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Stem cell therapy for voiding and erectile dysfunction

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Abstract

Voiding dysfunction comprises a variety of disorders, including stress urinary incontinence and overactive bladder, and affects millions of men and women worldwide. Erectile dysfunction (ED) also decreases quality of life for millions of men, as well as for their partners. Advanced age and diabetes are common comorbidities that can exacerbate and negatively impact upon the development of these disorders. Therapies that target the pathophysiology of these conditions to halt progression are not currently available. However, stem cell therapy could fill this therapeutic void. Stem cells can reduce inflammation, prevent fibrosis, promote angiogenesis, recruit endogenous progenitor cells, and differentiate to replace damaged cells. Adult multipotent stem cell therapy, in particular, has shown promise in case reports and preclinical animal studies. Stem cells have also enabled advances in urological tissue engineering by facilitating *ex vivo* construction of bladder wall and urethral tissue (using a patient's own cells) prior to transplantation. More recent studies have focused on bioactive factor secretion and homing of stem cells. In the future, clinicians are likely to utilize allogeneic stem cell sources, intravenous systemic delivery, and *ex vivo* cell enhancement to treat voiding dysfunction and ED.

Keywords

stem cell therapy; urology; tissue engineering; voiding; bladder dysfunction; urinary incontinence; erectile dysfunction; MSC; urothelium

Introduction

Voiding and erectile dysfunction (ED) constitute two of the most common, yet challenging, conditions managed by genitourinary specialists. Voiding dysfunction constitutes a wide range of pathologies, including stress urinary incontinence (SUI) and overactive bladder (OAB).¹ Collectively, these conditions affect millions of patients worldwide and have a significant impact on quality of life (QOL). ED also affects millions of men and is associated with substantial reductions in QOL. The pathophysiology of these conditions often involves acute injury (for example, following radical prostatectomy) and is worsened by chronic comorbid conditions, such as diabetes and obesity.^{2,3}

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To date, few treatments have been developed that target disease progression.⁴ Current standard-of-care therapies, such as the sling for SUI and phosphodiesterase type 5 inhibitors for ED, tend to focus on symptom relief rather than correcting pathophysiology. Surgical treatment is often indicated for these conditions and frequently involves the placement of a foreign body—such as a midurethral sling or inflatable penile prosthesis—which can lead to infection or erosion.^{5,6} Ideally, treatment would prevent disease progression and restore anatomy with disease-free tissue. Stem cell therapy holds great promise, in terms of both treating pathophysiology and urological tissue engineering and regeneration. Studies into the use of stem cell therapy to treat voiding dysfunction and ED have generally involved either embryonic or adult multipotent stem cells. Although induced pluripotent stem cells (iPSC) have been studied in the context of treating urologic malignancies, these cells have yet to be investigated for patients with other urological conditions. The use of adult multipotent, rather than embryonic, stem cells circumvents certain issues regarding the ethical use and acquisition of cells, their tumorigenic potential, and the potential rejection of cells by the recipients immune system.^{7,8} Mesenchymal stem cells (MSCs), a group of adult stem cells that can be obtained from almost every tissue of the body, hold particular promise for the treatment of voiding dysfunction and ED.⁹ These cells have great differentiation capabilities and are known to secrete a host of bioactive factors that are beneficial for tissue repair. Furthermore, MSCs, in conjunction with various scaffolds, can be used to regenerate tissues for reconstructive applications.

In this review, we provide an overview of the use of stem cell therapy for voiding dysfunction and ED. We identify stem cell sources and isolation techniques, and review several potential mechanisms of action. We provide a summary of major clinical studies, as well as preclinical animal studies, that illustrate potential mechanisms and identify opportunities for further investigation. Finally, we acknowledge hurdles in the transition from laboratory research to clinical practice as stem-cell-based therapies and tissue engineering techniques are incorporated into treatment algorithms for patients with these widespread and challenging disorders.

Key advances in urological stem cell therapy

Stem cell sources

Bone-marrow-derived MSCs (BMSCs) were the first stem cells to be described and are now the most well characterized.¹⁰ However, BMSCs are rare in bone marrow (approximately 1 per 10,000 cells) and harvesting these cells can confer significant morbidity to patients.¹¹ The primary source of stem cells for urological trials has been autologous muscle-derived stem cells (MDSCs). However, although these cells are a more accessible resource than other types of adult stem cells, they require a harvest procedure and ex vivo expansion process that lengths time to treatment.^{12–24} Investigators continue to identify other novel sources of stem cells for urologic applications, including placental and amniotic fluid stem cells,¹⁵ menstrual fluid stem cells,¹⁶ cells derived from umbilical cord,¹⁷ urine-derived stem cells,¹⁸ and stem cells derived from hair follicles.¹⁹ The abundance of adipose tissue and its dense vascularity has meant that adipose-derived stem cells (ADSCs) can be isolated in large quantities, making this cell type a suitable alternative for clinical application.²⁰

Although embryonic stem cells (ESC) and iPSC provide promising avenues for further research, neither have been used to treat voiding dysfunction or ED as yet. Very small embryonic-like (VSEL) stem cells—primitive adult stem cells that mobilize in the peripheral blood after injury or appropriate stimuli to participate in repair—have demonstrated potential as a novel source of cell-based therapeutics. However, these cells have yet to be utilized for urologic applications.²¹

Although significant progress has been made in terms of characterizing MSCs *in vitro*, the *in vivo* characteristics of MSCs remain elusive. Crisan *et al.* provide evidence to suggest that the location and cell surface marker expression of MSCs is similar to that of pericytes (perivascular cells) and that a subset of pericytes maintains the *in vitro* differentiation capacity of MSCs.²² MSCs could thus be a subset of pericytes, which would explain their presence in almost every organ of the body. However, contrary to this finding, other researchers have demonstrated that human BMSCs and ADSCs assume pericyte-like marker expression and phenotype in hypoxic culture and *in vivo* under inflammatory conditions.²³ This would suggest that MSCs attain pericyte-like functions when they are required to reduce inflammatory damage and improve vascularity.

Regardless of their exact location within tissues, MSCs retain niche specificity. MSCs from different tissues require different conditions for *in vitro* differentiation and have differing gene expression profiles.^{24–26} This variance (in terms of potential to differentiate and secrete bioactive factors) suggests that certain types of MSCs might be more appropriate for treating particular disorders.

Stem cell isolation

The procedure for collecting stem cells typically involves at least three stages.²⁷ The patient first undergoes a biopsy (if muscle then generally of the quadriceps) to harvest the cells. The cells are then transported to a regulated facility, usually off-site, where the stem cells are isolated from other cell types and grown until they reach adequate numbers. The process of *ex vivo* stem cell expansion, which involves cycles of differentiation and senescence, inherently alters the pathophysiology of the cells.^{28,29} Although repeat cell sorting immediately prior to delivery can filter out non-stem-cell populations, this process is expensive and time-consuming. Therefore, the cells that are ultimately delivered to the patient, generally via local injection, might be a heterogeneous combination of stem cells and differentiated cells.

