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Adipose-Derived Stem Cells — Are They the Optimal Cell Source for Urinary Tract Regeneration?

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1. Introduction

The urinary system consists of two kidneys, two ureters, urinary bladder and urethra. The main function of the urinary system is to eliminate wastes and harmful materials from the body. Additionally, it adjusts blood volume and pressure, regulates electrolytes and blood pH. The urine passes from the kidneys via the muscular tubes of ureters to the urinary bladder where it is stored until it is socially convenient to be expelled outside through the urethra by the act of micturition.

The urinary tract (ureters, bladder and urethra) is composed of two main functional tissues: epithelial coverage (urothelium) and muscular coat (smooth muscle). A highly impermeable urothelial layer is necessary to prevent reabsorption of noxious materials present in urine despite its large osmotic and chemical gradients. Muscular coat creates coordinated waves of contractions necessary for urine transport and expulsion.

Since birth, the urinary system is subjected to a variety of diseases and pathologies including congenital and acquired disorders that threaten the patient's life. Current therapeutic options to replace severely damaged urinary organs are associated with many complications and hazards on patient survival and quality of life. Regenerative medicine has emerged as potential replacement treatment. Although progenitor cells of the urinary tract have been used in experimental studies and clinical trials, however, they are not amenable options in cases of benign end-stage diseases and malignancy. Stem cells can be the source of cellular components in regenerating urinary organs. Among those stem cell types, Adipose-derived Stem Cells (ASCs) are the current most convenient source due to easiness of harvest in abundant quantity, potential differentiation into many cell types and lack of ethical problems.

ASCs had also been investigated to form matrix (scaffold) and differentiate into urothelial and smooth muscle cells. Both can be coupled together to form tissue-engineered constructs that can replace the wall of urinary tract including ureters, urinary bladder and urethra. When complete wall replacement is not needed in some situations, ASCs, as cellular therapy, had beneficial benefits reflected on pattern of voiding. Additionally, ASCs can enhance vascularity and improve survival of urinary flaps or grafts.

In this chapter, we will review the current and future applications of ASCs for regeneration and repair of urinary tract. Mechanism of actions, preclinical and clinical trials and forms for therapies will be addressed. We will highlight the challenges that face their use and potentials to overcome these obstacles.

2. ASCs characteristics and prospectives for regenerative medicine

In the past, little consideration has been given to adipose tissue since its main functions were mainly associated with the storage and release of lipids. For years, lipoaspirated material has been discarded as surgical waste. Over the past few years, scientists became interested in investigating this highly complex tissue. Adipose tissue is composed mainly of adipocyte cells organized into lobules. The most prominent fraction, volume-wise, are mature adipocytes, which store and hydrolyzed triglycerides in response to environmental signals. The stromal vascular fraction (SVF), which includes preadipocytes, endothelial cells, vascular smooth muscle cells, fibroblasts, resident monocytes/macrophages and lymphocytes [1] also, provides structural support to adipose tissue. In 2002, a novel adult stem cell population isolated from SVF was first identified by University of California, Los Angeles (UCLA) researchers and named processed lipoaspirate (PLA) cells [2,3].

A wide variety of terms have been used to describe the multipotent cells derived from white adipose tissues and there was no consensus on the nomenclature used, which can sometimes lead to confusion. At the Second Annual International Fat Applied Technology Society (IFATS) meeting in 2004, scientists concluded to refer to these cells as adipose-stem cells (ASCs) [53]. Accordingly, the term ASCs will be used throughout this chapter.

ASCs are mesenchymal stem cells (MSCs) and possess similar characteristics to those extracted from bone marrow or umbilical cord [4]. They are undifferentiated non-embryonic multipotent stem cells, which have the ability to divide and self-renew in undifferentiated state. When submitted to specific inductive signals, ASCs can mature into a broad spectrum of cell lineages. ASCs have been reported to express the MSCs surface protein markers CD10, CD13, CD29, CD44, CD54, CD73, CD90 and CD105 (Table 1) [5]. Major differences occur for the CD34 stem cell marker that is not expressed by MSCs, but is present in ASCs in early passages. Morphologically, ASCs are fibroblast-like and preserve their shape after expansion in vitro [6].

Cell surface markers	Level of expression
CD3	Low or negative
CD10	High
CD11b	Negative
CD14	Negative
CD29	High
CD34	Variable
CD44	High
CD45	Negative
CD73	High
CD90	High
CD105	High
HLA-DR	Negative

Table 1. Expression level of ASCs surface markers

2.1. Isolation of ASCs from adipose tissue

When compared to other stem cell populations and sources, ASCs can be easily harvested while providing higher yields upon processing of adipose tissue, with minimal discomfort under local anaesthesia [7]. The efficiency of the cell isolation process depends on the localisation of the white adipose tissue and the donor general condition. For example, ASCs from visceral deposits are more prone to apoptosis and, therefore, less proliferative than the same cells isolated from the subcutaneous deposits [8]. ASCs are usually isolated from subcutaneous adipose tissue samples removed during liposuction, lipoplasty or lipectomy procedures, which are minimally invasive or painful. Adipose tissue is one of the richest sources of MSCs. From 1 gram of adipose tissue, 5×10^3 colony-forming stromal cells can be isolated, which is 500 times more cells than from an equivalent amount of bone marrow [9]. Current protocols used for release the cells rely on enzymatic digestion with collagenase, dispase, trypsin or related enzymes. Following the centrifugation and neutralization of the enzymes, the SVF fraction sediment is separated from the floating mature adipocytes. When seeded in culture flasks, the ASCs lack the intracellular lipid droplets seen in adipocytes and adhere to the plastic surface where they can be purified from other SVF cells using a combination of washing steps and culture expansion. Several exogenous supplements have been shown to have a stimulatory effect on the proliferation of ASCs. Platelet-derived growth factor, sphingosylphosphorylcholine, oncostatin M, and fibroblast growth factor (FGF) 2 have all been shown to increase ASC proliferation [9, 10, 11, 12].

2.2. ASCs potentials for regenerative medicine

The potential use of stem cell-based therapies for the repair and regeneration of various tissues and organs offers alternative therapeutic solutions for a number of diseases. Developments in stem cell research provided new cell source for regenerative medicine. An emerging body of literature suggests that adipose tissue may provide an abundant, readily accessible source of cells with similar potential to that described for other adult stem cells.

Adult stem cells are far more plastic than had previously been imagined. ASCs have been described as being able to give rise to several quite different mesenchymal cell phenotypes including osteocytes, adipocytes, neural cells, vascular endothelial cells, cardiomyocytes, pancreatic β cells, and hepatocytes [13, 14, 15, 16].

