Review Article

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Prospects of stem cell treatment in benign urological diseases

Amjad Alwaal[1,2], Ahmed A. Hussein[1,3], Ching-Shwun Lin[1], Tom F. Lue[1]

[1]Department of Urology, University of California, San Francisco, CA, USA, [2]Department of Urology, King Abdul Aziz University, Jeddah, Saudi Arabia, [3]Department of Urology, Cairo University, Cairo, Egypt

Stem cells (SCs) are undifferentiated cells that are capable of self-renewal and differentiation and that therefore contribute to the renewal and repair of tissues. Their capacity for division, differentiation, and tissue regeneration is highly dependent on the surrounding environment. Several preclinical and clinical studies have utilized SCs in urological disorders. In this article, we review the current status of SC use in benign urological diseases (erectile dysfunction, Peyronie disease, infertility, and urinary incontinence), and we summarize the results of the preclinical and clinical trials that have been conducted.

Keywords: Erectile dysfunction; Infertility; Stem cells; Urinary incontinence

INTRODUCTION

Stem cells (SCs) are undifferentiated cells that are capable of self-renewal and differentiation and that therefore contribute to the renewal and repair of tissues [1]. Their capacity for division, differentiation, and tissue regeneration is highly dependent on the surrounding environment [1]. SCs are difficult to classify owing to a lack of defined morphologic and molecular characteristics. However, they can be classified according to their differentiation potential as follows [2]:

(1) Totipotent SCs: These SCs have the highest potential and can differentiate into any tissue type, regardless of origin. The zygote and morula are examples of this SC type.

(2) Pluripotent SCs: These SCs can differentiate into cells from the 3 different germ cell layers and gonadal ridge but not into extra-embryonic tissues. An example is embryonic SCs (ESCs), which are a derivative of the inner cell mass of the blastocyst.

(3) Multipotent SCs: These SCs are capable of self-renewal and can differentiate into organ-specific cell types. Examples include hematopoietic SCs, mesenchymal SCs, and neural SCs.

(4) Unipotent SCs: These SCs can give rise to only one defined cell type, epithelial cells.

(5) Induced pluripotent SCs: These SCs are "reprogrammed" cells, i.e., differentiated cells that are manipulated in the laboratory to express genes that are normally present in ESCs and that therefore behave like ESCs. Induced pluripotent SCs can differentiate into cells of all organs and tissues.

When tissue damage occurs, SCs, which are normally quiescent, become stimulated to undergo cellular division and enhance cellular regeneration. The microenvironment of SCs, also known as the niche, is crucial for this process. The niche properties, including proximity to the bloodstream, the presence of certain cytokines and growth factors, and low

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Corresponding Author: Amjad Alwaal
Department of Urology, University of California, 400 Parnassus Ave., Ste A-630, San Francisco, CA 94143-0738, USA
TEL: +1-415-476-3801, FAX: +1-415-476-3803, E-mail: amjadwal@yahoo.com

oxygen tension and other physiochemical properties, allow optimal interaction between SCs and their neighboring stromal or epithelial cells and the extracellular matrix [3].

ERECTILE DYSFUNCTION

Erectile dysfunction (ED) is defined as the inability to attain or maintain a penile erection for satisfactory sexual intercourse [2]. ED can significantly impair the quality of life and relationships of men and their partners [4]. The estimated prevalence of ED is about 20% of men aged 40 years and older, with a higher prevalence among older men [5]. There are several management options for ED, including lifestyle modifications, pharmacotherapy (including oral phosphodiesterase-5 inhibitors [PDE5Is], intraurethral alprostadil, intracorporal injections, vacuum devices, and surgery [including penile revascularization and penile implants]). Despite the efficiency of many of these modalities, limitations to their use exist, including different drug interactions (especially PDE5Is with cardiovascular medications), intolerance to side effects, cost, and that not all patients achieve a satisfactory outcome [6]. Apart from a successful revascularization, these modalities offer symptomatic relief rather than a cure for the disease, spurring interest in developing a curative treatment for ED, including SC therapy [7].

