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Adipose Derived Stem Cells for treatment of Lower Genitourinary Dysfunction

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Abstract

Tissue regeneration is the focal point of intensive research efforts that are supported by the increasing number of stem cell sources available. In particular, multipotent mesenchymal stem cells feature many functional properties attractive for regenerative medicine strategies, including their paracrine activity. Adipose-Derived Stromal/Stem Cells (ASCs) have been the focus of extensive work recently, in order to evaluate their efficacy both as cellular therapies and for tissue engineering-oriented applications. The lower genitourinary tract is subjected to many pathologic conditions necessitating repair and treatment. Stem cells freshly extracted from adipose tissue (SVF) or their expanded ASCs counterparts are quite widely studied because they are easily harvested in abundant amounts, making them an excellent source for functional restoration. The therapeutic value of these cells has been evaluated using specific *in vivo* animal models recapitulating various dysfunctions of the genitourinary system. The aim of this review is to discuss the current status and potential of ASCs for repair and treatment of lower genitourinary tract conditions. Work pertaining to bladder replacement and voiding dysfunction, urinary incontinence, erectile dysfunction and tunica albuginea reconstruction will be discussed. In addition, recent studies concerning urethral tissue engineering and regeneration will be described.

Keywords: Adipose derived Stem cells; Mesenchymal stem cells; Lower urinary tract; Erectile dysfunction; Self-assembly; Urethral replacement

Introduction

ASCs potentials for regenerative medicine

Many tissues have been investigated as a source of adult Mesenchymal Stem Cells (MSCs) including adipose tissue, bone marrow, periosteal tissue, peripheral blood, skeletal muscle and the synovium [1-5]. Of known MSC-containing tissues, adipose tissue is a particularly attractive source due to its availability and accessibility [6]. Adipose-Derived Stromal/Stem Cells (ASCs) have the advantage of being safely harvested in abundant quantity. Per gram of adipose tissue 5×10^3 colony-forming stromal cells can be isolated, which is estimated to represent up to 500 times more cells than for bone marrow stromal cells [5,7]. ASCs display a fibroblast-like morphology in culture and meet the minimal criteria for MSC definition, according to the International Society for Cellular Therapy. They express the cell surface markers CD73, CD90 and CD105 while lacking the expression of CD11b, CD19, CD45 and feature variable expression of CD34. A basic phenotyping for ASCs has been suggested to include at least two molecules acting as negative markers and at least two cell surface positive markers [8,9]. In culture, ASCs have displayed good proliferative capacities as well as an impressive developmental plasticity, including the ability to undergo multi lineage differentiation [10].

ASCs have been reported to exert strong anti-inflammatory and immunosuppressive effects *in vitro* through their production of various soluble factors. Such immunomodulatory activity in culture models has been correlated with the ASCs expression of molecules like prostaglandin E2 and indoleamine-2,3-dioxygenase (IDO) [11-13]. ASCs have been shown to inhibit the proliferation of activated T cells, production of inflammatory cytokines and stimulate the production of anti-inflammatory cytokines and antigen-specific Treg cells [14]. Furthermore, cultured ASCs would be immuno privileged due to lack of expression of class II Major Histocompatibilty Complex (MHC-II) and co-stimulatory molecules on the cell surface [15,16]. Whether allogenic ASCs would actually be immunoprivileged or immune evasive *in vivo* awaits further investigation along with other types of MSCs [17].

The functional properties of ASCs are greatly associated with their paracrine effects. They have been reported to secrete a wide range of molecules that modulate local cellular activity and promote tissue regeneration at the injury site. For example, their release of Hepatocyte Growth Factor (HGF), Insulin-Like Growth Factor-1 (IGF-1), Vascular Endothelial Growth Factor (VEGF) and Basic Fibroblast Growth Factor (bFGF), can promote angiogenesis and prevent cell death [10,18-21]. ASCs can be isolated easily from a donor's subcutaneous fat depots during liposuction, lipoplasty, or lipectomy procedures, which are minimally invasive or painful. Enzymatic tissue digestion with collagenase, dispase, trypsin or related enzymes are routinely used to release the cells defined as the Stromal Vascular Fraction (SVF) and centrifugation allows their separation from the mature adipocytes [22,23]. The SVF consists of a heterogeneous mesenchymal population of cells that includes not only adipose stromal and hematopoietic stem and progenitor cells but also endothelial cells, erythrocytes, fibroblasts, lymphocytes, monocyte/macrophages and pericytes, among others [24]. When seeded in culture flasks, the ASCs adhere to the plastic surface and can be enriched further using a combination of washing

