



## Advances in stem cell therapy for the lower urinary tract

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### Abstract

Lower urinary tract diseases are emotionally and financially burdensome to the individual and society. Current treatments are ineffective or symptomatic. Conversely, stem cells (SCs) are regenerative and may offer long-term solutions. Among the different types of SCs, bone marrow SCs (BMSCs) and skeletal muscle-derived SCs (SkMSCs) have received the most attention in pre-clinical and clinical trial studies concerning the lower urinary tract. In particular, clinical trials with SkMSCs for stress urinary incontinence have demonstrated impressive efficacy. However, both SkMSCs and BMSCs are difficult to obtain in quantity and therefore neither is optimal for the eventual implementation of SC therapy. On the other hand, adipose tissue-derived SCs (ADSCs) can be easily and abundantly obtained from "discarded" adipose tissue. Moreover, in several head-on comparison studies, ADSCs have demonstrated equal or superior therapeutic potential compared to BMSCs. Therefore, across several different medical disciplines, including urology, ADSC research is gaining wide attention. For the regeneration of bladder tissues, possible differentiation of ADSCs into bladder smooth muscle and epithelial cells has been demonstrated. For the treatment of bladder diseases, specifically hyperlipidemia and associated overactive bladder, ADSCs have also demonstrated efficacy. For the treatment of urethral sphincter dysfunction associated with birth trauma and hormonal deficiency,

ADSC therapy was also beneficial. Finally, ADSCs were able to restore erectile function in various types of erectile dysfunction (ED), including those associated with diabetes, hyperlipidemia, and nerve injuries. Thus, ADSCs have demonstrated remarkable therapeutic potentials for the lower urinary tract.

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### INTRODUCTION

The lower urinary tract can become dysfunctional due to aging, diabetes, obesity, and other factors. Although usually not life-threatening, problems of the urinary bladder, urethra, and penis can severely impact the patient's quality of life and impose heavy financial burdens on the individual and society. Current treatments for these diseases are ineffective or invariably temporary, symptomatic, and/or accompanied by adverse side effects. Therefore, stem cells (SCs), owing to their regenerative capacity, are considered promising curative agents for these urological diseases.

In this regard, several kinds of SCs have been studied, including embryonic SCs (ESCs), bone marrow SCs (BMSCs), skeletal muscle-derived SCs (SkMSCs), adipose tissue-derived SCs (ADSCs), and amniotic fluid SCs (AFSCs). While ESCs and BMSCs, owing to their earlier discoveries, are the best studied SCs in most medical disciplines, it is SkMSC research that has advanced ahead of other kinds of SC research and these cells have been used in clinical trials in urological research. However, ADSCs are

much easier than SkMSCs to obtain in quantity and have been shown to possess properties very similar to BMSCs. In several animal studies that are recently published or in press, ADSCs have demonstrated efficacy in the treatment of various types of dysfunctional bladder, urethra, and penis<sup>[1-4]</sup>. For detailed discussions on the general properties of ADSCs and other SC types mentioned above, several review articles are available<sup>[5-9]</sup>. In this editorial article, attention is focused on SC clinical and pre-clinical applications; it is organized into three sections according to diseases of the bladder, urethra, and penis.

## BLADDER

SC research has been conducted for two situations regarding the bladder. One is treatment of urge urinary incontinence (UUI), which is defined as the involuntary loss of urine associated with a strong sensation to void. Many risk factors are associated with UUI, one of which is hyperlipidemia. In a rat model of hyperlipidemia-associated UUI, ADSCs have recently been shown to improve continence<sup>[1]</sup>. Administration of ADSCs through intra-bladder injection or tail vein injection was equally effective. Functional improvement was accompanied with tissue improvement, as treated subjects showed enhanced muscle, vascular and nerve contents compared to controls. In preclinical studies of UUI related to diabetes or birth trauma/menopause, ADSC treatment was also effective (data not published).