1. Mechanism of ED varies according to the cause

Aging is associated with increased resistance to penile blood flow and diminished response to cavernosal nerve stimulation [8]. In addition, nitric oxide (NO) levels decrease as a result of high levels of reactive oxygen species, causing endothelial dysfunction [9]. Structural changes also may occur with aging, including replacement of smooth muscles with collagen fibers and degeneration of elastic fibers [10].

Metabolic syndrome constitutes diabetes mellitus (DM), hypertension, and dyslipidemia. DM is associated with decreased cavernosal NO, endothelial cells, and smooth muscles [11]. *Hyperlipidemia* is associated with lower levels of cavernosal NO, with subsequent neuronal and endothelial dysfunction [12].

Following radical prostatectomy (RP) for prostate cancer treatment, cavernous nerve injury may ensue. Although nerve-sparing RP results in a lower incidence of post-surgery ED, about 20% of patients still experience ED at 2 years following a nerve-sparing procedure [13]. This may be attributed to neurapraxia, diminished NO production, smooth muscle apoptosis, and penile fibrosis [14]. Radiation-based therapies are thought to cause ED via a similar mechanism [15].

2. Potential role of SC therapy in ED

Several cell types have been studied in the treatment of ED. ESCs improve erectile function in neurogenic ED [16]. However, ethical concerns have limited further research using this cell type. One study showed that vascular endothelial growth factor (VEGF)-transfected endothelial progenitor SCs improved erection in diabetic rats [17]. Similarly, several preclinical studies have shown the beneficial effect of bone marrow-derived SCs (BMSCs) on erectile function in different rat models, including models of DM, cavernous nerve injury, and aging [18-20]. Another SC type used in ED treatment research is skeletal muscle-derived SCs (SKMSC). These SCs can be easily obtained through muscle biopsy and have been shown to improve erectile function in cavernous nerve injury and aging ED rat models [21,22]. Neural crest SCs have shown the potential to differentiate into smooth muscle cells and endothelial cells in the rat penis [23]. Adipose tissue-derived SCs (ADSCs) are the most widely used type of SC in ED [7]. They improve erectile function by promoting angiogenesis and through direct transformation to endothelial cells, smooth muscle cells, and neurons and also through the release of stimulatory cytokines such as VEGF and fibroblast growth factor [24-26]. Testicular and human urine SCs have been also studied [27].

3. Methods of SC delivery

SC performance may be potentiated by modifying the characteristics of the cells by manipulating their genes or by incubating them with scaffolds, growth factors, or other substances. The therapeutic effect of SC injection may be via migration of these cells to the injury site [28]. Different routes have been suggested for delivery of SCs. Intravenous injection of ADSCs showed improvement of erectile function [28]. Moreover, intracorporal SC delivery for ED treatment is popular, being easy and successful. The regenerative effect of SCs is achieved by either secreting growth factors into the bloodstream or migrating to major pelvic ganglia [7]. Direct injection of SCs into the major pelvic ganglia has not been studied extensively despite their regenerative effect because of difficulties in the injection process [16,29]. Periprostatic injection with or without simultaneous intracorporal injection has also been tried [30-32]. Intraperitoneal injection of SCs was less effective than intracorporal injection in restoring erectile function in a cavernous nerve injury mouse model [33].

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PEYRONIE'S DISEASE

Peyronie's disease (PD) is an acquired connective tissue disease of the tunica albuginea of the corpus cavernosum, characterized by extensive fibrosis and plaque formation. PD can result in significant physical and psychological morbidity; men may suffer incapacitating pain and deformities that may prevent intercourse and reduce satisfaction, with adverse impacts on partner relationships [34].

1. Mechanism of PD

The exact pathogenesis of PD is unknown. The most widely accepted theory is repeated microvascular trauma to the erect penis resulting in inflammation, disruption of the elastic fibers, and deposition of fibrin [35]. Some studies related vascular trauma to osteoid formation via osteoblast-like cells originating from the vascular lumen [36]. More recent reports showed that upregulation of certain genes, namely, *osteoblast specific factor 1*, may be responsible for plaque calcification [37]. Another theory is cavernosal hypoxia, which induces collagen deposition and fibrosis. This may explain the penile morphological changes and the development of PD following RP [38]. Transforming growth factor (TGF)-β1 may play an important role in the induction of collagen production by fibroblasts and myofibroblasts in the development of PD plaques [35]. Prolonged inflammation causes the formation of dense fibrotic plaques, which may progress to calcification or ossification. The exact mechanism by which tissue mineralization occurs remains uncertain [39].