Cell lineage	Culture medium supplementation	References
Adipocyte	Insulin, dexamethasone, thiazolidinedione	17, 18
Chondrocyte	Ascorbic acid, BMP-6, FGF, IGF, dexamethasone, TGF- β	19, 20
Osteoblast	Ascorbic acid, BMP-2, valproic acid, dexamethasone	17
Myocyte	Dexamethasone, horse serum	21
Cardiomyocyte	Stem cell factor, transferrin, IL-3, IL-6, VEGF, TGF- β	22, 23
Neuronal-like	Valproic acid, insulin, butylated hydroxyanisole, hydrocortisone	24
Hepatocyte-like	HGF, FGF-1, FGF-4, oncostatin	24, 25

Table 2. Differentiation potential of ASCs

However, the ability to differentiate is not the only characteristic that makes these cells attractive for therapeutic purposes. Some reports have suggested that the therapeutic effects observed following ASCs administration, such as promotion of angiogenesis, reduction of inflammation, and functional recovery, are largely related to the trophic actions of their cytokines and growth factors secretion rather than by their differentiation into local tissue cell types [26]. The secretion of a broad range of bioactive molecules by ASCs, such as growth factors, cytokines and chemokines, constitutes their most biologically significant role under injury conditions [27]. Analyses of the soluble factors released from human cultured ASCs have revealed that, at relatively early passages, they secrete hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), transforming growth factor- β (TGF- β), insulin-like growth factor (IGF)-1, fibroblast growth factor (bFGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), tumour necrosis factor (TNF)- α , interleukin (IL)-6, 7, 8, and 11, adiponectin, angiotensin, cathepsin D, pentraxin, pregnancy zone protein, retinol-binding protein, and CXCL12 [28, 29, 30]. These factors promote an anti-inflammatory environment, angiogenesis and wound healing, which likely potentiate tissue repair.

ASCs possess immunomodulatory effects through their production of various soluble factors and rather low immunogenicity properties. In vitro, they have the ability to inhibit maturation of dendritic cells and suppress the proliferation of B cells, T cells, and natural killer cells following activation through both cell to cell binding and paracrine signalling [31, 32]. During

inflammation, ASCs release TGF- β that promotes premature helper T-cell differentiation toward T regulatory cells promoting anti-inflammatory environment [33]. Moreover, ASCs promote tryptophan degradation, limiting the bioavailability of a crucial molecule for T-cell proliferation and function [34]. Low expression levels of class I Major Histocompatibility Complex (MHC-I) and lack of MHC-II and co-stimulatory molecules on the cell surface (CD80 and CD86) makes ASCs immunoprivileged cells [35].

During the past decade, several studies have provided preclinical data on the safety and efficacy of ASCs administered in various animal models, supporting the use of these cells in future clinical applications. Furthermore, various clinical trials have shown the regenerative capability of ASCs in a variety of medical fields such as plastic surgery, autoimmune and inflammatory disorders, orthopedic surgery, oral and maxillofacial surgery, cardiac surgery and urology [36, 37, 38].

3. ASCs and kidney repair

Cell therapies using ASCs are highly promising in various clinical fields based on in vitro and in vivo research results. Important experimental findings in recent years suggest considerable therapeutic potential for cellular replacement in the context of kidney diseases (Figure 1).

3.1. Acute kidney injuries

Acute kidney injury (AKI), a multi-factorial syndrome that is common but often asymptomatic, is caused by such incidents as major infections, trauma, complications following major surgery and adverse reactions to drugs. It is characterised by the rapid loss of the kidney's functions, usually occurring within 48 hours or less, and represents a major treatment-resistant clinical problem with high mortality rates. In United States, AKI is an increasingly prevalent condition in hospitalized patients, a 10 percent increase per year, and the number of associated deaths has more than doubled over the last decade [39].

In the last few years, many studies have shown that stem cell transplantation is an efficient method for AKI repair. It was shown that ASCs drastically reduced mortality and improved renal structural and functional recovery using an animal ischemia-reperfusion model of AKI [56]. Indeed, intrarenal arterial injection of ASCs in ischemia-reperfusion-induced AKI rat model reduced blood urea nitrogen and creatinine levels [40]. Moreover, in a dose-dependent manner, ASCs reduced the tubular injury score 48 hours after ischemia-reperfusion [40]. The precise mechanism that attenuates kidney injury after ASCs injection remains to be clarified.

The renoprotective effects might be attributable to the replacement of damaged tubular cells by transdifferentiation of ASCs. The injured kidney expresses renotypic factors that may influence the differentiation of ASCs into a renal tubular epithelial lineage [41, 55]. A study using a murine model of AKI showed that differentiation and replacement of the dead cells at an early stage of injury by ASCs seems to be one of the major mechanisms in AKI kidney repair. However, most studies indicate that intravenously injected ASCs show minimal

homing to renal tubules and have limited survival in the kidney environment [42]. To bypass this problem, scientists tried to inject ASCs directly at the injury site. However, more than 80% of grafted cells die within the first week after injection and the majority of cells leak out from the injection site. Recently, it was found that using an injectable scaffold could improve the survival of cells as well as enhance retention of ASCs at the injury site [43].

There are increasing evidences that other differentiation-independent mechanisms of ASCs play key role in promoting tissue repair. Beneficial effects could be partially explained by the anti-inflammatory and anti-oxidative properties of ASCs, as revealed by their capacity to reduce apoptosis and inflammation in the injured kidney tissue [56]. Indeed, the expression of inflammatory, oxidative stress and apoptotic biomarkers such as IL-10, glutathione peroxidase and TNF- α at both gene and protein levels, were significantly decreased in ASCs treated group in ischemia-reperfusion rat model [45].

3.2. Chronic kidney disease

Twenty-six million american adults have chronic kidney disease (CKD) with progression of the disease leading to kidney failure, which requires dialysis or a kidney transplant to maintain life.

Positive effects of bone marrow MSCs treatment in CKD animal models have been reported [46, 47]. However, bone marrow MSCs derived from CKD patients failed to provide the same beneficial effect [48, 49]. A recent study has shown that ASCs did not seem affected by renal disease [50]. Thus, the impact of ASC therapy has been studied in different CKD animal models. In the majority of induced models investigated, ASCs administration has resulted in beneficial therapeutic effects, as evidenced by decrease the speed of renal fibrosis progression and improvement in renal functional parameters such as plasma creatinin, proteinuria and renal filtration. Indeed, intraperitoneal injection of ASCs in ischemia-reperfusion CKD mouse model reduced renal dysfunction and tubular injuries after 24 h post-reperfusion [44]. At 6 weeks, ASCs-treated animals showed reduced renal fibrosis [44].

