of its function using alternative mechanisms, of which, stem cell based engineered tissue is proposed as a possible future strategy.

## CONSIDERATIONS IN THE APPLICABILITY OF STEM CELL THERAPY

Stem cells are classically defined by their self-renewal based long-term survival and their flexible fate, being able to differentiate into other cell types. However, as simple as the concept seems to be, the accurate

identification, isolation and transplantation of stem cells could be more intricate than originally thought. For example, the functional distinction between stem cells and their progenitor cells remains ambiguous<sup>[4,5]</sup>. This should be taken in consideration when planning stem cell therapies; a successful therapy may require other progenitor cells to turn on differentiation and proliferation signals of the parent stem cell. As well, the transition of the stem cell between quiescent and active stages is a dynamic process that challenges the accurate prediction of stem cell behaviour in different environments<sup>[6]</sup>. The control of stem cell niche and microenvironment appears to be fundamental in the long-term success of stem cell therapy. Therefore, studying the stem cell niche could identify important biochemical and mechanical cues that need to be provided in addition to the cellular component to bridge the variability gap between the donor and host stem cells' niche and allow their effective engraftment and proper function *in vivo*<sup>[7]</sup>.

## BLADDER ADULT STEM CELLS

The ability to use someone's cells to repair another area of one's own body could indeed reduce risks of infectivity and immunogenicity. For that, adult stem cells maintained its key position among other stem cell types<sup>[8]</sup>. Tumorigenicity is also of much less concern. However, adult stem cells could be of less potential for long term survival due to inherent cell aging concerns<sup>[9]</sup>. Various adult stem cell types have been used in treating bladder dysfunction including muscle; bone marrow and adipose tissue derived stem cells<sup>[10]</sup>. In addition, urine stem cells have been isolated and differentiated into specialized cells to offer a readily accessible stem cell source for various applications<sup>[11]</sup>. Although a general depot of adult stem cells exists in fat and bone marrow, however, lineage specific stem cells are believed to dominate in specific organs such as skin and cornea<sup>[12,13]</sup>.

The urothelial adult stem cells are thought to be slow cycling *in vivo* (3-6 mo), clonogenic, highly proliferative and located in protected sites. These cells are commonly identified by their localization in the basal layer of the urothelium, and by being label-retaining cells with high expression of  $\beta$ -4 integrin<sup>[14,15]</sup>. The identification and isolation of these cells are important for tissue engineering of urothelium-lined organs including bladder, urethra and ureters.

## CLINICAL APPLICATIONS OF STEM CELLS IN BLADDER PATHOLOGIES

### *Stem cells for urinary bladder replacement*

Following cystectomy for benign or malignant bladder pathologies, bladder replacement or reconstruction is a critical step for maintaining patient's life. Whether ureterosigmoidostomy, ileal conduit or orthotopic

neobladder are used for reconstructing a new urinary reservoir, significant morbidity and mortality often occur due to the incorporation of intestinal segment into the urinary tract. This could result in recurrent urinary tract infection, metabolic and electrolyte disturbance, mucous retention and anastomotic site cancer. Moreover, the patient is left to deal with either an external draining bag through an opening on the skin called stoma or self catheterization with no external bag, both of which could interfere with body image and daily activities. Current *in situ* surgical bladder constructs are also unable to contract and squeeze the urine through the urethra since it lacks the muscle layer and the patient needs to adapt to techniques to push urine out such as contracting the abdominal muscles<sup>[16-18]</sup>. Therefore, seeking new therapies to provide optimal bladder reconstruction is of utmost clinical importance.

Ideally, a perfect bladder reconstruct should be made of low immunogenic or autologous tissue that contains all anatomical layers of the bladder wall (mucosa, submucosa and muscle layer). It should be designed to mimic the detrusor muscle mechanics and to provide significant dispensability. It has also to provide similar urothelial mucosal barrier and ultimately should be incorporating functioning neuronal elements. Accordingly, advanced and complex tissue engineering and regenerative models are required. Thus far, there has been no such comprehensive efficient model; however preliminary studies are ongoing worldwide to achieve such goals.