Transplantation

MSCs are known to induce a characteristically weak allogeneic immune response when transplanted from donor to recipient.³⁰ This response is thought to result from reduced major histocompatibility complex (MHC) class I expression, ablated MHC class II (or costimulatory molecule) expression, and suppression of immune cell function (by direct or indirect methods). Additionally, MSCs have been shown to actively suppress the proliferation of T-cells *in vitro*.³¹ MSCs can be easily xenotransplanted into unrelated, nonimmunodeficient animals.^{27,28} However, in humans, MSCs are generally cotransplanted with allogeneic haematopoietic stem cell transplants to suppress the immune response of the

recipient and prevent complications.³² In the future, it is feasible that preharvested cells might be suitable for 'off-the-shelf' allogeneic use, eliminating the need for separate harvest and delivery procedures. Already, allogeneic cells have been successfully used in human clinical trials of cardiology patients and in urological single case reports.^{33,34}

Given that cells isolated from patients with genetic predispositions to urological conditions (such as voiding dysfunction and ED) could function suboptimally when reimplanted, allogeneic cells could be of particular use for managing patients with genetic risk factors for these conditions.^{31,32,34} This hypothesis has been supported by several studies that have used preclinical animal models to show the beneficial effects of transplanting allogeneic MSCs into mice that are genetically predisposed to developing muscular dystrophy.³⁵ Case reports have hinted at the potential role of allogeneic MSCs for treating severe genetic disorders. In one study, Horwitz *et al.* treated six children with osteogenesis imperfecta (OI) with allogeneic BMSCs and reported a significant increase in growth velocity without significant adverse effects.³⁶ A similar result was reported by Le *et al.*, who performed an intrauterine transplant of allogeneic foetal-liver-derived MSCs into a foetus with severe OI.³⁷ These studies indicate that treating patients with allogeneic MSCs is a viable option that could eliminate the need for staged procedures and benefit patients with genetic predispositions to conditions such as voiding dysfunction or ED.

Homing and delivery route

In contrast to tissue-specific MSCs, such as ADSCs or MDSCs, stem cells that are derived from the bone marrow circulate throughout the bloodstream and migrate to areas of tissue injury and ischemia—directed via a gradient of cytokines released by damaged tissues.³⁸ Homing cytokines, such as stromal derived factor-1 (SDF-1), have been shown to improve functional outcomes—by attracting circulating haematopoietic stem cells—when delivered directly to the hearts of rats with ischaemic cardiomyopathy.³⁹ Within the context of urological research, the effects of homing cytokines on SUI have been studied most commonly in rats with vaginal distension (VD). Woo *et al.* showed that levels of these cytokines were significantly increased in the urethra and vagina of rats with VD.⁴⁰ This observation was supported by findings from Wood *et al.*, who noted that the upregulation of cytokine expression increased with the duration of VD.⁴¹ Vricella *et al.* reported similar results in obese mice with SUI, suggesting that obesity might also stimulate the secretion of homing cytokines.⁴² Cruz *et al.* used *in vivo* bioluminescence imaging to demonstrate homing of BMSCs (injected via tail vein) to the pelvic organs of rats in response to VD.⁴³ Lin *et al.* also showed that systemic injection of ADSCs was as effective as local injection of ADSCs in terms of functional outcomes, such as elastin organization in the urethra.⁴⁴

Dissaranan *et al.* suggested that the pelvic organs might not be the only sites at which stem cell homing occurs upon simulated childbirth injury.⁴⁵ In this study, a significant number of stem cells were also identified in the spleens of rats with VD. The study authors speculated that systemically injected cells might modulate the immune system via the spleen. Lin *et al.* also found that ADSCs injected into the corpus cavernosa of rats that have undergone cavernous nerve crush migrated to the bone marrow.⁴⁶ Injecting umbilical cord blood stem cells (uCBSCs) intracavernosally into men with diabetic ED has been shown to result in

improved blood glucose levels, and decreased medication dosages and glycosylated hemoglobin levels.³³ These preclinical and clinical data suggest that MSCs have an important systemic effect that can be exerted even when therapy is delivered locally.

Although local injection of stem cells results in immediate distribution to the affected area, the stem cell homing that accompanies systemic delivery has its own advantages—particularly in relation to targeting diseases with lower levels of focal injury and modulating the immune response locally and potentially via splenic localization. If a time gap exists between injury and treatment administration, homing can be impaired because of a lack of ongoing tissue damage and resultant cytokine expression. A similar obstacle has been circumnavigated in cardiology studies. The use of stem cell therapy to increase expression of SDF-1 following a myocardial infarction has a proven benefit in both rats and humans (based on data from a phase I clinical trial).^{39,47} Electrical stimulation has been shown to facilitate stem cell migration *in vitro* and could, theoretically, be applied to the target organ *in vivo* to increase the homing effect.^{48–50} Thus, novel techniques could improve homing in patients whose treatment is initiated long after injury has occurred.

Stem cell differentiation

Initially, the efficacy of stem cell therapy was attributed to the differentiation potential of stem cells. MSCs, for example, are defined by their ability to differentiate *in vitro* into chondroblasts, osteoblasts, and adipocytes.⁵¹ Stem cell therapy in urology has largely focused on the induced differentiation of stem cells into muscle tissue for urethral sphincter regeneration and urothelium tissue for bladder and urethral reconstruction.⁵² For example, pretransplantation enhancement of cells by induced differentiation with 5-azacytidin has been investigated.^{52,53} The differentiation of MSCs into urothelium is a somewhat controversial finding given the endodermal lineage of bladder and urethra urothelium. Nevertheless, several investigators have shown that MSC differentiates into urothelial-like cells under appropriate conditions^{54,55}—probably because the renal pelvis and ureter urothelium are derived from mesoderm⁵⁶—and the *ex vivo* differentiation of stem cells remains a vital step in the use of tissue engineering for reconstructive purposes.

Bioactive effects of stem cells

Recently, a paradigm shift has occurred in stem cell biology to focus attention on the paracrine, autocrine, and growth factor effects of stem cells.^{57,58} MSCs are known to have anti-apoptotic, anti-scarring, and neovascularization effects, as well as systemic and local immunomodulatory properties, including the inhibition of T-cell and B-cell proliferation. In addition to their role as effector cells, MSCs also activate and direct endogenous stem and progenitor cells to areas of injury by secreting cytokines and chemokines.⁵⁹

In support of this finding, many of the functional and histological improvements associated with stem cell therapy have seemed disproportionate to the number of cells that engraft to injured organs. Lin *et al.* showed that, although only a limited number of ADSCs engrafted into the urethra of rats subjected to VD and ovariectomy, these cells had a profound effect.⁴⁴ Dissaranan *et al.* reported similar results with BMSCs in response to simulated human childbirth injury.⁴⁵ A cardiology study illustrated this point by injecting BMSCs into the

hamstrings of hamsters with heart failure.⁶⁰ Although cells were unable to migrate out of the muscle, they had significant functional and histological benefit to the heart.