The mechanisms mediating the therapeutic effects described are still under investigation. ASCs would enhance the angiogenic process in CKD by increased expression of VEGF, a growth factor that stimulates new blood vessels formation, suggesting a paracrine mechanism. Moreover, the level of BMP-7 and Pax-2, two proteins that participate in cellular repair response to kidney damage, increased after ASCs administration in CKD models [52].

3.3. Graft tolerance

Kidney transplantation remains the best therapeutic option for patients with end stage renal disease. In 2012, the Scientific Registry for Transplant Recipients reported that approximately 17 000 kidney transplants were performed and over 92 800 patients were remaining on the waitlist at the end of the survey year [54]. Unfortunately, transplanted patients need life-long immunosuppressive drugs to prevent allograft rejection. This phenomenon may happen when transplantation of a kidney from a donor who differs genetically from the graft recipient induces an immune response in the recipient against alloantigens of the donor graft. Effector

T cells infiltrate the graft and orchestrate an inflammatory response leading to destruction of the tissue. The new immunosuppressive drugs have improved short-term patient survival but rejection remains a major problem. Effective treatments are necessary to effectively address the problem of transplanted organ rejection.

Because of their immunomodulatory properties, ASCs are believed to play a role in the induction of transplantation tolerance. Studies have shown that ASCs attenuated acute rejection in kidney transplantation by increasing graft survival and reducing rejection grade [60]. A kidney transplantation study in rats that received intravenous injection of autologous ASCs before intervention showed decreased infiltration of macrophages and lymphocytes into the allograft and suppression of alloreactive T cells [60]. Studies reported that graft tolerance can be induced by ASCs therapy. Indeed, donor-ASCs portal infusion in a 29 year-old recipient before renal transplantation induced production of T-regs cells [59]. At 2-years post-transplant, immunosuppression weaning was started and 6 months later, anti-rejection therapy was completely stop with normal graft function. An Indian study in 90 patients showed similar results [57]. Consequently, ASCs may serve as effective immunomodulators in clinical transplantation. Long-term studies in larger populations are required to confirm efficacy.

Indication	Disease model	Cells used	Functional Assessment	Ramakes	References
Acute renal injury (AKI)	Ischemia-reperfusion rat model	Human ASCs	Urea and creatinine level. Tubular injury score.	Improved renal recovery.	40
	Ischemia-reperfusion mouse model	Human ASCs	Dead cells replacement	Improved renal recovery.	51
	Ischemia-reperfusion mouse model	Mouse ASCs	-	Improved renal recovery.	58
	Ischemia-reperfusion rat model	Rat ASCs	Inflammation, oxidative stress and apoptose	Reduce inflammatory and oxidative stress reaction.	45
Chronic kidney disease (CKD)	5/6 NPX rat	Human ASCs	Creatinine level, structural analysis, angiogenesis	Improved renal recovery.	52
	Ischemia-reperfusion mouse model	Human ASCs	Renal fibrosis, inflammation	Improved renal recovery.	44
Graft Tolerance	Rat kidney transplantation	Rat ASCs	Inflammation, rejection	Improved graft tolerance	60
	Human kidney transplantation	Human ASCs	Inflammation, rejection	Improved graft tolerance	57

Table 3. Shows the potential of ACSs as Kidney cellular therapy.

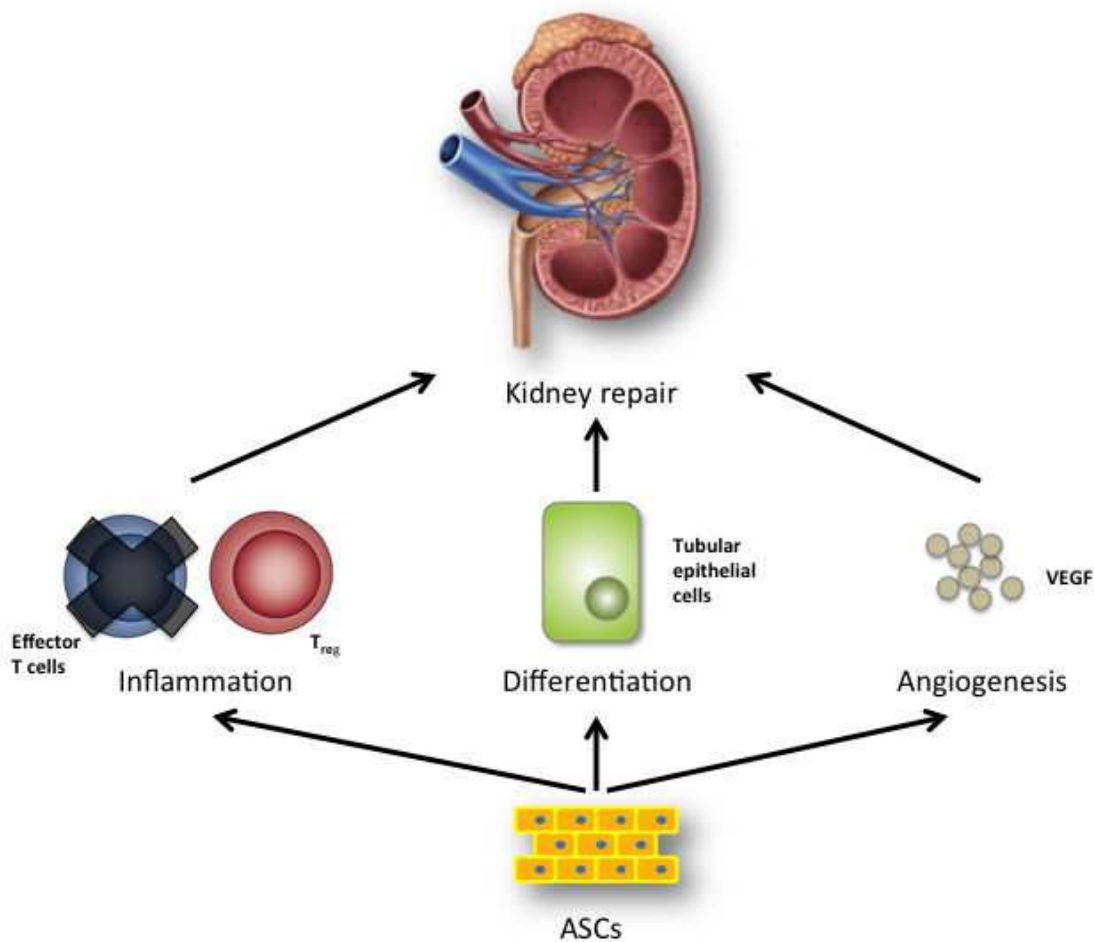


Figure 1. ASCs reduce inflammation by direct (cell–cell contact) and indirect (paracrine) actions, by suppression of T-cell and induction of T_{reg} . The differentiation of ASCs into tubular epithelial cells can contribute to kidney recovery. Microvascular damage is reduced by the secretion of the growth factor VEGF, which promotes angiogenesis.