The other is bladder restoration or augmentation; that is, the need to replace part of or the entire bladder or to increase the size of the bladder. Bladder restoration or augmentation requires tissue engineering to recreate the native bladder milieu. Currently, the favored approach involves seeding a scaffold, usually an acellular matrix, with autologous bladder smooth muscle and epithelial cells. However, a significant drawback of this approach is the risk of reintroducing the pathologic condition (e.g. cancer) to the engineered tissue. For this and several other reasons, SCs are considered as an ideal alternative to using autologous bladder cells. To this end, acellular matrix seeded with embryoid body-derived SCs has been shown to facilitate the complete regeneration of partially cystectomized bladder<sup>[10]</sup>. However, whether the seeded cells had differentiated into bladder cells remained unclear. On the other hand, possible differentiation of SkMSCs into bladder smooth muscle cells (SMCs) has been reported<sup>[11]</sup>, and this was followed by a related report demonstrating contractility of seeded SkMSCs<sup>[12]</sup>.

Possible differentiation of BMSCs into bladder SMCs has also been reported in several studies<sup>[13-17]</sup>. One of these studies<sup>[13]</sup> showed that BMSCs or AFSCs transplanted into cryo-injured rat bladder underwent limited SMC differentiation. Although differentiation of ADSCs into bladder SMCs has been reported<sup>[18,19]</sup>, we found this to be a rare occurrence and suggested that ADSC therapeutic effects were principally mediated by paracrine actions<sup>[1]</sup>. Urothelial differentiation of SCs is expected to be more

difficult than SMC differentiation because the urothelial cells are highly specialized entities both in structure and in functionality; and this adds to what is already a challenging task of transdifferentiating from the mesenchymal to the epithelial lineage. However, despite these tremendous odds, a recent study showed that ADSCs were able to express certain urothelial markers when co-cultured with preexisting urothelial cells<sup>[20]</sup>. Interestingly, this probable urothelial differentiation of ADSCs required direct cell-cell contact with the pre-existing urothelial cells.

Thus, it appears that conventional strategies, such as growth factors and gene transfer, will not be sufficient to direct differentiation of ADSCs, and possibly other SCs, into functional urothelial cells. Nevertheless, this latest study reiterates the remarkable differentiation potential of ADSCs (at least, *in vitro*) and hopefully, with continued research efforts, it may be possible someday to convert ADSCs into a useful urothelium.

## URETHRA

The urethra is the most studied urological organ as far as SC therapy is concerned. This is perhaps due to the assumption that restoration of the urethral musculature alone would be sufficient to correct the most frequently encountered urethral problem; i.e. sphincter deficiency, which manifests symptomatically as stress urinary incontinence (SUI). While primarily a female concern, because of pregnancy and parturition-associated injuries to the urethra, SUI can also occur in men due to prostate surgeries.

Initial cell-based experimental therapy for SUI involved the injection of autologous skeletal myoblasts into the vicinity of the urethral sphincter<sup>[21]</sup>. It then progressed to substituting myoblasts with SkMSCs, and eventually several clinical trials with SkMSCs were conducted, resulting in 3 publications from an Austrian group<sup>[22-24]</sup> and one from an American research team<sup>[25]</sup>. Although clinical outcomes of these studies are generally favorable, a clear disadvantage of SkMSCs is the requirement for complicated isolation procedures and long-term culturing, as skeletal muscle cannot be practically obtained in quantity from the patient.

Application of other types of SCs, including BMSCs, may also pose the same problem if cells are to be employed autologously. The only exception is ADSCs because, in our increasingly obese society, adipose tissue is often considered dispensable, and the commonly performed liposuction procedure is capable of safely isolating large quantities of adipose tissue. Furthermore, it has been shown that ADSCs can be isolated and injected back into the same patients for successful breast augmentation in approximately 4 h<sup>[26]</sup>; therefore, it is reasonable to expect that ADSCs can be used to treat the much smaller urethra on a same-day basis without the need for culturing. Thus, as a first step toward this goal, we recently demonstrated the efficacy of ADSCs in treating SUI in an animal model<sup>[2]</sup>. We showed that tail vein injection of ADSCs was equally effective as intra-urethral injection, thus pointing to the possibility of using the convenient

intravenous route for administering ADSCs clinically. We also showed that ADSC treatment restored not only the cellular (SMC) but also the extracellular (elastin) components in the experimentally injured rat urethra. Thus, it appears that ADSCs have the potential to “cure” SUI by correcting the underlying cellular and extracellular defects in the injured urethral sphincter.