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steps and culture expansion [25,26]. Both SVF and ASCs are been used in clinical trials ranging from myocardial infarction to perianal fistulas treatments [27]. Their efficacy in preclinical studies for a range of urologic conditions will be described later in the corresponding sections.

ASCs suitability for the regeneration of genitourinary system

There are a number of conditions affecting the genitourinary system which can lead to loss of function. Congenital disorders, cancer, trauma, infection, inflammation, iatrogenic injuries or other conditions of the genitourinary system require extensive reconstructive procedures. However, current techniques may lead to a number of complications [28]. Tissue engineering and stem cell therapy is promising alternatives to current methods to perform genitourinary reconstruction. In addition to the previously mentioned advantages, ASCs do not express HLA-DR, which reduces their immunogenicity and render them more suitable for allogenic transplantation. ASCs express different biomarkers typical of smooth muscle and endothelial cells, which make them easily differentiated into these cell types which are major constituents of genitourinary system [29,30]. Moreover, ASCs secrete many potentially synergistic proangiogenic and antiapoptotic growth factors that are important for vascularization of ex vivo formed tissue constructs and restoring the erectile function [31]. The presence of automated commercially available devices that can isolate ASCs in sufficient numbers over short period of time should also be considered [29]. Lastly, ASCs can secrete and assemble/deposit extracellular matrix components, which can be used as a scaffold for tissue engineering of genitourinary structures [32]. As a result, ASCs could act at multiple levels in order to achieve tissue regeneration and restoration of function of the lower genitourinary tract including the formation of the scaffolds, specialized cell contribution and vascularization promotion (Figure 1).

The aim of this review is to discuss the current status and potential of ASCs for repair and treatment of lower genitourinary tract dysfunction and also to highlight present obstacles and prospective on this topic.

Urinary Bladder

Urinary bladder replacement

Urinary bladder substitution/augmentation is needed in many disease conditions. The current treatment options compromise the use of gastrointestinal segments, which results in numerous complications that affect the health and quality of life of the patients [28]. Tissue engineering approaches for urinary bladder rely on cell-seeded scaffolds with autologous urinary tract cells [33]. Clinical trials using autologous urothelial and smooth muscle cells along with exogenous biomaterials have been performed [34]. Urinary bladder specimen was the source of urinary tract cells in most of bladder regeneration researches. However, it cannot be used in case of bladder cancer and end-staged bladder [35]. Stem cells derived from many tissues including bone marrow, muscle and adipose tissue are possible sources for urinary tract cells in these conditions. Among those, ASCs are more favorable due to their previously mentioned advantages. ASCs have been differentiated into urothelial-like cells using coculture technique [36]. The urothelial differentiated cells exhibited urothelial biomarkers including cytokeratin 18 and uroplakin II. Also, ASCs were differentiated into smooth muscle cells, which showed SMCs markers including smooth muscle actin, myosin, calponin and caldesmon [37,38]. Both differentiated cell types survived and maintained their phenotype when implanted in vivo [38,39]. Unmodified cultured ASCs were seeded on bladder acellular matrix to replace bladder defects in rabbits. At 24weeks, the engineered bladders had a better bladder capacity and regeneration than the control group [40]. However, the lack of well-formed stratified urothelial layer in the graft would allow the urine leakage in large bladder defects as in human.