4. ASCs and urinary tract

4.1. Urinary tract reconstruction

The urinary tract is anatomically composed of the renal pelvis, ureters, urinary bladder and urethra. It is concerned with urine transport from the kidneys, storage and expulsion outside of the body. Histologically, it is formed of 3 different layers including epithelium, connective tissue forming the submucosal layer and smooth muscle on the outside. The epithelial coverage is composed of transitional epithelium called urothelium, the function of which is to prevent the reabsorption of noxious materials present in urine despite its large osmotic and chemical gradients. Muscle layer creates coordinated waves of contractions necessary for urine transport and expulsion. The submucosa is formed of extracellular matrix containing blood vessels, lymphatics, nerves and variety of cells; predominantly fibroblasts.

The urinary tract is the target of many congenital and acquired insults that affect certain or all layers of the organ involved, resulting in partial or complete loss of function. This influences the patient survival and quality of life.

Although the urinary tract is naturally equipped with progenitor cells in the urothelium and muscle layers that help the regeneration of the urinary tract after any insult, severe disruption of the involved layer or the whole structure may exceed the capacity of the local progenitors or stem cells to compensate for the cellular loss. Hence, a necessity for repair with exogenous therapies emerges to restore the structure and hence the function, partially or completely.

Replacing the damaged urinary organs with non-native tissues is related to many problems including the lack of available tissues and postoperative complications including metabolic derangements. Regenerative medicine has evolved as a potential solution for replacing the damaged urinary tract. The patient's own cells that can be used in regenerating urinary tract are either local progenitors or stem cells. Local progenitor cells cannot be utilized in many instances as cancer or end stage benign diseases being the reasons for the defect. Consequently, stem cells should be harvested to build up new tissues for replacement.

Ideally, for clinical use, stem cells should be harvested in abundant quantities, by a minimally invasive procedure, and differentiated along multiple cell lineage pathways in a reproducible manner. Also, it can be manufactured in accordance with current Good Manufacturing Practice guidelines [61]. For these reasons, ASCs represent an ideal source for stem cell therapy especially for repairing the urinary system [62]. Additional advantages for the urogenital tract, ASCs populations likely contains vascular stem cells at various stages of differentiation toward becoming smooth muscle cells (SMCs) and endothelial cells (ECs), which are required components for regenerating the urinary tract [63]. Furthermore, ASCs have the ability to secrete many potentially synergistic proangiogenic growth factors delineating their angiogenic and antiapoptotic potential.

4.2. ASCs Contribution to urinary tract reconstruction

ASCs can be the source for epithelial and muscle cells through many established protocols of differentiation. Also, they can provide extracellular matrix (ECM) scaffold that sustains mechanical stress and supports the formation of epithelial coverage.

4.2.1. ASCs as cell source

1. Undifferentiated mesenchymal stem cells:

Unmodified ASCs can be seeded on different scaffolds to replace the urinary tract. In vivo differentiation of ASCs into SMCs has been observed after delivery of unmodified human ASCs within the bladder wall of nude mice [64], or autologous rat ASCs seeded on scaffold [65]. However, this phenotypic conversion occurs for a small percentage of the delivered ASCs.

2. Urothelial cell differentiation:

The main challenge in differentiating ASCs into urothelial cells (UCs) is due to the fact that the urothelium is derived from the endoderm [66], but ASCs are derived from the mesoderm,

making cross-blastoderm induction difficult. In vitro differentiation of ASCs into UCs was achieved using either conditioned medium [67], growth factors as all-transretinoic acid [68] or direct (seeding both cell types together) or indirect (using Transwell system { Corning-Costar}) coculture with UCs [69,70]. Lineage specific markers such as keratin 18 and uroplakins were detected with high percentage among differentiated cells. ASCs were labeled with CM-DiI and mixed with immortalized human bladder urothelial cells in a collagen matrix to be implanted in subcutaneous tissue of athymic mice. After 4 weeks, the expression of uroplakin-Ia was 70% and the expression of uroplakin-II was 65%. However, there was a lack of organized stratified urothelium [70].

The difficulties of final phenotypic conversion of ASCs into UCs may be attributed to the lack of epithelial-specific microenvironment including 3D biomimetic culture conditions, mesenchymal component with its cross talk with UCs (organ-specific ECM and fibroblasts). Identifying the key factors responsible for induction of urothelial markers in previous experiments is another difficulty [71].

3. Smooth muscle cell differentiation:

ASCs are easier to differentiate into smooth muscle cells. A subpopulation of adipose SVF was isolated and proved to be different from other classes of adipose-derived cells after expanding SVF cells with DMEM (Dulbecco's Modified Eagle Medium) plus 10% FBS (fetal bovine serum). They were called adipose-derived SM-like cells as they consistently express SMCs markers (Smooth muscle alpha actin, Smooth muscle myosin Heavy chain, Myocardin, SM22 and calponin), independent of donor site and across multiple passages [72]. SMC differentiation could be achieved either with chemical induction using different protocols [73, 74] or with coculture with SMCs [64]. Mechanical extension stimulation could improve the feasibility of ASCs induction into SMCs [75]. When mature SMCs are seeded on collagen scaffolds and implanted in vivo, they not only regenerate SMCs needed for contraction but they also reduce inflammation and promote neovascularization [76].

4.2.2. ASCs as a possible source of scaffolds

The use of acellular matrices or synthetic scaffolds can be associated with immunologic reactions and possibility of infection transmission as exogenous ECM materials still retain a significant portion of residual DNA. Therefore, a new method was developed; the self-assembly method; to produce a tissue built by the cells themselves where a dense ECM is completely produced by fibroblasts. In opposition to all exogenous scaffold models, these models are autologous, which is a real advantage by eliminating the biocompatibility concerns. The absence of immunological response should reduce the inflammatory and fibrotic reactions and consequently improve the success rate of the procedure. Although this method has been developed for skin engineering in case of severe burn, however, its use has been extended to a wide variety of applications ranging from skin to blood vessels [77-79].