The importance of bioactive factors secreted by stem cells unveils the possibility of harnessing these factors in the laboratory and utilizing them as cell-free treatments. These treatments can be produced *in vitro* and stored as 'off-the-shelf' therapeutics for immediate delivery without the need for harvest and expansion. Furthermore, they are potentially cell-free, thus eliminating the risk of rejection, immune reaction, and tumourigenic potential. *In vitro* stem cell enhancement—for example, by genetically modifying cells to enhance production of bioactive factors—is another potential focus for future research.

Stem cell therapy for voiding dysfunction

Stress urinary incontinence

Several studies have demonstrated the short-term safety and efficacy of stem cell therapies for SUI. Carr *et al.* showed that injecting autologous MDSCs into the urethral sphincters of eight women with SUI resulted in no significant adverse effects and five of the eight women experienced improvement in their symptoms one year after treatment.⁶¹ The same group later confirmed the safety of this treatment approach in a larger blinded study and reported significant improvements in the frequency of stress leak episodes, as well as improvements in quality of life indicators, one year after periurethral injection of autologous MDSCs.⁶² Peters *et al.* presented supportive data to show the safety of autologous MDSC injections in a large, multicentre trial of patients with SUI.⁶³ Although the study was underpowered to determine the exact efficacy of each treatment dose, the data suggest a general correlation between increasing stem cell dose and benefit to the recipient. However, these clinical studies failed to elucidate the mechanism of action of stem cells, partly because, in humans, we are unable to track the injected cells *in vivo* and it can be difficult to acquire human tissue for analysis. Thus we rely upon animal models to supplement our understanding of how stem cells might alter the disease process.

Numerous animal models have sought to address the various causes of SUI, including mechanical injury during vaginal childbirth, pudendal nerve injury, external urethral sphincter (EUS) injury, and the effects of hormonal changes, such as those associated with the menopause.^{64–67} Local injections of BMSCs, MDSCs, and ADSCs have all demonstrated therapeutic potential in animal models that have sustained either mechanical, nerve, or EUS injury—improving both functional and anatomical outcomes.^{44,68,69} However, levels of stem cell viability and engraftment following delivery are uncertain and have been the focus of some debate. A recent study by Corcos *et al.* reported an association between BMSC differentiation, smooth muscle antigen upregulation, and desmin expression in a rat model of SUI.⁷⁰ In an effort to enhance stem cell retention, cells were delivered concomitantly with either poly lactic-coglycolic acid (PLGA), nerve growth factor (administered via controlled release), brain-derived neurotrophic factor, or calcium concentration microdomains, resulting in significant improvements in leak point pressure, muscle mass, and neuronal density compared to delivery of stem cells alone.⁷¹

Coinciding with the general paradigm shift in stem cell biology towards utilizing MSCs as vehicles for the delivery of bioactive factors, some researchers have begun to investigate the *in vitro* products of these cells. In a rat model of postpartum SUI, we have shown that administering either a systemic injection of BMSCs or a concentrate of the conditioned media (CCM) produced *in vitro* results in functional and histologic improvements compared to controls.⁴⁵ Our data, suggest that paracrine secretions have an important role in the recovery process. A key function of stem cells might thus relate to stem cell homing to areas of injury to stimulate the local secretion of bioactive factors.

Bladder dysfunction

With regards to other forms of voiding dysfunction, such as OAB and underactive bladder (UAB), preclinical studies of stem cell therapy have also demonstrated success. Nishijima *et al.* showed that presumptive BMSCs injected into the bladder walls of rats with bladder outlet obstruction (BOO) restored bladder contractility.⁷² These investigators also observed the differentiation of GFP-labelled cells into smooth muscle tissue. However, the isolation techniques used in this study did not specifically sort for BMSCs, suggesting that other (more downstream) muscle progenitor cells, such as satellite cells, could have differentiated into smooth muscle tissue *in vivo* instead.

Huang *et al.* showed that injecting ADSCs locally (via the tail vein) into hyperlipidaemic rats with OAB improved bladder function by enhancing angiogenesis and cholinergic innervation.⁷³ The study authors tracked cells to the bladder and noted that more ADSCs collect within the bladder following local injection compared to systemic injection, although few differentiated into smooth muscle tissue. ADSCs were shown to improve nerve and blood vessel density—overcoming, in part, one of the key challenges associated with tissue engineering. However, it is unclear whether the increased number of neurons results from increased levels of cell survival or regeneration. Woo *et al.* delivered BMSCs intravenously into mice with BOO and observed evidence of histological and functional improvement.⁷⁴ Both these studies illustrated the ability of stem cells to initiate bladder remodelling 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.^{73,74}

Preclinical animal studies continue to provide the impetus and rationale for performing clinical trials to analyze the efficacy of systemic cell delivery and cell-free CCM delivery. Future research aims include determining the duration of time after injury (or disease onset) that equates to optimal release of homing cytokines and developing innovative methods for upregulating the expression of these cytokines, such as electrical stimulation.

Stem cell therapy for erectile dysfunction

Very few clinical trials have used stem cells to treat ED. One study of note demonstrated that treating patients with type 2 diabetic ED ($n = 7$) with human uCBSCs via intracavernosal injection resulted in some short-term benefit (over a period of several months).³⁰ Importantly, no significant adverse effects were observed. Furthermore, a significant glucose-lowering effect was observed in all patients that persisted over several

months, resulting in substantial decreases in diabetes medication dose. Encouragingly, stem cell therapy for ED has also proven successful in preclinical animal models and the results of these studies support the theory that bioactive factors confer an important mechanism of action. For example, Huang *et al.* showed that, although numbers of ADSCs injected intracavernosally into rat penises decreased significantly after 14 days they exerted a functional effect for up to 1 month compared to controls.⁷⁵ In a rat model of cavernosal nerve (CN) crush, Albersen *et al.* compared the intracavernosal injection of whole ADSCs versus ADSC lysate (osmotically ruptured cell contents that are isolated from sediment and insoluble material).⁷⁶ Administering either cell lysate or whole ADSCs significantly improved functional outcomes and histology after 4 weeks. Furthermore, no differences between cell lysate and ADSC treated groups were seen. This suggests that ADSCs influenced the repair of injury through the paracrine-mediated release of bioactive factors that remained present and active in the cell lysate.