## PENIS

Phosphodiesterase type-5 (PDE5) inhibitors are currently the first-line treatment of choice for men with erectile dysfunction (ED). However, PDE5 inhibitors are strictly contraindicated in men taking nitrate therapy and are known to cause a variety of adverse side effects that may reduce their suitability for some patients. More importantly, PDE5 inhibitors are ineffective in treating certain types of ED including those associated with diabetes, hyperlipidemia, and surgery-induced penile nerve injuries. As such, alternative treatments, particularly those that can treat the underlying disease process of ED, would be preferable to currently available interventions.

In this regard, one of the strategies currently being evaluated is stem cell therapy. In 2003, Deng *et al*<sup>[27]</sup> showed that BMSCs transduced with endothelial nitric oxide synthase (eNOS) were able to improve erectile function in aged rats. In 2004, Bochinski *et al*<sup>[28]</sup> showed that ESC transduced with brain-derived neurotrophic factor improved erectile function in a rat model of post-prostatectomy ED. In 2007, Bivalacqua *et al*<sup>[29]</sup> demonstrated that BMSCs alone or transduced with eNOS were able to reverse age-associated ED. Also in 2007, Song *et al*<sup>[30]</sup> showed that immortalized human BMSCs (by v-myc transfection) transplanted into rat corpus cavernosum could differentiate into endothelial cells and SMCs. In 2008, Fall *et al*<sup>[31]</sup> reported that intracavernous injection of BMSCs improved erectile function in a rat model of post-prostatectomy ED. Also, in 2008, Nolzco *et al*<sup>[32]</sup> indicated that intracavernous injection of SkMSCs could restore cavernous SMCs and erectile function in aged rats.

The therapeutic potential of ADSCs for ED was recently reported<sup>[33]</sup>. Specifically, we showed that *in vitro* endothelial differentiation of ADSC, mediated by FGF2 and ADSC injected into rat penis, appeared to have differentiated into endothelial cells. Since the cavernous endothelium plays a key role in penile erection and is often damaged in disease processes such as diabetes and hyperlipidemia, it is important that we provide further evidence that ADSC can restore endothelial function in the erectile tissue. To this end, we recently demonstrated the efficacy of ADSCs to treat diabetes and hyperlipidemia-associated ED, respectively<sup>[3,4]</sup>. In both studies, functional improvements were accompanied with restoration of the crucial endothelial and neural components in the penis, suggesting the curative prospect of ADSC treatment. In another study of postprostatectomy ED, ADSCs were also effective in restoring the damaged nerves and improving erectile function (data not published). Thus, it appears that ADSC has the potential to treat various forms of ED.

## CONCLUDING REMARKS

Several types of SCs have been investigated for the treatment of lower urinary tract diseases. Specifically, BMSCs, SkMSCs, and AFSCs have been tested in preclinical studies for bladder augmentation and detrusor regeneration with various degrees of efficacy. In addition, clinical trials on SkMSC therapy for SUI have produced favorable outcomes. Moreover, ESCs, BMSCs, and SkMSCs were shown to improve erectile function in animal models of age-related and postprostatectomy ED. However, the afore-mentioned SC types suffer from ethical and/or availability concerns. Conversely, ADSCs are an abundant cell source and have been shown to possess similar biological properties and therapeutic potentials as BMSCs. In particular, ADSCs seemed able to, at least partially, differentiate into the complex and highly specialized urothelial cells. In regard to ADSC therapeutic potential for lower urinary tract diseases, recent pre-clinical studies have produced favorable results. Specifically, ADSCs were able to restore near normal function in animal models of hyperlipidemia-associated overactive bladder, birth trauma-induced SUI, hyperlipidemia-associated ED, and diabetic ED.

Despite these advances, however, challenges facing urology and other medical disciplines are numerous. In regard to ESC, ethical and tumorigenicity concerns are paramount. In regard to adult SCs, can they really transdifferentiate *in vivo* and thereby replenish the degenerated tissue? Or, do they simply secrete certain growth factors that help the host tissue to regenerate? More importantly, how “translatable” are pre-clinical studies? That is, can animal models, which are designed to display a specific human disease entity, faithfully represent human patients who are prone to having co-morbidity? In any event, answers to these questions are probably easier to find in urological research than in other disciplines because the lower urinary tract organs are relatively simple in structure and are easily accessible. Thus, advances in SC therapy for the lower urinary tract are the forerunners of SC research.

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