Recently, tissue engineering using cell seeded scaffolds has been investigated in urinary bladder bioengineering studies<sup>[19]</sup>. This method includes the seeding of a scaffold with autologous bladder muscle and epithelial cells. The use of autologous cells, however, may not be available as in cases of cancer<sup>[20]</sup> or benign end-stage bladder diseases<sup>[21]</sup>. Alternatively, stem cells can be derived from other sources including adipose tissue, bone marrow or amniotic fluid cells. They can be seeded on scaffolds and transplanted for *in vivo* differentiation. However, current data shows that such differentiation occurs only in a small percentage of the delivered cells<sup>[22]</sup>. Another method is to differentiate stem cells into urothelial and smooth muscle cells *in vitro*. Stem cells have shown a good potential for urothelial differentiation. This was achieved by using either conditioned medium<sup>[23,24]</sup> with or without growth factors such as all-transretinoic acid<sup>[25]</sup>. Both direct culture (seeding stem cells with urothelial cells) and indirect co-culture (using trans-well system) have been attempted with variable outcome, though cell-to-cell contact in direct culture appears to be an enhancing factor for stem cell differentiation<sup>[26,27]</sup>. Stem cell differentiation into smooth muscle cells is more feasible and can be achieved either by chemical induction<sup>[28]</sup> or co-culture with smooth muscle cells<sup>[22]</sup>.

Induced pluripotent stem cells (iPS) reprogrammed

from adult tissue such as skin fibroblasts, urinary tract stromal cells and urine-derived cells have been also used and were subsequently differentiated into urothelial and smooth muscle cells<sup>[29-31]</sup>. However, urinary tract-derived iPS cells are believed to be of superior differentiation properties to other sources, which should emphasize the epigenetic differences between individual iPS cell lines and stress on the importance of organ-specific iPS cells for tissue-specific studies<sup>[29-31]</sup>.

### **Stem cells for voiding dysfunction**

Voiding dysfunction (VD) can affect the patient's quality of life and interfere with social activities. It can be manifested clinically as various disorders of urine storage or emptying. Current therapies of VD are generally inadequate and often fail to target the actual pathophysiology of the disease. Stem cell therapies have been also investigated in this field and were shown to cause some positive response either due to differentiation or more likely due to indirect paracrine effect associated with the release of growth factors and cytokines. The later mechanism could lead to modulation of local and systemic inflammatory responses and mobilization, stimulation and differentiation of native stem cells in addition to the enhancement of vascularization of regenerating tissues and reduction of fibrosis<sup>[32]</sup>. A variety of stem cell types have been explored for the treatment of VD, including bone marrow, skeletal muscle and adipose derived stem cells<sup>[33-35]</sup>. Adipose stem cells was most popular due to their easy harvest, high yield of stem cells and easier smooth muscle differentiation compared to other types<sup>[35]</sup>. They could improve VD in animal models of bladder overactivity or hypoactivity associated with different etiologies such as diabetes mellitus, post radiation and hyperlipidemia<sup>[32,35]</sup>. Nevertheless, more studies are needed to determine the efficiency of such therapy, the form of transplanted cells (unmodified or differentiated cells), the method of cell delivery (systemic or local injection into the bladder) and the appropriate cellular dose (number of cells per injection and frequency of treatment).

### **Stem cells for stress incontinence**

Stress urinary incontinence (SUI) is a widespread disorder, particularly in women<sup>[36]</sup>, due to inherent predisposing anatomical and physiological factors specific to the female urethra. In addition, SUI is commonly initiated or aggravated by female specific physiological stages such as pregnancy, vaginal birth and menopause or pathological conditions such as uterine fibroid tumors<sup>[37]</sup>. The current treatment of SUI relies on pelvic floor exercises in mild cases and surgical interventions in severe cases. The surgical methods aim to provide support to the urethra using various artificial tapes or autologous tissue slings. Each one of these methods has its adverse effects and post-operative complications<sup>[38,39]</sup>. More recently,

**Table 1** Examples of clinical studies of stem cell therapy used in the treatment of stress urinary incontinence showing a comparison of stem cell source, the duration of *in vitro* expansion and the follow up of patients post treatment

Origin of stem cells and duration of <i>in vitro</i> expansion	Number of women and route of injection	Follow up (yr)	Success rate (improvement and cure)	Ref.
Autologous muscle derived stem cells (SC), expanded 8-10 d	16 female patients, transurethral approach	2	Up to 75%	[76]
Muscle derived SC	222 male patients, transurethral injection	1	Up to 54%	[77]
Minced autologous muscle cells, no <i>in vitro</i> expansion	35 female patients	1	Up to 63%. Improvement (clinical, diary, and ICIQ-SF scores)	[78]
Muscle derived SC, expansion duration NA	8 female patients, transurethral injection	1	Significant improvement in 5 women (pad-weight, bladder diary and QOL assessment)	[79]
Muscle derived SC, expanded <i>in vitro</i> for 7 wk	20 female patients	2	Significant improvement (clinical, QOL and cystometry). Therapy based on this method is now licensed in Europe	[80]
ASCs combined with bovine collagen	5 female patients	1	2 out of 5 patients were satisfied with treatment with negative cough test	[81]
Autologous ASCs with and without fat	11 male patients with post-prostatectomy incontinence	1	60% improvement in urine leakage, frequency and amount of incontinence in 8 patients with one patient achieved total continence	[82]