Our group has explored this unique technique for the reconstruction of urethra, urinary bladder and tunica albuginea [80-86]. As ASCs were able to lay down collagen-based matrix under the influence of ascorbic acid using the self-assembly technique [87], they would form

another alternative for scaffolds for urinary tract regeneration. Additionally, they have been shown to allow the growth and stratification of epithelial cells when seeded on top [88]. ASCs have been utilized to form vesical equivalents and showed that there was no significant structural difference between a ASCs and a dermal fibroblasts equivalents. When compared to matrix synthesized from fibroblasts cultured under the same conditions, ASCs matrix was thicker and showed comparable failure strain [89].

4.3. Reconstruction of urinary tract with ASCs

4.3.1. Tissue engineering of the ureter

Ureteral damage may result from trauma or pathologies that lead to stricture formation obstructing the urine flow from the kidneys to the urinary bladder. The resulting ureteral defects may be short or long. While bridging short defects is usually surgically feasible, the reconstruction of long defects require extensive surgical repair that is not always possible and may carry complications including metabolic derangements and tissue harvest problems. Accordingly, new therapeutic options incorporating urothelium and avoiding ample tissue harvest are required.

Tissue engineering can offer these new therapeutic options. Unlike urethra and bladder, ureteral tissue engineering is only at its beginning. Unseeded synthetic or naturally derived biomaterials were used in few animal studies and resulted in ureterohydronephrosis due to the lack of normal tissue formation [90]. Cell seeded scaffolds present the ideal template for tubular ureteral regeneration. The seeded cells should include urothelial cells to prevent early contact of the scaffold with harmful urine and smooth muscle cells to regenerate the muscular layer necessary for urine transport through the ureter. Whenever autologous urinary tract cells are not available, ASCs can be the source of these cells. ASCs were differentiated into UCs through indirect coculture protocol and seeded on tubular polylactic acid (PLA)/collagen scaffolds [91]. Strong evidence of differentiation into urothelial lineage was detected with CK-18 and UP2. When implanted subcutaneously in athymic mice, the differentiated cells in the graft survived, stratified and exhibited urothelial markers. In another study, when ASCs were differentiated into SMCs and seeded onto decellularized rabbit aortas, at 16 weeks after implantation, radiography revealed patent ureter with no stricture associated with evidence of stratified epithelium and organized muscle bundles similar to the native tissue [92].

4.3.1.1. Tissue engineering of the urinary bladder

Cell-seeded scaffolds with autologous urinary tract cells represent the current strategy for tissue engineering of the urinary bladder [93]. Clinical trials using autologous urothelial and smooth muscle cells along with exogenous biomaterials have been performed [94]. Although organ specific cells would be an ideal cell source, however they are not available in many situations as in bladder cancer or benign end-stage bladder [95]. Stem cells derived from many tissues including bone marrow, muscle and adipose tissue are possible options to bridge the defective cell source in these situations. ASCs are more encouraging due to their previously mentioned advantages. ASCs can form ECM matrix and can be differentiated into urothelial

cells and SMCs (Figure 2). Both the matrix and the differentiated cells can be used together or separately to reconstruct the urinary bladder for in vivo implantation.

ASCs have been used for in vivo studies for urinary bladder replacement or augmentation whether unmodified [96, 65] or after differentiating them into SMC [73]. Unmodified autologous ASCs were seeded on acellular matrices (bladder acellular matrix in rabbits or prepuce matrix in rats) and showed increase in smooth muscle content in the seeded group and improved bladder functional evaluation when compared to non-seeded scaffolds. However, ASCs were not labeled; therefore the origin of the regenerated muscle cells at the site of the seeded matrices was not known whether it is differentiated ASCs or growth of local bladder cells during normal healing process. In another study, ASCs were differentiated into SMCs and seeded on synthetic scaffold PLGA and implanted in rats to replace 50% of the bladder. Labeled SMC-differentiated ASCs persisted in vivo, became more organized with time and led to better bladder compliance when compared to non-seeded scaffolds. However, in either of the previous studies, the urothelial cell layer was lacking, exposing the scaffold to urine irritation in the early healing period in case of large defects, which may promote fibrosis and scarring of the graft highlighting the importance of the urothelial layer.

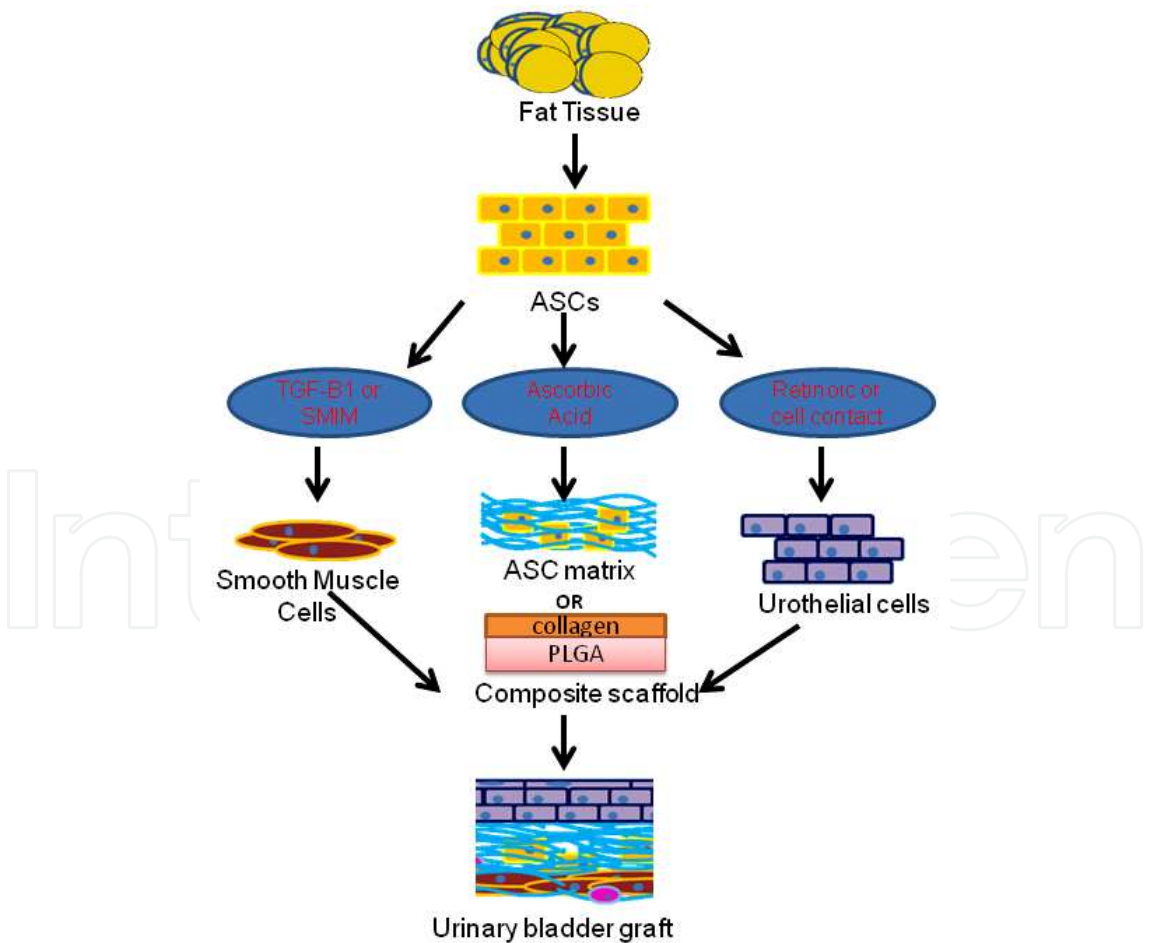


Figure 2. Shows the possible contributions of ASCs in tissue engineering of urinary bladder