The use of exogenous biomaterials (synthetic and or acellular matrices) is frequently associated with inflammation, immune responses and foreign body reaction. This may ultimately lead to fibrosis and contracture of the implant. That is why a biomatrix made from autologous cells and featuring favorable requirements (sufficient burst pressure, tensile strength and elasticity) can avoid these problems. Our team was able to construct a bladder equivalent made from dermal fibroblasts [41]. As ASCs showed advantageous matrix deposition during the self-assembly approach compared to dermal fibroblasts [42], it would represent another option as scaffold for urinary tract regeneration. ASCs are cultured with ascorbic acid to enhance the deposition of the collagen in the matrix (Figure 2). An *in vitro* study performed in our lab for reconstruction of vesical equivalent showed

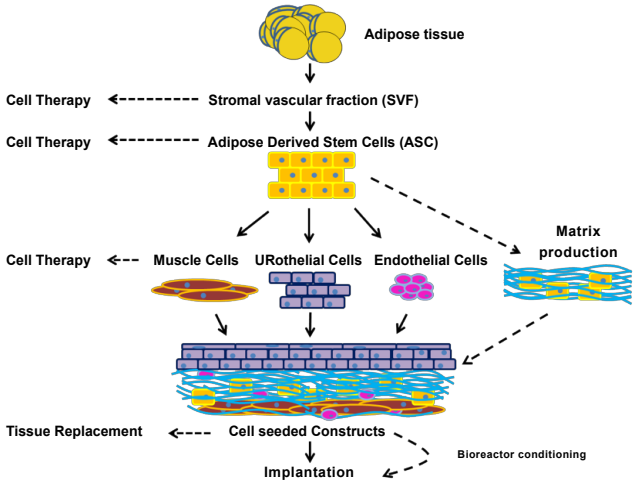

Figure 1: Possible applications of ASCs for treatment of genitourinary tract diseases.

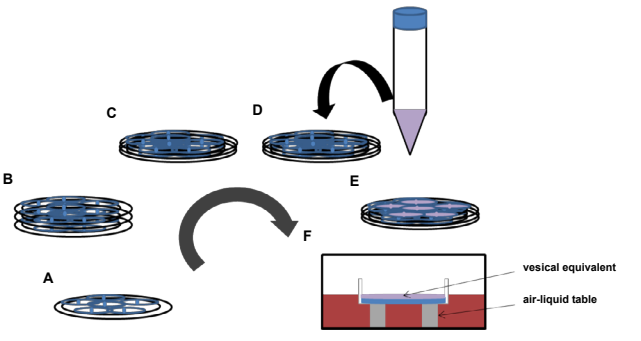

Figure 2: Schematic representation of the different steps for 3D reconstruction of bladder equivalent from ASCs.
A: Adipose derived stem cells (ASCs) are cultivated with Ascorbic acid for 21 days. B: Superimposition of cell sheets follows C: 4 days are allowed for fusion of the cell sheets to form the of the reconstruct. D: Urothelial cells are seeded on top of the reconstruct E: The reconstruct is cultured submerged for 7 days to give time for urothelial cells to proliferate. F: The reconstruct is placed at the air-liquid interface for urothelial differentiation.

that there is no significant structural difference between ASCs and fibroblasts Extracellular Matrix (ECM). Those cells were both able to produce a dense and well-organized ECM. When compared to matrix synthesized from fibroblasts cultured under the same conditions, ASCs matrix was thicker but displayed similar failure strain (Figure 3). However, ASCs matrix alone was not able to support the formation of a well-differentiated urothelium under the culture conditions used. When a layer of fibroblasts was added to ASCs matrix, a well-stratified epithelium was developed [32]. This is in contrast another study from our group where ASCs have been shown to support other type of epithelial cells [43]. Enhancement of urothelial and smooth muscle attachment to ASCs matrix without the use of any additional cell layer is our next goal. Additionally, ASCs promote vascularization of the grafts [19] which adds to their advantages for urinary bladder reconstruction.

Bladder voiding dysfunction

The inadequate efficiency of current pharmacological treatment and invasiveness of other modalities has supported the search for new stable therapeutic modalities for Bladder Voiding Dysfunction (BVD) including bladder overactivity or underactivity. Additionally, none of current treatments change the pathologic effects in the diseased bladders. Bladder Outlet Obstruction (BOO) causes bladder voiding dysfunction through increased collagen deposition, detrimental changes in ultrastructure of bladder smooth muscle cells and decrease blood flow [44]. All lead to impaired smooth muscle function and decreased bladder compliance. ASCs could potentially reverse many of the bladder pathologic changes in different animal models [45]. ASCs alleviated the symptoms of bladder overactivity in various animal models [46,47] or underactivity [48] or variable spectrum of voiding dysfunction [49].