2. Potential role of SC therapy in PD

Regenerative urology represents a novel method with potential benefits in the treatment of PD with the use of mesenchymal SC therapy [40]. The external location of the penis makes administration of local SC therapy technically feasible and easy. Moreover, pluripotent mesenchymal SCs are readily available, and their use avoids the ethical issues associated with the use of embryonic SCs. Also, autologous cells may be used, avoiding the issue of antigenic incompatibility [41]. ADSCs may be the most widely used of the mesenchymal SCs, as they are abundant and easily accessible [41]. The exact mechanism of action of ADSCs remains unclear; SCs may differentiate and replace the damaged tissue, increase the local production of cytokines and growth factors, decrease inflammation and oxidative stress, or modulate the extracellular matrix [42]. One interesting finding is that ADSCs seem to migrate to the site of injury, probably in response to cytokine signaling [43]. In rats treated with intratunical injections of TGF-β1, an established model for PD, ADSCs inhibited the development of PD. ADSCs decreased disordered collagen type III and elastin tissues (common in PD plaques) [44], which could be the basis for future research for their use in the treatment of PD in humans and the hope of interrupting the disease pathogenesis before it actually manifests.

INFERTILITY

1. Mechanism of infertility

Anticancer treatment, in the form of surgery, cytotoxic chemotherapy, novel targeted therapy, immunological therapy, and radiotherapy, may cause persistent damage to germ cells, somatic cells critical to germ cell survival and maturation such as Sertoli cells, and Leydig cells, which are critical for testosterone production. The extent of damage depends on the type of cancer, age, and treatment modality [45]. Cytotoxic therapy disrupts spermatogenesis by targeting spermatogonial SCs [46].

2. Potential role of SCs in the treatment of infertility

Isolation and cryopreservation of spermatogonial SCs from the prepubertal testicle prior to cytotoxic therapy may provide hope for children facing a sterilizing therapy. This technique requires a testicular biopsy followed by cryopreservation. Afterwards, spermatogonial SCs may be used for induction of *in vitro* spermatogenesis or autologous transplantation into the patient's own testes. This procedure was successfully replicated in many animal models [47].

URINARY INCONTINENCE

Urinary incontinence (UI) is defined as the involuntary loss of urine. It affects nearly 200 million people around the world. UI affects women 2 to 3 times more than men until the age of 80 years, after which the prevalence becomes equal in men and women. Nearly 50% of women above the age of 20 years will experience UI, and 50% of those will suffer from stress urinary incontinence (SUI). Other types of incontinence include urge UI and mixed UI. Oral pharmacotherapy usually fails in ameliorating SUI, and more effective, although invasive, surgical options such as a urethral sling may become necessary. Therefore, there is a need to develop less invasive alternative treatments for this common condition, and SC therapy represents a promising avenue [48].

The urethra is a multilayered structure composed of the epithelium, connective tissue, striated and smooth muscles,

and small blood vessels [49]. Striated and smooth muscle cells were found to be markedly reduced in animal models of SUI [49], and because SCs can differentiate into either muscle type, several studies have utilized SCs in the treatment of SUI to replenish those cells [50]. Furthermore, SCs secrete musculogenic and angiogenic growth factors that can further enhance their regenerative effect [50]. ADSCs were also found to improve urethral connective tissue, likely through the production and processing of elastin and collagen [51].

1. Preclinical studies

The initial concept behind cell-based therapy for SUI involved the use of skeletal myoblasts to replace the deficient urethral sphincter [52]. The idea then evolved into the use of SCs to substitute for myoblasts. Yiou et al. [53] are credited with the first utilization of SKMSCs in SUI in 2002. From there, SKMSCs were utilized exclusively in SUI preclinical studies until 2010 (Table 1). Since then, five preclinical studies have utilized BMSCs [54-58]. One of those studies was not a typical SC study in that the SCs were seeded in a degradable silk scaffold, which was then used as a sling for the urethra [57]. Umbilical cord blood SCs were also used in one preclinical trial [59]. More recently, seven preclinical studies utilized ADSCs in SUI [51,60-65], including one study in which ADSCs with silk fibroin microspheres were used as a bulking agent [65]. Human amniotic fluid-derived SCs have been used in 3 studies of mouse SUI models [66-68]. All SC types used have produced improvement in SUI.