the injection of bulking agents around or through the urethra to treat SUI has gained some popularity. Many agents have been used with variable success rates and complications were reported. Collagen, fat, Teflon, silicon or carbon coated beads are common examples of various agents employed<sup>[40]</sup>. A recent systematic review in the Cochrane database showed that current data is insufficient to prove benefit of these therapies especially that saline injection was of similar effectiveness to bulking agents, with some reports of serious side effects associated with some of these agents<sup>[41]</sup>. Consequently, stem cell therapies appeared as the next generation of therapies in SUI, which gained recent attention. Animal studies showed potential benefit in treating SUI. The animal model of SUI is usually induced by cutting the pudendal nerves. Muscle derived stem cells have been the most widely applied source and are believed to provide stem cells that are able to differentiate into committed striated muscle cells more than other stem cell sources. However, many other sources have been attempted with comparable success. To evaluate the success of stem cell therapy in SUI animal models, multiple outcome measures were used including leak point pressure, intra-bladder pressure, maximum bladder volume, urethral functional length, maximum urethral closure pressure, morphological examination of sphincter muscle and matrix<sup>[42]</sup>.

Adipose derived stem cells have been injected in rat models of stress incontinence using intravenous or trans-urethral routes, and showed significant improvement in terms of increased elastin content and voiding function measured by cystometry<sup>[43]</sup>. Adding nerve growth factor and poly (lactic-co-glycolic) acid to the adipose stem cells when injected in the rat urethral sphincter improved stem cell proliferation *in vivo* and that was increasing in a dose dependant pattern. Such factors appear to improve stem cell survival and

functional performance of the urethra compared to using adipose stem cells alone<sup>[44]</sup>. Furthermore, human amniotic fluid stem cells seem to be of potential benefit and favourable safety profile in restoring normal urethral function in the animal models of SUI due to their low immunogenicity and tumorigenicity<sup>[45]</sup>. A triple stem cell therapy approach used human amniotic stem cells that were processed to the stage of early differentiation into three lineages *in vitro* (myogenic, neurogenic and endothelial). This approach was able to improve SUI signs in the animal model compared to using only one or two types of differentiated cells<sup>[46]</sup>. A combination of gene therapy strategy by inducing urine derived stem cells to over express VEGF showed improvement of the sphincter composition especially the nerve fibres, muscle cells and vascularisation<sup>[47]</sup>.

The reconstruction of stem cell tissue engineered based slings to support the urethra was also investigated. A silk scaffold covered with bone marrow derived mesenchymal stem cell sheet has been implanted as a sling to support the rat urethra showing a better matrix deposition compared to using a silk sling alone<sup>[48]</sup>. Likewise, adipose tissue derived stem cells and silk fibroin microspheres were combined together and they were able to retain improvement in SUI for longer duration than the silk fibroin microspheres alone<sup>[49]</sup>. Following animal studies, a number of clinical trials have been attempted; examples are shown in Table 1.

#### **Urinary sources and applications of iPS**

iPS can be generated from somatic cells by inducing the expression of different transcription factors, originally Oct4, Sox2, Klf4 and Myc (OSKM). The induced pluripotent stem (iPS) cells have been considered as the stem cell that could offer several advantages over the adult stem cells while maintaining the plasticity of embryonic stem cells<sup>[50]</sup>. However,

close examination of their possible adverse outcomes, such as the reported immunogenicity, should be undertaken prior to wide application<sup>[51]</sup>. The urinary bladder and prostate cells were also reprogrammed successfully to iPS cells, offering a new method of the urinary system disease modeling<sup>[29]</sup>. Likewise, urine cells were reprogrammed into iPS cells that were differentiated successfully into cardiac cells *in vitro*<sup>[52]</sup>. Moreover, a model of personalized medicine was introduced by reprogramming urine cells of a haemophilia A patient into liver cells that express the traits of the disease<sup>[53]</sup>. Conversely, the urinary bladder cells were obtained from iPS cells *in vitro*<sup>[54]</sup>. Therefore, theoretically, the use of urine cells to produce urinary bladder wall cells for bladder reconstruction purposes would be attractive as they are originating in the same system<sup>[55]</sup>.