Unmodified ASCs are thought to exert their beneficial effects mainly through paracrine action and less through cell engraftment and differentiation. In a rat model of BOO, human ASCs increased sequence-specific transcription of Oct4, Sox2, and Stella in the submucosal and muscle layer of the rat bladders. These are markers for primitive pluripotent stem cells. In addition, ASCs enhanced the expression of several genes, responsible for stem cell trafficking, including SDF-1/CXCR4, HGF/cMet, PDGF/PDGFR, and VEGF/VEGFR signaling axis. Through these paracrine effects, ASCs caused the stimulation and mobilization of endogenous stem cells [47]. Also, ASCs seemed to preserve the bladder vascularity and decrease apoptosis [49]. Human ASCs decreased the frequency and irregularity of detrusor contractions and slightly increased their amplitude when injected into the rat bladders subjected to outlet obstruction [47]. This suggests the possibility of transfer of allogenic stem cells for people with perturbed stem cell depot as in diabetic or geriatric populations. There is no known human trial incorporating the use of ASCs for treatment of BVD.

ASCs differentiated into SMCs before local injection have been shown to survive and increase SMCs content at the injury site. However,

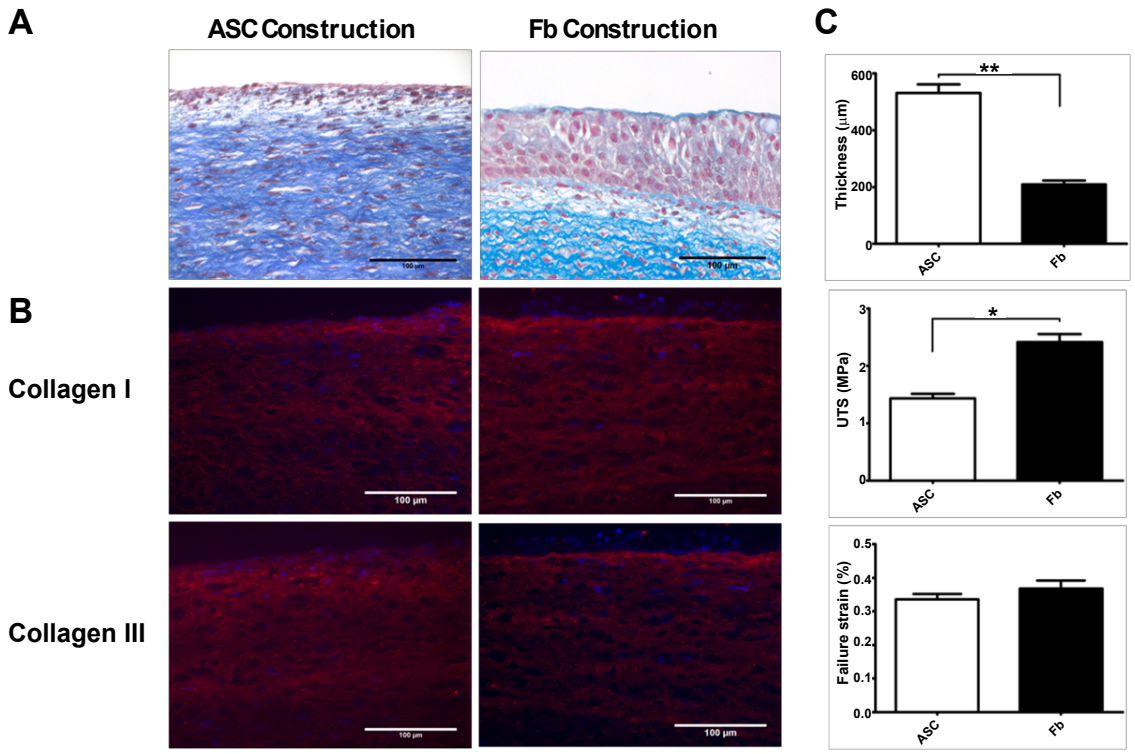

Figure 3: Histological cross-sections of the human tissue-engineered, characterization of the ECM and mechanical properties of vesical equivalents. **A**: Samples stained with Masson's trichrome show urothelial cells (purple) firmly anchored to the underlying stroma composed of ECM (blue) of the ASC and Fb constructions. Scale bars: 100 µm **B**: Expression of type I and III collagens. Scale bars: 100 µm **C**: Stromal thickness of the Fb was found to be significantly smaller than for ASC in presence of urothelial cells. The UTS of the Fb group was significantly higher compared to the ASC. The failure strains were not significantly different between the two constructions. Tests were performed using 3 different cellular populations (N) for Fbs and ASCs and each construct was produced in triplicate (n). Each column represents mean +/-standard error of the mean, with $p<0.05$ indicating significance (*$p<0.05$, ** $p<0.005$).

no record on the improvement of bladder function after injection was reported [38]. Although systemic injection of ASCs has improved BVD in animals, as seen with local injection into urinary bladder, like other MSCs, it may have serious side effects such as hemodynamic compromise, respiratory distress and impeding of pulmonary gas exchange that hinder its adoption as a regular route of delivery [50].

It is important to note that ASCs can be useful in early stages of BVD before severe affection of the bladder wall happens. This beneficial effect may be preventive (arrest of further pathologic effects) or ameliorative (correct existing pathologic effects) or both. The exact underlying mechanisms, the magnitude and type of positive outcomes and durability need to be further investigated.