Researchers have also attempted to genetically modify stem cells prior to penile injection to specifically target different components of the erection pathway. With the aim of targeting a neural pathway component, Bivalacqua *et al.* engineered MSCs to express endothelial nitric oxide synthase (eNOS), which dilates cavernous smooth muscle tissue and engorges the penis.⁷⁷ When injected into 25-month old rats with age-induced ED, the modified MSCs were associated with a more rapid improvement in erectile function compared to wild type MSCs. Qiu *et al.* attempted to target the vascular pathway component of erectile function by injecting MSCs engineered to express vascular endothelial growth factor (VEGF) into diabetic rats with ED.⁷⁸ Although injecting mice with either *VEGF*-transfected MSC or wild-type MSC resulted in functional and histological improvement, benefits were more pronounced in the *VEGF*-transfected MSC group.

However, the safety concerns associated with genetically modifying cells, especially after viral transduction, cannot be ignored when such technology is applied to humans. Clinical trials are being conducted that investigate the effect of intracoronary injection of adeno-associated viral vectors into patients with heart failure.⁷⁹ Ultimately, the added benefits of enhanced bioactive factor production must be weighed against the risk of contamination and unanticipated genetic modification.

Stem cell use in urological tissue engineering

The use of tissue engineering in urology has largely focused on the construction of bladder and urethral wall for augmentation. Popular strategies have utilized free (scaffold-free) grafts from engineered cells, as well as grafts with scaffolds (with and without cell seeding). Cell-seeded scaffolds are thought to provide better outcomes than bare scaffolds, for which tissue regeneration is dependent on in-growth of the surrounding tissue.^{80,81}

Studies of bladder or urethral reconstruction with free grafts have focused primarily on tissue engineering with urothelial cells (UC) or smooth muscle cells (SMC). UC can be isolated from bladder washings and cultured to produce bioartificial urothelium, which has been used to reconstruct urethral damage, such as strictures.^{82,83} Currently, the multilayer urothelium transplant is generally not stable enough for suturing and handling during the

surgical approach, but this obstacle could potentially be overcome in the future with the use of fibrin glue or scaffolds (pending testing in animal models).⁸⁴

Scaffolds destined for cell seeding should promote growth and proliferation of either the transplanted or host cells (or both), enabling them to support the structure and function of engineered tissue. In addition, these scaffolds must be biocompatible and should possess the mechanical and physical characteristics required to function properly at the target site.⁸⁰ Taking these requirements into account, naturally derived biomaterials—such as collagen, fibrin, or acellular tissue matrices—are advantageous compared to most synthetic materials, which often lack adequate cell-binding properties. Naturally derived scaffolds can be modified by molding, extrusion, phase separation techniques, or electrospinning to create an optimal structure and surface (in terms of porosity and polarity) and ratio of surface area to volume. These techniques can also be used to change the degradation (reabsorption) time associated with scaffolds and the degradation products that are released.⁸⁵

Studies in bladder tissue engineering

In one of the first clinical studies of bladder tissue engineering, Lai *et al.* demonstrated that transplanting either healthy or diseased bladder tissue could give rise to functional muscle and urothelium tissue *in vitro*.⁸⁶ Atala *et al.* later reported on the progress of seven patients (age 4–19 years) with poorly compliant bladders (mean preoperative capacity was 207 mL) secondary to myelomeningocele treated with augmentation cystoplasty using autologous bladder biopsy-derived smooth muscle and urothelial cells.⁸⁷ The authors reported improved bladder function after cystoplasty over a follow-up period of up to 5 years. However, patients were recruited over three years and details regarding the surgical implantation technique—including the type of acellular scaffold used and the use of an omentum wrap to aid revascularization—were modified as the study progressed. Although this approach enables the incorporation of new techniques, it limits the clinical utility of the study findings. However, contrary to these early findings, research by Subramaniam *et al.* in pediatric patients has indicated that diseased urothelium displays reduced proliferative and differentiation capacity *in vitro*.⁸⁸ Regardless, bladder augmentation and replacement is critical to improve current outcomes for patients requiring partial cystectomy and cystectomy with neobladder construction for oncologic indications. Given the risk of harvested autologous bladder tissue harbouring microscopic disease, alternative sources of cells, such as allogenic stem cells or autologous stem cells from other organs, should be considered.

The optimal scaffold type for bladder tissue engineering remains unknown. Results associated with the use of stem-cell-seeded scaffolds for cystoplasty vary and are probably dependent on the way in which the small intestine submucosa (SIS) scaffold is set up.⁸⁹ Chung *et al.* reconstructed rat bladder with SIS seeded with BMSCs and demonstrated formation of organized urothelium, discrete muscle fascicles, and positive nerve staining.⁹⁰ Sharma *et al.* performed bladder augmentation using MSC-seeded SIS in baboons and reported typical bladder histoarchitecture.⁹¹ Quantitative morphometric analyses revealed muscle-to-collagen ratios of approximately 32% and 52% in animals treated with unseeded and seeded SIS scaffolds, respectively, 10 weeks after augmentation.

Synthetic scaffolds have been utilized to avoid issues such as structure variability and host response that are commonly associated with biological alternatives. Sharma *et al.* utilized poly(1,8-octanediol-co-citrate) (POC) to reconstruct partially cystectomized athymic nude rats.⁹² POC scaffolds, seeded with human MSCs and UCs, supported partial regeneration of bladder tissue *in vivo*. Similarly, other researchers have shown that predifferentiated BMSCs seeded onto 3D nano scaffolds can be used to regenerate bladder tissue⁹³ and ADSCs seeded onto synthetic bladder composites⁹⁴ also have potential for future use.

Studies in urethral tissue engineering

Urethral tissue construction for the repair of urethral defects has undergone similar preclinical investigations to bladder construction and has been associated with many of the same challenges. However, urethral defects are generally smaller and more accessible to native cells migrating across unseeded acellular scaffolds. Preclinical animal studies and clinical trials have shown success using different types of bioscaffolds without stem cell seeding.^{86,88} However, it is worth noting that bare scaffolds depend on native tissue for regeneration, which can only migrate distances of up to 0.5 cm.⁹⁵

Stem-cell-seeded scaffolds could potentially overcome this limitation. Preclinical studies in rabbits have shown that a tubularized, acellular collagen scaffold seeded with autologous cells from bladder biopsies can be used to repair and regenerate the urethra, generating tissue with a normal histological appearance.⁹⁶ Other investigators have also used rabbit models to show that the use of unseeded scaffolds with growth factor supplementation can negatively affect outcomes (resulting from urethral narrowing and dilation) compared to scaffolds alone.⁹⁷ These data suggest that enhanced tissue growth *per se* does not necessarily equate to favourable outcomes. Instead, a critical balance must be gained in a controlled manner.