4.3.1.2. *Tissue engineering of the urethra*

Tissue engineering of urethra, using seeded or unseeded scaffolds has been used with success in preclinical studies and clinical trials [93]. Among the available models, a biomaterial made by the self-assembly technique was fabricated from dermal fibroblasts and seeded with urothelial cells [85]. As preliminary formation of UC-seeded ASCs matrices for bladder replacement was achieved [89], it is expected that the same approach in tubularized form can be used for urethral regeneration.

ASCs have been employed to constitute urinary tract epithelium [97] and SMCs [75] in urethral tissue engineering. In the first study, ASCs were differentiated into urothelial cells with DMEM (supplemented with 2% FBS, all-trans retinoic acid, epidermal growth factor, hepatocyte growth factor, keratinocyte growth factor, hydrocortisone) at the air liquid interface to promote urothelial differentiation. Then, they were seeded on bladder acellular matrix to be implanted in rabbits. The urethral continuity was maintained with large caliber and the labelled differentiated urothelial cells survived and formed a stratified epithelial layer. In the second study, ASCs were induced chemically with 5-azacytidine (5-AZA) to differentiate into SMCs under mechanical extension stimulation. The autologous induced cells together with oral mucosal epithelium cells were seeded on PGA mesh to replace urethral defects in dogs. The mechanical extension-stimulated engineered urethras were developed into more normal architectures resembling nearby native urethra. Whether they are source of SMCs or UC, ASCs, with their advantages, can contribute significantly in urethral tissue engineering

4.3.1.3. *Tissue engineering of urinary conduit*

Urinary diversion after cystectomy with gastrointestinal segments is the current treatment for patients with bladder cancer or benign end-stage bladder diseases as bladder exstrophy or neurogenic bladder due to spina bifida. It can be either continent or incontinent diversion depending on the patient ability to control urine evacuation. Incontinent urinary diversion with ileal conduit is common; however it still carries the complications of the use of gastrointestinal segments. That is why a new approach for construction of tissue engineered urothelium-lined urinary conduit was developed.

A limited number of studies incorporated the use of adult cells from the urinary tract to construct neourinary conduit (NUC) [98]. Nevertheless, autologous cells in these disease states are not appropriate source for formation of NUC. Stem cells differentiated to mature urinary tract cells may be a suitable alternative for the development of NUC. ASCs as a source of easily obtained autologous stem cells can be an ideal option. A study led by Tengion™ [99] used tubular scaffolds made of PLGA seeded with adipose-derived SMCs in a porcine cystectomy model. Implantation of these constructs led to the formation of patent tubular neo-organ that was histologically comparable to native urinary bladder. They had well developed urothelial and smooth muscle layers with minimal collagen deposition. As a result, Tengion has started Phase I clinical trials of NUC constructs in human patients requiring urinary diversion. This Phase I study “Incontinent Urinary Diversion Using an Autologous NeoUrinary Conduit” (<http://www.clinicaltrials.gov/ct2/show/NCT01087697>) is currently recruiting patients using autologous adipose-derived SMC with biodegradable biomaterial.

4.3.1.4. Tissue engineering of sling materials for urinary incontinence and pelvic organ prolapse

Synthetic slings used in treatment of urinary incontinence (SUI) and pelvic organ prolapse (POP) have serious complications such as tissue erosion [100]. Biodegradable biological materials; whether xenografts or allografts; are more likely to undergo tissue remodeling and less likely to cause erosion [101]. They are designed to provide a scaffold of acellular material to facilitate autologous cell infiltration and subsequent replacement of the graft tissue with regenerated functional host tissue. However, they have the drawbacks of poor integration into the host and rapid degradation with early recurrence [102]. A new approach was developed to use a cell-seeded bioabsorbable material to attain a long-term repair. Addition of autologous cells will help the formation of new ECM, leading to long-term mechanical integrity while the degradable nature of the scaffold should avoid a harmful chronic inflammatory response, allowing integration, neovascularization, and remodeling.

In two in vitro studies, ASCs were seeded onto biodegradable materials and the mechanical properties and cell attachment were observed. In the first one, human ASCs were seeded on a collagen mesh from porcine dermis. An increase in the mechanical properties after cell seeding was observed [103]. In the second study, the authors compared the use of human oral fibroblasts (OF) and ASCs seeded on poly-L-lactic acid (PLA) scaffolds as candidate cell types for the development of a pelvic floor tissue engineered repair material (TERM). They found that both cells were well attached and proliferated on scaffolds. The addition of any of the two cell types led to improvement in the mechanical properties compared to non-seeded scaffolds in vitro. In addition, ASCs produced more total collagen and a denser homogenous ECM than OF with unrestrained scaffolds. The authors concluded that OF and ASC both appeared to be suitable cell types to combine with biodegradable scaffolds, in the development of a TERM for the treatment of SUI and POP [104]. Moreover, autologous ASCs have the other advantages of modulating the inflammatory response and promoting a weaker foreign body reaction, which could have a beneficial impact in patients treated with cellularized meshes. Also, ASCs decrease the degradation rate of the collagen meshes and enhance angiogenesis. However, it remains to examine the in vivo response to these cell seeded meshes in animal studies as well as their degradation rate and changes in mechanical properties over time.

Preclinical studies for urinary reconstruction are included in table 4.