Urethra

Urethral replacement

Multiple urethral illnesses including congenital, traumatic and inflammatory pathologies require extensive urethral reconstructive surgeries, which are limited by the availability of donor tissues. Tissue engineering, using scaffolds or cell seeded constructs, has been used with success in preclinical studies and clinical trials [33]. This is based mainly on the use of acellular matrices or synthetic scaffolds alone or seeded with urinary tract cells. However, this may carry the risk of transmission of infection or immunologic reaction with fibrosis. That is why a scaffold made from the patient's cells would obviate these problems. A biomatrix made by the self-assembly technique of tissue engineering from dermal fibroblasts was fabricated and seeded with urothelial cells [51]. Based on the successful production of biomaterials from human ASCs using the self-assembly technique with favourable mechanical characteristics for bladder replacement [32], ASCs-based scaffold is another appealing alternative for urethral replacement.

As a cell source for urethral engineering, ASCs have been used to replace urinary tract epithelium [39] and smooth muscle [52]. In the former study, ASCs were differentiated into urothelial cells and seeded on bladder acellular matrix to be implanted in rabbits. The urethral continuity was preserved with wide calibre and the labelled differentiated urothelial cells survived and formed a multilayer structure. In the latter study, ASCs were used to enhance and increase the uptake and survival of implanted urethral grafts [53]. This may be attributed to in situ differentiation of ASCs into endothelial cells and increased growth factors secretion by ASCs, such as VEGF and TGFβ3 that enhance angiogenesis and wound healing.

Urinary incontinence

Stress urinary incontinence affects both males and females and decrease quality of life [54]. Many injectable bulking agents are minimally invasive but have a poor long-term efficacy [55]. More invasive approaches, like sling procedures or artificial urinary sphincter implantation are more effective but have a higher morbidity [56,57]. More importantly, none of these therapies replace the deficient urethral sphincter. The ideal strategy for treating SUI using stem cell therapy besides being a bulking agent would be to allow for the regeneration of functional periurethral tissue, providing adequate mucosal coaptation and to restore resting urethral closure pressures [58]. ASCs carry future special importance in this regard due to its reported myoblast and neuronal-like differentiation capacity and neovascularization potential beside their ease of harvest and high stem cell content. Lin et al. [59] showed that therapeutic effects of unmodified ASCs were attributed to trophic factors that support host tissue regeneration as most of the delivered ACSs remained undifferentiated after injection.