Most preclinical studies on SC treatment for SUI used rats as an animal model. However, several studies utilized mice [66-68], including the first study by Yiou et al. [53], and one study used monkeys [69]. Different techniques have been performed to establish SUI animal models. A sphincteric injury model has been developed using cauterization, injection of myotoxin, or electrocoaugulation. Pudendal or sciatic nerve injury models have also been developed by using crush injury or transection. The delivery, vaginal distension, and ovariectomy animal model is the most widely used animal model for birth injury [48,70-72]. All these models suffer from their short durability of 2 to 3 weeks [73-75]. Administration of SCs in SUI preclinical studies has been through periurethral injection. In one study by Lin et al. [51], both periurethral and intravenous routes were utilized, and both routes demonstrated improvement of urinary continence.

Functional and histological assessments are used to assess the outcome of SC use in SUI. Functional assessment is typically achieved by either measuring leak point pressure by use of the Crede or vertical tilt table method or through electrical stimulation of the urethral sphincter neurovascular bundle [48]. The purpose of histological assessment is to locate the SCs, identify SC differentiation, and assess for tissue improvement. Histological assessment is typically done by sacrificing the animal and harvesting the urethral tissue, followed by staining with H&E or trichrome. To identify possible differentiation of the transplanted SCs, immunohistochemical and immunoelectron microscopy were done in several studies [48].

2. Clinical studies

Five clinical trials, done by the same group of researchers, have examined the effect of injected SKMSCs in male and female UI between 2007 and 2008. Those studies reported 80% to 90% improvement in UI [61,76-80]. However, two of those trials were later retracted, citing ethical concerns [79,80]. Carr et al. [81] showed that 5 out of 8 women with SUI achieved total continence using SKMSCs. Lee et al. [59] demonstrated 70% to 80% improvement of continence in 39 female patients with SUI by using cord blood SCs . A small case series utilizing ADSCs for SUI was later retracted for unknown reasons [82]. Using SKMSCs in 12 female patients with SUI, Sebe et al. [83] showed improvement in 10 of 12 women, but worsening of SUI in 2 patients. The typical injection method in clinical trials has been transurethrally, although Carr et al. [81] utilized both transurethral and periurethral routes and showed improvement in incontinence with both routes. In a small pilot study of 3 male patients with SUI, Yamamoto et al. [84] showed an improvement in SUI by using ADSCs at 6 months. Another study using ADSCs showed 60% improvement in SUI in 8 of 11 male patients at 1 year [85]. A Polish study with a longer follow-up of 2 years reported 75% improvement in 16 female patients with SUI with the use of SKMSCs, with 50% of patients achieving complete continence [86]. Most recently, Kuismanen et al. [87] showed improvement of SUI in 3 of 5 female patients at 1 year of follow-up with the use of ADSCs with collagen gel as a bulking agent. Functional assessment in clinical trials has been through measuring pad weights, bladder diaries, and quality of life assessment, in addition to urodynamic study findings such as peak flow rate, postvoid residuals, and maximal urethral closing pressure [48].

3. Future directions

ADSCs represent an easier SC type to obtain given the availability of adipose tissue and ease of acquisition. Therefore, future use of SCs in UI would probably uti-

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Table 1. Stem cell studies for urinary incontinence