In a clinical study by Raya-Rivera *et al.*, five boys with posterior urethral defects underwent repair with tubularized collagen–polyglycolic acid scaffolds seeded with autologous cells.⁹⁸ All five patients had functional urethras—determined by uroflowmetry, voiding cystourethrogram, and cystoscopy—and were both continent and voiding without difficulty after a median of 71 months follow-up (although one patient required a pubovesical sling after surgery for daytime SUI). However, despite the success of this clinical series, tissue engineering of the urethra remains a significant engineering challenge and further clinical studies are needed to validate these findings.⁹⁹

Studies that have considered the longer-term follow-up of patients implanted with seeded scaffolds have provided some encouraging preliminary safety data regarding the fate of stem cells.^{87,98} However, many of the *in vivo* studies that have demonstrated functional improvement with delivery of stem cells have also encountered difficulties determining the ultimate fate of these cells.^{44,45,75} The *in vivo* effects of stem cells probably extend beyond cell engraftment, differentiation, and retention. Potential tumourigenesis remains a common concern and, therefore, a potential barrier to widespread clinical adaptation. A shift in focus, from the short-term functional and histological outcomes to the longer-term potential for tumor formation, might help to address this issue.

Conclusions

Although preclinical data and clinical case reports have suggested a potential role for stem cells in the treatment of voiding dysfunction and ED, as well as in urological tissue engineering, more research is needed before stem cells can be fully integrated into clinical practice for urology. The potential role of stem cells in aiding functional recovery in patients with SUI, bladder dysfunction, and ED is incompletely understood. Clinical studies have not yet elucidated mechanisms of action and data from animal models is conflicting. With a few notable exceptions, most studies have established that stem cells do not persist in the target organ for extended periods of time after acute injury. Differentiation is not consistently present during periods of repair. Bioactive factors are thus credited with improvements in functional parameters. Furthermore, it has been suggested that bioactive factors are systemic in nature, which could explain reports of increased homing to the spleen and migration of cells to bone marrow. Nonetheless, the transplantation of stem cells that have been genetically modified to enhance delivery to the target organ can improve patient outcomes compared to transplantation with unmodified cells, confirming the importance of a localized effect. It seems feasible that the role of stem cells in functional recovery is dependent, not only on inherent cellular and regional characteristics, but also on the nature of the pathology. A comprehensive understanding of the mechanism by which stem cells exert benefit should enable researchers to further refine and enhance therapies.

The use of allogeneic stem cell sources could circumvent some of the challenges associated with conventional stem cell therapy, such as the time and expense linked to multiple procedures for cell harvest and delayed delivery. Larger clinical trials of stem cell use in urology have generally utilized autologous stem cell sources while trials outside the field have taken advantage of 'off-the-shelf' allogeneic options with significant success.³⁴ Despite concerns regarding immune rejection and related adverse reactions, the biology of MSCs—as it is currently understood—suggests that these cells are immune privileged. Thus, immunosuppression (either during or after transplantation) might not always be strictly necessary for allogeneic stem cell therapy. Xenotransplantation of MSCs is standard practice in animal models and small case reports of allogeneic MSC transplantation are surfacing in the published urological literature.³³ Investigators coordinating upcoming clinical trials should consider allogeneic stem cells for treating urological conditions.

In terms of stem cell delivery, homing continues to be investigated as a novel delivery mode, especially for targeting disorders that are nonfocal in a noninvasive manner. The major limitation of homing is the delay between injury (for example, caused by vaginal delivery or prostatectomy) and the initiation of treatment, which means that homing signals are often reduced. In order to prevent unnecessary treatment, research is needed to improve the accuracy of predictions regarding disease progression. Alternatively, methods such as electrical stimulation or local injection of homing cytokines could help direct systemically delivered stem cells to the target organ.

Neovascularization and re-innervation of engineered tissues for bladder and urethra reconstruction are critical hurdles to overcome for the application of tissue engineering in urology. The ingrowth of native vessels and nerves—which is stimulated by MSC bioactive

factors—is acceptable for smaller grafts. However, full regeneration with functional integration is more challenging. Studies have shown the feasibility of nerve regeneration via the overexpression of neurotrophins, transcription factors, and cytoskeleton-related genes in patients who have sustained peripheral nerve injury.¹⁰⁰ These approaches should be extended to urological practice, potentially in combination with cellular therapy.^{101,102} Animal models offer the most promising tool for studying techniques to improve neovascularization and reinnervation.

In summary, stem cell therapies for voiding dysfunction, ED, and urological tissue engineering have shown promise in early trials and these approaches continue to advance. Findings from clinical case reports and larger ongoing clinical trials have supported early evidence from pre clinical animal studies. Current challenges include the modification of harvest, isolation, and delivery techniques. In the near future, clinicians could have ‘off-the-shelf’ MSCs prepared for single-stage delivery at their disposal, as well as the option to systemically deliver cells and the ability to optimize treatment by selecting niche-specific stem cells for certain pathologies. Ultimately, stem cells could be the next minimally invasive pathophysiology-based therapy for targeting challenging urologic disorders.

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Key Points

Voiding and erectile dysfunction can significantly diminish quality of life and progress over time; optimizing the time at which therapy is initiated is thus of great importance

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, stem 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

Table 1

Clinical trials of stem cell therapy for SUI

Study	Stem cell source and type	Study population	Study design	Outcomes
Carr <i>et al.</i> ⁶¹ (2008)	Autologous MDSC	8 women with SUI who experienced no improvement of symptoms for 12 months following prior non-invasive therapy	Pad weights, bladder diaries, and QOL measurements were assessed at 1, 3, 6, and 12 months after treatment.	<p>No adverse effects reported</p> <p>Treatment did not have a negative impact on subsequent midurethral sling placement chosen by two patients</p> <p>62.5% (5 of 8) patients demonstrated improvement in pad weight and diaries after 12 months</p> <p>One patient achieved total continence</p> <p>Three patients elected to have a second injection</p>
Carr <i>et al.</i> ⁶² (2010)	Autologous MDSC	29 women with SUI who experienced no improvement in symptoms for 12 months following standard therapy	<p>Outcomes - stress leaks and improvement in quality of life (by IIQ-7 and UDI-6) - assessed at 1, 3, 6, and 12 months post-treatment.</p> <p>Patients could choose a second injection of the same dose after the 3-month follow-up visit.</p>	<p>No serious adverse effects reported</p> <p>86% (25 of 29) patients elected to receive double injections after 3 months of follow-up</p> <p>All 4 patients in single treatment group reported >50% reduction in stress leaks.</p> <p>12 months after second injection 40% of the patients experienced 50% improvement in quality of life</p>
Peters <i>et al.</i> ⁶³ (2011)	Autologous MDSC	64 women with SUI symptoms for at least 6 months who had failed prior treatment	24-hr pad weight, 3-day diary of incontinence episodes, and UDI-6 and IIQ-7 scores were assessed at 6 months	<p>No adverse effects reported</p> <p>58% (37 of 64) patients completed 6 month follow-up (with dose escalation)</p> <p>Increased dose was associated with greater improvement: decreased average number of stress leaks and pad weight; improved UDI-6 and IIQ-7 scores)</p>