In another study, ASCs were differentiated into myoblasts using 5-AZA and injected in the posterior urethra after induction of SUI in rats. Maximal bladder capacity and Abdominal Leak Point Pressure (ALLP) significantly increased 1 and 3 months after implantation with unmodified and differentiated rat ASCs with better results in case of differentiated ASCs [60]. ASCs coupled with biodegradable microbeads as carriers improved in Abdominal Leak Point Pressure (ALPP) and Retrograde Urethral Perfusion Pressures (RUPP) in a rat model of SUI [61]. ASCs in combination with Nerve Growth Factor (NGF) and PLGA resulted in significant improvements in ALPP and RUPP as well as the amount of muscle and ganglia when compared to ASCs alone [62]. Few clinical trials are incorporating the use of ASCs for treatment of SUI (www.clinicaltrials.gov). In a clinical trial, 11 male patients with persistent post-prostatectomy SUI received ASCs in 2 fractions; ASCs alone and mixed with fat. SUI improved progressively in eight patients during the 1-year follow up, as determined by a 59.8% decrease in the leakage volume in the 24h pad test, decreased frequency and amount of incontinence, and improved quality of life. One patient achieved total continence up to 12 months after stem cell injection [63].

Penis

Tunica albuginea reconstruction

The tunica albuginea is an important penile structure, which necessitates reconstruction in many diseases such as congenital penile curvature, hypospadias and Peyronie's Disease (PD). It allows tunical expansion and help to determine stretched penile length. It protects erectile tissue, promotes penile rigidity and length and participates in veno-occlusive mechanism [64]. ASCs, with their advantages previously mentioned, can be an alternative therapeutic option. ASCs were injected intratunically during acute phase in a PD rat model. They prevented fibrosis and elastosis and maintained erectile function [65]. Current therapeutics for tunical replacement include either the use of autologous grafts (commonly fascia lata, tunica vaginalis and saphenous vein) or non-autologous materials (porcine Small Intestinal Submucosa (SIS), human dura mater and porcine and human dermis) [66]. However, both are associated with many problems including harvest-related complications with the former and possibility of transmission of infection and immunologic reactions with the latter. ASCs, being easily harvested, were amplified in culture and seeded on SIS and implanted in rats. This cell-seeded graft was recorded to result in considerable cavernous tissue preservation and maintained erectile responses better than SIS alone [67]. Innovative treatment choices include the autologous self-assembly technique which was developed to avoid the use of any exogenous material. We developed endothelialized self-assembled grafts for tunical replacement from Dermal Fibroblasts (DF) featuring adequate mechanical resistance [68]. Adipose stromal cells can also be stimulated with ascorbic acid to form the self-assembled graft instead of DF. Moreover, ASCs could be a source of endothelial and smooth muscle cells for restoring erectile dysfunction, which may be associated with PD. Therefore, a single source (SVF or cultured ASCs) for both matrix and effective cells (endothelial and/or SMC) would be ideal to avoid multiple biopsies and steps needed for isolation of different cells for creation of optimal tunical graft.

Erectile dysfunction

Erectile Dysfunction (ED) is defined as the persistent inability to attain and maintain penile erection sufficient for sexual intercourse [69].

A prevalence of ED of no less than 52% was reported [70]. ED causes major morbidity and distress for men and their partners [71]. The main etiologies for ED include aging, diabetes mellitus and Cavernous Nerve Injury (CNI) during radical prostatectomy [72]. The insufficiencies and complications of the existing therapies for ED have urged many scientists to search for new modalities including stem cell replacement. All available therapies for ED tend to alleviate the symptoms rather than correcting the existing pathology. Stem cell therapy aims to replenish the damaged endothelial and smooth muscle cells and prevent further apoptosis and fibrosis. Among the different types of stem cells tested for ED treatment, ASCs were the most frequently investigated, due to easy harvest in abundance, established efficiency in other medical venues, the availability of separation devices. Both SVF and ASCs have been employed in ED research with success [73]. In an *in vitro* model of cavernous tissue, ASCs contributed to the repair of endothelial damage and decrease apoptosis resulting from Diabetes Mellitus (DM). ASCs showed the ability to undergo differentiation toward ECs and SMC [74]. When employed for treatment of ED due to type 1 or type 2 DM in rats, ASCs show increase in intracavernous pressure and improvement of ED, together with improvement in blood glucose level [75,76]. In crush injury of Cavernous Nerve (CNI), autologous ASCs were able to treat both acute (immediate) and chronic (4 weeks) CN injury-induced ED [73]. ASCs when used in combination with PDE-5 inhibitors or growth factors had additional intensity of therapeutic efficacy [77,78]. In case of resected CNI model, ASCs were seeded on autologous vein graft or adipose tissue biomatrix and had beneficial effect on penile histology and functional outcome [79,80].