Involved organ/ disease	Nature of the study/ disease model	Cells used	Functional Assessment	Notes	References
Ureteral replacement	Athymic mice	Human ASCs differentiated into urothelial cells	Not available	The grafts was implanted subcutaneously for 14 days	91
	Normal rabbits	Autologous rabbit ASCs differentiated into smooth muscle cells	Intravenous Urography (IVU)	IVU demonstrated no ureteral stricture or hydroureteronephrosis	92

Involved organ/ disease	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 with good mechanical properties	89
	Normal rabbits	Autologous cultured ASCs were seeded on bladder acellular matrix.	Cystography. Normal bladder capacity was acquired.	Exogenous scaffold was used.	96
	Normal Rats	Autologous rat ASCs were seeded on decellularized prepuce dynamically	Cystometry. It showed better parameters with seeded group.	ASCs were not labeled. Small number of animals were used.	65
	Athymic rats	Human ASCs differentiated into SMCs were seeded on PLGA	Cystometry. It showed better compliance with ASCs-SMC seeded group.	Labeled ASCs survived and formed organized muscle tissue.	73
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 urothelial- differentiated cell seeded constructs	BrdU-labeled cells survived in vivo transplantation.	97
	Normal canine model	Autologous SMC- differentiated ASCs and oral epithelial cells were seeded on PGA scaffolds	Urethrography. It showed slight strictures at the site of implantation	The use of bioreactor improved the characters and outcome of engineered graft	75
Urinary conduit	porcine cystectomy model	Autologous Adipose-derived SMCs were seeded on PLGA scaffolds	Not available	Urothelial and smooth muscle regeneration with no fibrosis	99
Pelvic sling material	In vitro study	Human unmodified ASCs seeded on porcine dermis	Not available	Increase in mechanical properties after cell seeding	103
	In vitro study	Human oral fibroblasts and	Not available	Both cell types had good results regarding	104

Involved organ/ disease	Nature of the study/ disease model	Cells used	Functional Assessment	Notes	References
		unmodified ASCs were seeded on PLA scaffolds		cell attachment and mechanical properties	

Table 4. Shows the different studies involving the use of ASCs in urinary tract reconstruction.

5. Cellular therapy

5.1. Voiding dysfunction

There are approximately 400 million patients with bladder diseases, and a lot of them might eventually need bladder reconstruction [66]. The International Continence Society and International Urogynaecological Association define voiding dysfunction as abnormally slow and/or incomplete micturition (voiding) based on symptoms and urodynamic investigations [105]. Voiding dysfunction usually presents in 2 forms; lower urinary tract symptoms (LUTS) and urinary tract decomposition [106]. Both affect the patient’s quality of life and survival. It can be classified functionally into failure to store or failure to empty urine or both; and anatomically, bladder dysfunction and bladder outlet dysfunction or both [107]. Bladder outlet obstruction (BOO) causes voiding dysfunction through increased collagen deposition, detrimental changes in ultrastructure of bladder SMCs and decrease blood flow [108]. All lead to impaired smooth muscle function and decreased bladder compliance. The inadequate efficacy of current pharmacological treatment and invasiveness of other modalities has supported the search for new stable therapeutic modalities for voiding dysfunction including bladder overactivity or underactivity. Additionally, none of the current treatments are able to modify the pathologic effects in the diseased bladders.

Possible mechanisms for a role of ASCs for the treatment of voiding dysfunction

1. Engraftment:

When delivered either locally or systemically, ASCs are recruited to the affected tissue with the effect being more pronounced with the local delivery. Homing cytokines, such as stromal derived factor-1, has been shown to attract ASCs to the site of injury [109]. After an acute injury, however, homing of transplanted stem cells may not be achieved because of the minimal amount of tissue damage, which subsequently leads to less cytokine expression [110].

2. Differentiation

Few studies have shown in vivo differentiation of ASCs after transplantation [64, 65]. SMC differentiation may occur due to the plasticity of ASCs and the effect of local bladder micro-environment rather than cell fusion [64]. The low evidence of in vivo SMC differentiation can be explained by the fact that labelled DNA in dividing cells is quickly diluted by cell divisions whereas dilution takes much more time for slow dividing stem cells [111] and the rapid wash

of the cells from the desired sites into circulation with lack of time needed for phenotype change. That is why *in vitro* differentiation of ASCs before delivery may be needed if SMC regeneration *in vivo* is strongly required.

3. Paracrine effect

Growing evidence has been shown that the beneficial effects of ASCs are largely due to paracrine actions with the release of cytokines and growth factors by the transplanted cells or neighbouring cells [112]. These cytokines and growth factors result in anti-inflammatory, musculotropic, angiogenic, antifibrotic and antiapoptotic actions. These actions cause modulation of local and systemic inflammatory responses and mobilization, stimulation and differentiation of local stem cells, promotion of vascularisation of regenerating tissues and reduction of fibrosis [113]. The secretome of ASCs includes: hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) [114]. 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 [115].

5.2. Preclinical studies

ASCs could potentially reverse many of the bladder pathologic changes in different animal models [113]. ASCs alleviated the symptoms of bladder dysfunction in various animal models of detrusor overactivity [115, 116] or underactivity [117] or variable spectrum of voiding dysfunction [112]. Also, ASCs seemed to preserve the bladder vascularity and decrease apoptosis. 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 [112]. This suggests the possibility of allogenic stem cells transfer 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 voiding dysfunction.

ASCs differentiated into SMC before local injection; have been shown to survive and increase SMC content at the injury site. However, no record on the improvement of bladder function after injection was reported [118]. Although systemic injection of ASCs has improved voiding dysfunction 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 [119].

It is important to note that ASCs can be useful in early stages of voiding dysfunction 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 furtherly investigated.

5.3. Urinary incontinence

There are 17 million people in the USA and more than 200 million people worldwide who live with urinary incontinence [120, 121]. Stress urinary incontinence (SUI) is the most common type of urinary incontinence. SUI is involuntary leakage of urine with sudden increase in the intra-abdominal pressure. It happens when intra-abdominal pressure causes the bladder pressure to exceed the urethral closure pressure. SUI is classified into three conditions, including intrinsic sphincter deficiency (ISD), urethral hypermobility, or a combination of both [122]. Most of the patients have both disorders in varying degrees [123]. SUI affects both males and females and decreases quality of life [124]. Many injectable bulking agents are minimally invasive but have a poor long-term efficacy and complications such as chronic inflammatory reactions, particle migration, periurethral abscess and erosion [125]. More invasive approaches, like sling procedures, bladder neck suspensions or artificial urinary sphincter implantation are more effective but have higher morbidity [126, 127]. 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 tissues, provide adequate mucosal coaptation and restore or improve resting urethral closure pressures [128].