Abbreviations: MDSC, muscle-derived stem cell; QOL, quality of life; IIQ-7, incontinence impact questionnaire 7; UDI-6, urogenital distress inventory

Table 2

Selected preclinical studies of stem cell therapy for SUI

Study	Stem cell source and type	Study population	Study design	Functional outcomes	Histological outcomes
Lin <i>et al.</i> ⁴⁴ (2010)	Autologous ADSC (derived from peri-ovary adipose)	12 injured female rats treated with urethral injection of EdU-labeled ADSC 6 injured female rats treated with BrdU-labeled ADSC via tail vein injection 10 injured female rats treated with urethral injection of saline	Balloon dilation used to induce SUI CMG analysis at 4 weeks post-treatment Histological analysis with IF, Masson's trichrome, and Hart's stains	80% (8 of 10) control rats had abnormal voiding 33.3% (3 of 12) urethrally injected rats had abnormal voiding 33.3% (2 of 6) tail-vein-injected rats had abnormal voiding	EdU- and BrdU-labelled cells present in urethral connective tissue Urethras of ADSC-treated rats had increased elastin compared to control animals Rats in treated groups with normal voiding had increased elastin compared to treated rats with abnormal voiding
Kinebuchi <i>et al.</i> ⁶⁸ (2010)	Autologous BMSC (derived from iliac bone)	6 injured female rats treated with GFP-labeled BMSCs 6 injured female rats treated with CFM injections 6 sham-operated female rats	Urethrolisis and cardiotoxin injection used to induce SUI LPP and bladder capacity assessed before injury (or sham injury), before treatment, and 1–4, 6, 8, and 12 weeks post-treatment	Injection of either BMSCs or CFM gradually improved LPP No difference between rats treated with BMSCs and CFM in terms of LPP No change in bladder capacity for any group	No difference in smooth muscle between groups Proportions of skeletal muscle cells and peripheral nerves were increased in BMSC-treated rats compared to controls Rats treated with CFM showed no increase in skeletal muscle and peripheral nerves compared to controls
Kwon <i>et al.</i> ⁶⁹ (2006)	Autologous MDSC	6 injured female rats treated with MDSC 6 injured female rats treated with fibroblasts 6 saline-treated injured female rats 6 untreated uninjured female rats	Bilateral sciatic nerve transection used to induce SUI Comparison of LPP and tissue contractility 4 weeks after treatment	All treated groups exhibited significantly greater LPP than saline-treated rats (and no difference compared to uninjured controls)	MDSC treatment reduced striated muscle fibre atrophy MDSC treatment improved fast twitch muscle contraction
Corcos <i>et al.</i> ⁷⁰ (2011)	Allogeneic BMSC	12 injured female rats received BMSC 6 injured female rats received plasma-lyte (placebo) 6 untreated injured female rats	Bilateral pudendal nerve transection used to induce SUI Recording of Valsalva leak point pressure (VLPP) and histological changes 4 weeks after injection	Outcomes assessed 4 weeks after treatment LPP significantly improved in BMSC-treated group compared to controls	IHC showed BMSCs (labeled with PKH26) at injection site Positive staining for myosin heavy chain and desmin in BMSC-treated group

Study	Stem cell source and type	Study population	Study design	Functional outcomes	Histological outcomes
Zhao <i>et al.</i> ⁷¹ (2011)	Autologous ADSC	8 injured rats received ADSC 8 injured rats received ADSC + PLGA 8 injured rats received ADSC + NGF 8 injured rats received ADSC + PLGA/NGF 8 injured rats received PBS	Bilateral pudendal nerve transection used to induce SUI Bladder capacities, abdominal LPP, and RUPP were reassessed 2, 6, and 8 weeks after injection. Evaluation of regeneration of muscles and peripheral nerves	Outcomes assessed 1, 2, 4, and 8 weeks after treatment Rats treated with ADSC + PLGA/NGF showed significant improvements in LPP and RUPP compared to other groups at 8 weeks post-treatment Administering NGF alongside ADSC improved survival	PLGA/NGF increased urethral muscle layer distribution and neuronal density at 8 weeks compared to other groups
Dissaranan <i>et al.</i> ⁴⁵ (2011)	Allogeneic BMSC	Experiment 1: 7 injured rats treated with IV BMSC 6 injured rats treated with IV saline 5 sham-injured rats treated with IV saline Experiment 2: 5 injured rats treated with local CCM injection 4 injured rats treated with local CM injection 5 sham-injured rats treated with local CM injection	VD used to induce SUI Sham-injured rats underwent anesthesia and insert of foley into vagina without inflation of balloon. LPP and EUS EMG were performed 1 week after injury <i>Ex vivo</i> fluorescent, immunofluorescent imaging, and Masson's trichrome staining at 1 week after injury	No difference in LPP between injured rats treated with IV and sham-injured saline treated controls No difference in LPP between injured CCM-treated and sham-injured CM treated controls EUS EMG was not improved by IV MSCs or CCM	More GFP-labelled cells were present in the smooth muscle of the urethras and vaginas of injured treated rats than sham-injured treated rats Histology improved with IV BMSC and CCM treatment compared to IV saline and CM controls, respectively

Abbreviations: ADSC, adipose-derived stem cells; EdU, 5-ethynyl-2'-deoxyuridine; BrdU, 5-bromo-2'-deoxyuridine; SUI, stress urinary incontinence; CMG, cystometrogram; IF, immunofluorescence; BMSC, bone marrow derived stem cells; GFP, green fluorescent protein; CFM, cell-free media; LPP, leak point pressure; MDSC, muscle-derived stem cell; IHC, immunohistochemistry; PLGA, poly(lactic-co-glycolic acid); NGF, nerve growth factor; phosphate buffered saline; RUPP, retrograde urethral perfusion pressure; IV, intravenous; CCM, concentrated conditioned media; CM, control media; VD, vaginal distension; EUS, external urethral sphincter; EMG, electromyogram