Intracavernous injection of ASCs is the preferred method for stem cell delivery especially in case of CNI, however, it is associated with the rapid disappearance of the injected stem cells from penis, minimizing therapeutic efficiency in chronic disease model as DM [81]. Other routes of delivery include periprostatic injection [82], subtunical implantation [83] or coupled with biomaterial as nerve or tunical graft [67,79]. Although IV route of ASCs has shown efficacy in ED after irradiation [84], however, it may be associated with severe adverse effects. The main mechanism of ASCs-mediated repair in treating ED is largely dependent on paracrine actions with scarce evidence of cell engraftment [76].

Currently, there is only one registered clinical trial for use of ASCs for treatment of ED registered in USA (identifier NCT01601353).

Hurdles and Future Directions

In spite of the great advantages of ASCs, there many challenges that face their wide spread use in clinical applications. Among those is the lower therapeutic efficacy of ASCs in case of chronic pathologies in comparison to their efficacy in case of acute injuries. This is may be explained by the fact that in the absence of an acute illnesses, ASCs are less likely to be attracted to the diseased tissues and therefore lower efficiency and less involvement in the regenerative process [85]. Additionally, the process of ASCs engraftment within the desired tissues needs to be enhanced. It would be interesting to investigate whether pre-differentiation of ASCs into the targeted tissue cell types would increase their benefits and help engraftment without affecting their secretomes. Moreover, there is no final agreement on the preferred form of cells to use (SVF cells or cultured and purified adipose-derived stem cells), number of cells per treatment or number of cell injections. Hence, more chronic animal models, consistent protocols and many clinical trials are required to make sure of ASCs therapeutic efficacy and safety.

	Nature of the study; disease model	Cells used	Functional Assessment	Notes	References
Bladder replacement	In vitro study	Human cultured unmodified ASCs	Not available	ASCs formed matrix graft	Rousseau et al., 2013 [32]
	Normal rabbits	Autologous cultured ASCs were seeded on bladder acellular matrix.	Cystography. Normal bladder capacity was acquired .	Exogenous scaffold was used.	Zhu eta al., 2010 [40]
Bladder voiding dysfunction	BOO	Cultured Human ASCs injected into rat bladder wall	UDS. Decrease bladder overactivity (frequency and irregularity of contractions) with increase in bladder voiding pressure.		Song et al., 2013 [47]
		Autologous cultured ASCs and muscle precursor cells (MPCs) injected into rat bladder.	UDS. Micturiting pressure (maximum and threshold) and voided volumes increased.		Tremp et al., 2013 [48]
	Diabetes Mellitus	Autologous cultured ASCs injected in bladder wall or tail vein of diabetic type II rats.	UDS . It showed Diabetic Voiding dysfunction improvement in 40-60 %.	Improvement with local (bladder) injection is more effective than systemic (tail vein) injection	Zhang et al., 2012 [49]
	Hyperlipidemia	Autologous cultured ASCs injected into bladder or tail vein of hyperlipdemic rat	Improved micturition frequency and voided volumes	Improvement with direct(bladder) injection is more efficient than systemic (tail vein) injection	Huang et al., 2010 [46]
	Cryo-injury	Human ASCs differentiated into SMCs and injected into cryo-injured bladder wall of mice.	Not available	There was an Increase in the ASMA positive area of injured Bladder. The injected labeled cells were detected in vivo.	Sakuma et al., 2009 [38]
Urethral replacement	Normal rabbits	Autologous cultured ASCs and urothelial -differentiated ASCs were seeded on bladder acellular matrix.	Urethrography. It revealed restoration of urethral continuity with only urotheklial-differentiated cell seeded constructs	BrdU-labeled cells survived in vivo transplantation.	Li et al., 2014 [39]
	Normal canine model	Autologous SMC-differentiated ASCs and oral epithelial cells were seeded on PGA	Urethrography. It showed slight strictures at the site of implantation	The use of bioreactor improved the characters and outcome of engineered graft	Fu et al., 2014 [52]