Source	Year of publication	Animal model/patients	Stem cell type	Injection method
Yiou et al. [53]	2002	Sphincter injury mice	Autologous SKMSC	Periurethral
Lee et al. [88]	2003	Sciatic nerve transection rats	Allogeneic SKMSC	Periurethral
Yiou et al. [89]	2003	Sphincter injury rats	Autologous SKMSC	Periurethral
Cannon et al. [90]	2003	Sciatic nerve transection rats	Allogeneic SKMSC	Periurethral
Chermansky et al. [91]	2004	Sphincter cauterization rats	Allogeneic SKMSC	Periurethral
Lee et al. [92]	2004	Pudendal nerve transection rats	Allogeneic SKMSC	Periurethral
Yiou et al. [93]	2005	Sphincter injury rats	Autologous SKMSC	Periurethral
Kwon et al. [94]	2006	Sciatic nerve transection rats	Allogeneic SKMSC	Periurethral
Kim et al. [95]	2007	Sciatic nerve transection nude rats	Human SKMSC	Periurethral
Mitterberger et al. [76]	2007	123 Female patients	Autologous SKMSC	Transurethral
Mitterberger et al. [77]	2008	63 Male patients	Autologous SKMSC	Transurethral
Mitterberger et al. [78]	2008	20 Female patients	Autologous SKMSC	Transurethral
Carr et al. [81]	2008	8 Female patients	Autologous SKMSC	Transurethral/ periurethral
Hoshi et al. [96]	2008	Periurethral injury rats	Allogeneic & xenogeneic rodent SKMSC	Periurethral
Furuta et al. [97]	2008	Pudendal nerve transection nude rats	Human SKMSC	Periurethral
Lin et al. [51]	2010	Vagina distension rats	Autologous ADSC	Periurethral & IV
Fu et al. [60]	2010	Vagina distension rats	Allogeneic ADSC	Periurethral
Kinebuchi et al. [55]	2010	Sphincter injury rats	Autologous BMSC	Periurethral
Lim et al. [98]	2010	Sphincter injury rats	Human CBSC	Periurethral
Lee et al. [59]	2010	39 Female patients	Allogeneic CBSC	Periurethral
Zou et al. [57]	2010	Sciatic nerve transection rats	BMSC on scaffold	Sling surgery
Xu et al. [99]	2010	Pudendal nerve transection rats	Allogeneic SKMSC	Periurethral
Zhao et al. [63]	2011	Pudendal nerve transection rats	Autologous ADSC	Periurethral
Kim et al. [56]	2011	Pudendal nerve transection rats	Allogeneic BMSC	Periurethral
Corcos et al. [54]	2011	Pudendal nerve transection rats	Allogeneic BMSC	Periurethral
Wu et al. [62]	2011	Pudendal nerve transection rats	Allogeneic ADSC	Periurethral
Watanabe et al. [61]	2011	Pelvic nerve transection rats	Allogeneic ADSC	Periurethral
Sebe et al. [83]	2011	12 Female patients	Autologous SKMSC	Endourethral
Yamamoto et al. [84]	2012	3 Male patients	Autologous ADSC	Transurethral
Kim et al. [66]	2012	Pudendal nerve transection mice	Human AFSC	Periurethral
Li et al. [64]	2012	Vagina distension rats	Autologous ADSC	Periurethral
Chun et al. [67]	2012	Pudendal nerve transection mice	Human AFSC	Periurethral
Badra et al. [69]	2013	Pudendal nerve transection monkeys	Autologous SKMSC	Periurethral
Stangel-Wojcikiewicz et al. [86]	2014	16 Female patients	Autologous SKMSC	Transurethral
Dissaranan et al. [58]	2014	Vagina distension rats	Allogeneic BMSC	Periurethral
Gotoh et al. [85]	2014	11 Male patients	Autologous ADSC	Transurethral
Shi et al. [65]	2014	Pudendal nerve transection rats	Autologous ADSC with silk fibroin microspheres	Periurethral
Chun et al. [68]	2014	Pudendal nerve transection mice	Human AFSC	Periurethral
Kuismanen et al. [87]	2014	5 Female patients	Autologous ADSC with collagen gel	Transurethral

SKMSC, skeletal muscle-derived stem cell; ADSC, adipose tissue-derived stem cell; BMSC, bone marrow-derived stem cell; CBSC, umbilical cord blood stem cell; AFSC, amniotic fluid-derived stem cell; IV, intravenous.

lize ADSCs more than other SC types. Current SUI animal models have the disadvantage of short durability. Development of more durable, chronic-type SUI animal models is important to accurately determine the therapeutic effects of SCs. The development of induced pluripotent SCs represents a milestone in SC research, and utilization of this

technology in urology should be a future goal. More clinical trials recruiting a larger number of patients are needed, and they should adhere to the highest standards of ethical considerations.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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