Table 3

Selected preclinical studies of stem cell therapy for BD

Study	Stem cell source and type	Study population	Study design	Functional results	Histological results
Nishijima <i>et al.</i> ⁷² (2007)	Allogeneic BMSC (GFP-labelled)	6 injured female rats treated with BMSC 6 injured female rats treated with saline 6 uninjured untreated female rats	Underactive bladder induced by PBOO (created by urethral ligation) Outcomes assessed 1 month after treatment Isovolumetric cystometry parameters and histology of bladder were assessed.	Residual urine was lower for injured rats treated with BMSC compared to injured rats treated with saline, but greater than uninjured controls Bladder contractility was greater and contractions were shorter in injured rats treated with BMSC compared to injured rats treated with saline and no different from controls	Some labelled cells present 1 month after treatment (associated with anti-alpha SMA antibody)
Huang <i>et al.</i> ⁷³ (2010)	Autologous ADSC (derived from peri-gonadal fat)	10 OH male rats treated with bladder injection of ADSC 10 OH male rats treated with tail vein injection of ADSC 10 OH male rats treated with PBS 10 normal male rats fed normal diet	OAB induced by obesity and hyperlipidaemia Bladder function (24-hour voiding behavior study and conscious cystometry) and histology (immunostaining and trichrome staining) assessed.	Micturition intervals shorter in OH rats treated with PBS than all other groups	No difference in smooth muscle content in bladder wall between OH rats Nerve content and blood vessel density lower in controls than in ADSC-treated OH rats
Woo <i>et al.</i> ⁷⁴ (2011)	Allogeneic BMSC (GFP-labelled)	15 ovariectomized C57BL/6 female mice treated with BMSC 12 injured untreated female mice 15 uninjured untreated female mice (age-matched controls)	Underactive bladder induced by PBOO (created by urethral ligation) Performance of urodynamic and histological evaluation 4 weeks after injection.	100% (10 of 10) mice with positive cell engraftment showed decreased hypoxia, hypertrophy, and fibrosis 90% (9 of 10) mice with positive cell engraftment showed improvements in compliance	66.6% (10 of 15) of treated mouse bladders included GFP-labeled cells Twofold increase in CCL2 chemokine expression (determined by RT-PCR analysis)

Abbreviations: BD, bladder dysfunction; C57BL/6, inbred strain of mice; CCL2, chemokine ligand 2 (monocyte chemotactic protein-1); GFP, green fluorescent protein; OAB, overactive bladder; OH, obese hyperlipidaemic; PBOO, partial bladder outlet obstruction; PBS, phosphate buffered saline; RT-PCR, real time-polymerase chain reaction; SMA, smooth muscle actin.

Table 4

Selected preclinical and clinical stem cell trials for ED

Study	Stem cell source and type	Study population	Study design	Functional results	Histological results
Bahk <i>et al.</i> ³³ (2010)	Allogeneic UCBSC	7 male patients who failed to achieve an erection for at least 6 months despite therapy	Type 2 diabetic ED Outcomes assessed on followup visits	86% (6 of 7) patients regained morning erections within 3 months 86% (6 of 7) patients reported increased desire 29% (2 of 7) patients returned for prosthesis 57% (4 of 7) patients were without erections by 9 months 14% (1 of 7) maintained erections with medication for 11 months Blood glucose levels improved in all patients	None
Huang <i>et al.</i> ⁷⁵ (2010)	Autologous ADSC (EdU-labelled) derived from perigonadal fat	18 hyperlipidaemic male rats treated with IC ADSC 10 hyperlipidaemic male rats treated with IC PBS 10 normal male rats treated with IC PBS	Hyperlipidaemic ED outcomes assessed 28 days post-treatment with ICP and IHC	Hyperlipidaemic rats treated with ADSC had a greater mean ICP/MAP ratio than hyperlipidaemic rats treated with PBS (similar to that of normal rats treated with PBS)	Greater concentration of nNOS-positive nerve fibres in hyperlipidaemic rats treated with PBS compared to ADSC Significantly more EdU-labelled ADSC cells visualized after 2 days compared to 14 days in hyperlipidaemic ADSC-treated rats EdU-labelled cells were rarely visualized at 28 days
Albersen <i>et al.</i> ⁷⁶ (2010)	Autologous ADSC (EdU-labelled) (derived from perigonadal fat)	8 injured male rats treated with ADSC 8 injured male rats treated with lysate 8 injured male rats treated with vehicle 8 sham-injured male rats	ED induced by bilateral CN crush ICP, nNOS content, apoptotic index, and histology assessed at 4 weeks post-treatment	Improved erectile function in cell and lysate treated groups compared to controls Increased nNOS in rats treated with ADSC and lysate compared to vehicle-vehicle-treated rats	Less fibrosis and more smooth muscle in rats treated with ADSC and lysate compared to vehicle-treated rats
Bivalacqua <i>et al.</i> ⁷⁷ (2007)	Allogeneic BMSC or eNOS-modified BMSC	5 five-month old male rats treated with BMSC 5 five-month old male rats treated with	BMSCs were harvested from six-week old rats ICP, eNOS protein, NOS activity, cGMP levels, and histology	Modified BMSC induced an earlier response to treatment than WT BMSC	BMSC survived up to 21 days after treatment and reduced inflammatory response Treatment with BMSC (modified or WT) resulted in increased

Study	Stem cell source and type	Study population	Study design	Functional results	Histological results
		eNOS-modified (AdeNOS) BMSC 5 five-month old male rats treated with eNOS-modified (AdntlacZ) BMSC 5 five-month old male rats treated with PBS	assessed at 7 and 21 days post-treatment		eNOS protein, NOS activity, and cGMP levels
Qiu <i>et al.</i> ⁷⁸ (2011)	Allogeneic BMSC or VEGF-modified BMSC	15 male rats with DM treated with BMSC 15 male rats with DM treated with VEGF-modified BMSC 15 male rats with DM treated with PBS 10 normal male rats treated with PBS	Type 1 diabetic ED (induced by streptozotocin) Erectile function (measured by CN electrostimulation) assessed 4 weeks post-treatment ELISA to evaluate VEGF concentration IHC for histological analysis	Increased erectile function in BMSC-treated (VEGF-modified or WT) DM rats compared to PBS-treated rats DM rats treated with VEGF-modified BMSC showed greater improvements than DM rats treated with WT BMSC	Significantly greater smooth muscle and endothelial content in corpus cavernosum of BMSC-treated versus PBS-treated groups Greater improvements were seen in DM rats treated with VEGF-modified BMSC versus WT BMSC

Abbreviations: UCBSC, umbilical cord blood derived stem cells; ED, erectile dysfunction; ADSC, adipose-derived stem cells; EdU, 5-ethynyl-2'-deoxyuridine; IC, intracavernosal; PBS, phosphate buffered saline; ICP, intracavernosal pressure; IHC, immunohistochemistry; MAP, mean arterial pressure; nNOS, neuronal nitric oxide synthase; CN, cavernosal nerve; BMSC, bone-marrow derived stem cells; eNOS, endothelial nitric oxide synthase; NOS, nitric oxide synthase; WT, wild type; VEGF, vascular endothelial growth factor; DM, diabetes mellitus; ELISA, enzyme linked immunoassay