Urinary incontinence	Stress urinary incontinence (SUI) -rat model	Autologous cultured ASCs	UDS with different measures including ALPP, RUPR and bladder capacity	SUI was mostly induced by vaginal balloon dilation and bilateral Ovariectomy.	Lin et al., 2010 [59]
		Unmodified ASCs and ASCs differentiated into myoblasts			Fu et al., 2010 [60]
		Cultured ASCs with PLGA microbeads		SUI was induced by urethrolysis.	Zheng et al., 2006 [61]
		Autologous cultured ASCs with PLGA or NGF or both		SUI was induced by bilateral pudendal nerve transection	Zhao et al., 2011 [62]
	Postprostatectomy urinary incontinence –clinical trial	11 patients received autologous ASCs with and without fat.	Frequency and amount of incontinence, daily leakage volume, UDS and ICIQ-SF	The cells were injected endoscopically into the region of external urethral sphincter and submuocal space.	Gotoh et al., 2013 [63]
Tunica Albuginea (TA) reconstruction	Peyronie's disease (PD)	Human ASCs injected in TA during acute phase of PD	Measurement of Intracavernous pressure (ICP)		Castiglione et al., 2013 [65]
	Normal rats	Syngeneic cultured ASCs seeded onto SIS			Ma et al., 2012 [67]
Erectile dysfunction	Cavernous Nerve crush injury	Autologous Stromal vascular fraction	Measurement of Intracavernous pressure (ICP)	IC injection was done immediately and after 4 weeks.	Qiu et al., 2012 [73]
		Cultured Human ASCs with NGF-incorporated hydrogel			Kim et al., 2012 [77]
		Cultured ASCs and BDNF with or without udenafil			Jeong et al., 2013 [78]
		Cultured Human ASCs		Delivered by IC injection or periprostatic implantation	You et al., 2013 [82]
	CN resection	Cultured allogenic ASCs seeded on fat matrix		Variable but substantial improvement of erectile function	Lin et al., 2011 [79]
		Cultured ASCs with autologous saphenous vein			Ying et al., 2014 [80]
	Diabetes mellitus	Autologous cultured ASCs		Type II DM rats	Garcia et al., 2012 [75]
		Stromal vascular fraction		Type I DM mice	Ryu et al., 2012 [67]
	Radiation injury	Autologous cultured ASCs		Delivered by tail injection	Qiu et al., 2012 [84]
	Normal rats	Autologous ASCs differentiated into SMC and EC	Not available	The labeled implanted cells survived for 2months after implantation.	Orabi et al., 2012 [83]

Abbreviations: BOO: Bladder outlet obstruction; SMC: Smooth muscle cells ; ASMA : Smooth muscle α-actin ; PGA: Poly-Glycolic acid
PLGA: Poly(lactic-co-glycolic acid) ; NGF: Nerve growth factor
ICIQ-SF : The International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF)
UDS: Urodynamic study; ALPP : Abdominal leak point pressure; RUPP : Retrograde urethral perfusion pressure
SIS: Small intestinal submucosa; EC: Endothelial cells; BDNF: Brain-derived neurotrophic factor

Table 1: Different applications, studies and clinical trials of Adipose Derived Stem Cells (ASCs) in therapy of lower genitourinary dysfunction.

Conclusions

In the search of new therapeutic options for lower genitourinary tract disorders, both SVF and cultured ASCs have been the focus of numerous studies *in vitro* and using various animal models. In addition, depending on the type of dysfunction to be treated, these cells can be used either as cellular therapies or combined with biomatrix for tissue-engineering applications. Few clinical trials showed promising results (Table 1), however, more future clinical trials will ensure proof of their efficacy for particular applications while shedding more light on the mechanisms ensuring their functional activity.

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