### **Bladder cancer stem cells**

On the other end of the stem cell paradigm, adult cancer stem cells (CSC) are implicated in cancer development, progression and recurrence after conventional therapy. CSC are practically identified and confirmed by their ability to initiate tumours in immunocompromised mice and by their *in vitro* clonogenicity. The cancer stem cell theory was a build up on older clinical observations that related cancer to stemness<sup>[56]</sup>. Starting with blood cancers that showed strong representation of the cancer stem cell model<sup>[57]</sup>, most solid cancers were studied for their CSC population<sup>[58-62]</sup>. Several groups were able to isolate cells with CSC features from urinary bladder cancers. These cells were identified by several markers as being CD44+, negative for EMA, high expression of 67LR, low expression of CD66C, strong association with ALDH1A1 expression or as a side population based on the efflux of the Hoechst dye<sup>[63,64]</sup>. It appears that CD44+ is one of the reproducible markers and its expression on bladder CSC was commonly supported by several groups<sup>[65]</sup>.

The value of such findings is to tailor specific target based therapies. Unlike traditional bladder cancer therapies, a cancer stem cell targeted therapy is postulated to be able to eradicate the resistant cancer initiating cells and minimize risk of recurrence. It is also important to understand bladder carcinogenesis and recognize how risk factors initiate cancer. For instance, activation of the transcription factor stat3 led to transformation of CK14+ urothelial stem cells into invasive cancer cells in a stat3 transgenic mouse model<sup>[66]</sup>. Also, arsenic could transform normal prostate stem cells into cancer stem cells; this may explain its known detrimental effect on the urogenital system<sup>[67]</sup>.

One of the promising molecular targets include the CD47 which is an integrin associated protein known for its "do not eat me" effect on the cancer cell, leading to less recognition by cell mediated immunity especially

macrophages. The CD47 was found to be significantly more expressed in CSC compared to normal cells<sup>[68]</sup>. A CD47 antibody labeled with fluorescence was used to distinguish human bladder cancer cells from normal cells with a specificity of more than 90%<sup>[69]</sup>. A number of clinical trials have started to use the antibody against CD47 as a model of immunotherapy in leukemia and solid tumors<sup>[70]</sup>. The bladder CSC niche and the microenvironment are also believed to significantly protect CSC from immunity and therapeutic factors and to maintain its carcinogenic behavior. Therefore, understanding the composition of this niche could highlight other potential targets for new therapies<sup>[71]</sup>.

### **Safety and quality**

It is obvious that the use of stem cells in clinical investigations and therapeutics of bladder and urethral pathologies will be of potential importance in the near future. Nonetheless, the rapidly gained popularity of these interventions should be cautiously perceived and publicized in scientific and non-scientific media. Premature application of such therapies in human subjects without matching scientific evidence as off label treatment in research studies or their unregulated use in clinical practice should be truly concerning. In underdeveloped settings, such unregulated practices are quite common. Stem cell therapy tourism is also expanding and could expose patients to considerable health hazards<sup>[72]</sup>. Factors such as sterility and viral screening of the introduced cell therapy, the lack of contaminants and toxic molecules, cell viability and cell karyotyping should be all included in stem cell clinical studies and should be reported clearly by researchers before being accepted in formal scientific publications<sup>[73]</sup>. Tumorigenicity of stem cell therapies is another factor that should not be overlooked and available tumorigenicity assays should be routinely used<sup>[74]</sup>. Awareness about current good manufacturing practices in stem cell therapy should be widely raised<sup>[75]</sup>. Nonetheless, this would not be adequate in the absence of strict regulations enforced on using such therapies in human especially in underdeveloped settings where baseline research ethics and quality control are not well developed or monitored. Wide international collaboration is, therefore, strongly needed to come up with a global consensus and standards that allow the safe use of stem cell therapies in human subjects without ignoring the cost and training involved to implement these standards in poor settings.

## **CONCLUSION**

Stem cell therapies will be in the clinical setting in the near future. At present, their use in bladder dysfunction and bladder reconstruction is under intense investigation. Various sources of stem cells

have been attempted with comparable success. More complex tissue engineered models that mimic the original bladder anatomy are awaited. Bladder cancer stem cells are investigated as a potential target for cancer therapies.

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