Table 5

Selected stem cell trials of bladder and urethral TE

Study	Stem cell source and type	Scaffold	Study population	Study design	Functional results	Histological results
Atala <i>et al.</i> ⁸⁷ (2006)	Autologous smooth muscle and urothelial cells cultured and expanded from bladder biopsies	Collagen or composite (collagen and PGA) with or without omental wrap	7 patients (3 male, 4 female) with myelomeningocele Mean age = 11 years 3 patients with collagen scaffolds without omental wrap 1 patient with collagen scaffold with omental wrap 3 patients with composite scaffold with omental wrap	Bladder capacity, LPP, and bladder compliance were assessed at follow up visits. Mean follow-up = 46 months (range 22–61 months)	No metabolic abnormalities reported <u>Compared to pre-operative values:</u> Decreased LPP in all but one patient Maximum bladder capacity increased in all patients but one who received collagen-only scaffold Increased compliance in all patients Greatest improvements were seen in patients with composite scaffold with omental wrap	No histological differences at margin between engineered and native bladder IHC analysis confirmed smooth muscle and urothelium in engineered bladder
Chung <i>et al.</i> ⁹⁰ (2005)	Allogeneic BMSC	SIS	8 rats underwent sham-cystectomy 8 rats underwent partial (40%) cystectomy and treated with unseeded SIS scaffold 8 rats underwent partial cystectomy and treated with BMSC-seeded SIS scaffold 8 rats underwent partial cystectomy and treated with oversewing the defect	TE used to repair 40% partial cystectomy in treatment animals 4 rats in each group were harvested at 1 and 3 months after treatment Outcomes were limited to histological examine of tissue	None	Improved bladder regeneration in BMSC-seeded SIS group versus unseeded and control groups RT-PCR analysis showed increased collagen type I and III in unseeded SIS groups than seeded SIS and control groups Seeded SIS group showed greater expression of cytokeratins and smooth muscle myosin heavy chains than unseeded SIS and control groups
Sharma <i>et al.</i> ⁹¹ (2011)	Autologous BMSC (GFP-labelled)	SIS	1 baboon treated with primary closure without scaffold	TE used to 40–50% partial cystectomy in all baboons	Cystometry studies revealed reduced bladder compliance in all bladders after treatment	GFP-labelled BMSC present and proliferating in both augmented bladders with BMSC-seeded scaffolds

Study	Stem cell source and type	Scaffold	Study population	Study design	Functional results	Histological results
			1 baboon treated unseeded SIS scaffold 2 baboons treated with BMSC-seeded SIS scaffold	Cystometry performed and histology assessed at 10 weeks post-treatment		BMSC-seeded SIS scaffold augmented bladders revealed typical histological architecture
Sharma <i>et al.</i> ⁹² (2010)	Allogeneic human BMSC	POCf	5 female athymic nude rats treated with mCherry fluorescent protein labeled SMC/UC-seeded scaffold 5 female athymic nude mice treated with BMSC/UC-seeded scaffold 5 female athymic nude mice treated with unseeded scaffold	TE used to repair 40–50% partial cystectomy in all mice Histological analysis was performed at 4 and 10 weeks after treatment	None	Typical histological architecture seen in MSC/UC-seeded POCf augmented bladders (determined by trichrome and IF staining) Greater muscle-to-collagen ratios in MSC/UC-seeded versus SMC/UC-seeded POCf groups
Tian <i>et al.</i> ⁹³ (2010)	Allogeneic human BMSC Myogenic induction by growth factors (GFs, PDGF-BB and TGF-beta1)	In vivo studies: PLLA	In vitro studies: Induction of myogenic phenotype by growth factors or in combination with bladder ECM or 3D dynamic culture In vivo studies: BMSCs seeded on PLLA scaffold implanted in nude athymic mice (undisclosed number of mice)	In vitro studies: RT-PCR, immunoblotting, IF at 3, 7, and 14 days In vivo studies: Histology and IHC	None	ECM coating or 3D dynamic culture improve cell proliferation and myogenic differentiation in vitro Subcutaneously implanted PLLA scaffold maintained myogenic differentiation and improved neovascularization in vivo
Jack <i>et al.</i> ⁹⁴ (2009)	Allogeneic human ADSC	Poly-lactic-glycolic acid bladder dome composite (85:15 ratio)	15 athymic nude rats treated with ADSC-seeded composite augmentation 15 athymic nude rats treated with matched acellular composite 15 athymic nude rats treated with suture closure	TE used for bladder augmentation following partial (50%) cystectomy	Improved compliance capacity in augmented groups versus controls No difference in capacity between augmented groups ADSC-seeded composite group showed improved compliance compared to acellular composite group	ADSCs were present in bladder tissue up to 12 weeks after treatment Smooth muscle increased in ADSC-seeded composite group overtime

Study	Stem cell source and type	Scaffold	Study population	Study design	Functional results	Histological results
De Filippo <i>et al.</i> ⁹⁶ (2002)	Autologous smooth muscle cells from bladder biopsies	Acellular collagen matrix	12 rabbits treated with cell-seeded scaffold 12 rabbits treated with unseeded scaffold	TE used to repair 1cm anterior penile urethral defect Urethrography and histology performed at 1, 2, 3, and 6 months post-treatment	All unseeded grafts collapsed or developed fistulas after 1 month Cell-seeded grafts remained patent	Cell-seeded groups had normal urethral architecture and improved muscle fiber organization after 1 month
Raya-Rivera <i>et al.</i> ⁹⁸ (2011)	Autologous smooth muscle and urothelial cells derived from bladder biopsies	Tubularized PGA (sized for each patient)	5 male patients Median age = 11 years (range 10–14 years)	TE used to repair urethral defect Questionnaires, cystourethroscopy, and cystourethrogram at 3, 6, 12, 24, 36, 48, 60, and 72 months after treatment Biopsies of grafts at 3, 12, and 36 months after treatment Median follow-up = 71 months (range 36–76 months)	No urinary tract infections or fistulas reported on follow-up Questionnaires confirmed overall satisfaction with procedure 20% (1 of 5) patients required transurethral resection for proximal stenosis 20% (1 of 5) patients required pubovesical sling for daytime SUI All patients had patent urethras and maintained continence	IHC confirmed normal urethral architecture by 3 months after treatment

Abbreviations: PGA, polyglycolic acid; LPP, leak point pressure; IHC, immunohistochemistry; BMSC, bone-marrow derived stem cells; SIS, small intestine submucosa; TE, tissue engineering; RT-PCR, real-time polymerase chain reaction; GFP, green fluorescent protein; POCf, poly(1,8-octanediol-co-citrate) elastomeric thin films; SMC, smooth muscle cells; UC, urothelial cells; PDGF-BB, platelet derived growth factor; TGF-beta1, transforming growth factor; PLLA, poly L-lactic acid; ECM, extracellular matrix; IF, immunofluorescence; ADSC, adipose derived stem cells; SUI, stress urinary incontinence