5.4. Cell source for injection therapy

The ideal cells for cell therapy should be easily procured from minimally invasive procedures, proliferate quickly in a well-controlled manner, provide sufficient quantities of cells, exhibit capabilities of differentiation to regenerate multiple tissues, and be able to be transplanted into an autologous host [129]. Currently, bone marrow-derived stem cells (BMSCs), adipose-derived stem cells (ASCs), and muscle-derived stem cells (MDSCs) are the stem cell sources applied in SUI therapy. ASCs carry future special importance in this regard due to its reported myoblast and neuronal-like differentiation capacity and neovascularization potential in addition to their ease of harvest and high stem cell content [130].

5.5. Preclinical studies and clinical trials

ASCs were used in many animal studies as SVF or cultured ASCs or differentiated into myoblasts or coupled with biomaterials or with growth factors. Unmodified ASCs were labelled and injected periurethrally and systemically into female rats after induction of SUI postpartum vaginal balloon dilation and bilateral ovariectomy. There was improvement in cystometric parameters in 2/3 of the rats treated locally or systemically in relation to control animals with increase in muscle and elastin content. The ASCs beneficial effects were attributed mainly to growth factors that supported host tissue regeneration as most of the delivered ACSs remained undifferentiated after injection [131]. ASCs were also used in another model of SUI after injury to pudendal nerve injury. ASCs were labelled and injected at 3, 9, and 12 o'clock around the mid and distal urethra. Urodynamic evaluations revealed considerable improvements in maximum bladder capacity, abdominal leak point pressure, maximum urethral closure pressure and functional urethral length. Morphologic changes and significant im-

provement in urination control were consistent over time. Labeled cells gradually migrated in vivo toward the urethra and its lumen from urethra's edges [132].

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 [133].

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 [134]. 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 [135].

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 [136].

In a pilot study, 5 female patients with SUI were included to be treated with ASCs combined with bovine collagen gel and saline. The ASCs mixture with collagen was injected endoscopically through the urethra. The effect of the treatment was assessed objectively with cough test as a primary end point and subjectively with validated questionnaire. At 1 year, the cough test was negative for three patients; two of them were satisfied with the treatment and did not wish further treatment for SUI. Validated questionnaires showed some subjective improvement in all five patients [137].

Preclinical studies and clinical trials for cellular therapy are included in table 5.

Involved disease	Nature of the study/ disease model	Cells used	Functional Assessment	Notes	References
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.		115
		Autologous cultured ASCs and muscle precursor cells	UDS. Micturiting pressure (maximum and threshold) and		117

Involved disease	Nature of the study/ disease model	Cells used	Functional Assessment	Notes	References
	Diabetes Mellitus	(MPCs) injected into rat bladder.	voided volumes increased.		
		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	112
		Autologous cultured ASCs injected into bladder or tail vein of hyperlipidemic rat	Improved micturition frequency and voided volumes	Improvement with direct (bladder) injection is more efficient than systemic (tail vein) injection	117
	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.	118
Urinary incontinence	Stress urinary incontinence (SUI) -rat model	Autologous unmodified ASCs	UDS with different measures including ALPP, RUPR and bladder capacity	SUI was induced by vaginal balloon dilation and bilateral Ovariectomy.	131
		Unmodified ASCs and ASCs differentiated into myoblasts			133

Involved disease	Nature of the study/ disease model	Cells used	Functional Assessment	Notes	References
		Autologous unmodified ASCs		SUI was induced by pudendal nerve injury.	135
		Cultured ASCs with PLGA microbeads		SUI was induced by urethrololysis.	134
		Autologous cultured ASCs with PLGA or NGF or both		SUI was induced by bilateral pudendal nerve transection	135
	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.	136
	Female SUI-clinical trial	5 female patients received ASCs combined with bovine collagen gel and saline	Cough test and questionnaire	The mixture was injected transurethraly via cystoscope	137

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

Table 5. Shows the different animal studies and human trials with ASCs as a cellular therapy for lower urinary tract.

6. Hurdles and future directions

There are many hurdles that face the broad utilization of ASCs in clinical therapies for urinary tract diseases. First of all, is the limited ASCs efficiency for the treatment of chronic diseases. This can be explained by the fact that in chronic pathologies, there are less release of cytokines and so minimal attraction of ASCs to the site of the disease [138]. Research is needed to improve the accuracy of predictions regarding disease progression. Similarly, the time delay between injury and the initiation of treatment represents an obstacle as after an acute injury, homing signals are often diminished with time. Therefore, it is important to determine the duration of time after injury that equates to the optimal release of homing bioactive factors and to develop innovative methods for up-regulating the expression of these cytokines. Methods such as electrical stimulation or local injection of homing cytokines could help the direct recruitment of systemically delivered stem cells to the target organ [139].

The second is the form of delivery of ASCs; whether differentiated or undifferentiated cells would allow better regeneration of the urinary tract. 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. A mix of differentiated and undifferentiated cells may be a good option.

Moreover, there is no final agreement on the preferred type of cells to use (SVF cells or cultured ASCs), total number of cells for the treatment or the number of cells for single injection. Although recent studies showed that after cell labelling, ASCs migrate into the bone marrow after systemic or local delivery [140,109,141], however, more precise imaging studies are required to observe the fate of the implanted ASCs regarding cell survival, proliferation, migration and formation of functional tissues [142]. Live image tracing might be a good choice for that purpose [143]. Neovascularization and reinnervation of engineered tissues are critical obstacles to overcome for future application to bladder reconstruction. Ingrowth of native vessels and nerves—which is stimulated by MSCs bioactive factors—is achievable for smaller grafts; however, complete regeneration with functional integration is far more challenging.

ASCs are able to secrete some cytokines, which can modulate the host inflammatory response, and guards against severe inflammatory and fibrotic responses damage in the urinary tract [144]. The contributions of each of the cytokines and growth factors to the repair of urinary tract need to be evaluated. Finally, the safety of ASCs-based treatment needs to be carefully checked as ASCs have the potential risk of tumorigenic transformation [145]. Hence, more chronic animal models, consistent protocols and many clinical trials are required to make sure of ASCs therapeutic efficacy and safety.

7. Conclusions

Tissue Engineering and Regenerative Medicine is an emerging field of research for organ and tissue replacement to compensate for the deficiency of organ donation and complications of

immunosuppression. In the search of new therapeutic options for urinary tract disorders, both SVF and cultured ASCs have been the focus of numerous in vitro studies, various animal models and few clinical trials. Depending on the type of dysfunction to be treated, ASCs can be used either as cellular therapies or combined with scaffolds for tissue-engineering applications. Although few clinical trials showed promising results, however, more future clinical studies are required to prove their efficacy for those particular applications while exploring the mechanisms ensuring their functional activity. Clinical trials are listed for the use of ASCs in different diseases including urinary incontinence, renal failure, ischemic nephropathy and urethral strictures in males (<http://www.clinicaltrials.gov/>).

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