Multipotent Stromal Cell Therapy for Cavernous Nerve Injury-Induced Erectile Dysfunction

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ABSTRACT

Introduction. Erectile dysfunction (ED) following radical prostatectomy (RP) is a result of inadvertent damage to the cavernous nerves that run close to the prostate capsula. The mechanisms behind the development of post-RP ED are increasingly recognized and include cavernosal fibrosis and cavernosal smooth muscle apoptosis, resulting from cavernous nerve degeneration due to neuropraxia. In recent years, cell-based therapies have received increasing attention regarding their potential for recovery of erectile function following cavernous nerve injury (CNI). Multipotent stromal cells (MSCs) are an attractive cell source for this application based on their regenerative potential and their clinical applicability.

Aim. To review available evidence on the efficacy and mechanisms of action of MSC application for the treatment of ED, with an emphasis on ED following CNI.

Methods. A nonsystematic review was conducted on the available English literature between 1966 and 2011 on the search engines SciVerse-sciencedirect, SciVerse-scopus, Google Scholar, and PubMed.

Results. MSCs from both bone marrow and adipose tissue have shown beneficial effects in a variety of animal models for ED. While MSC application in chronic disease models such as diabetes, aging, and hyperlipidemia may result in cell engraftment and possibly MSC differentiation, this observation has not been made in the acute CNI rat model. In the latter setting, MSC effects seem to be established by cell recruitment toward the major pelvic ganglion and local paracrine interaction with the host neural tissue.

Conclusions. While the type of model may influence the mechanisms of action of this MSC-based therapy, MSCs generally display efficacy in various animal models for ED. Before translation to the clinic is established, various hurdles need to be overcome. **Albersen M, Kendirci M, Van der Aa F, Hellstrom WJG, Lue TF, and Spees JL. Multipotent stromal cell therapy for cavernous nerve injury-induced erectile dysfunction. J Sex Med 2012;9:385–403.**

Key Words. Adipose Tissue-Derived Stem Cells; Bone Marrow-Derived Stem Cells; Cavernous Nerve Injury; Recruitment; Erectile Dysfunction; Paracrine

Introduction

Prostate cancer is the most commonly diagnosed and treated solid malignancy in adult males. It has a significant impact on men's health, with 217,730 new cases diagnosed each year and 32,050 annual deaths attributed to the disease in the USA [1]. The lifetime probability of developing prostate cancer is estimated to be 11–20% [2]. In a recent U.S.-based study, 36–41% of men chose radical prostatectomy (RP) to control their localized prostate cancer [3]. Erectile dysfunction (ED) following RP remains a frequent consequence with a significant impact on quality of life in spite of development of novel laparoscopic and robot-assisted operative techniques [4,5]. As prostate cancer is detected at an increasingly younger age and lower stage, patients undergoing RP

generally have better erectile function and have higher expectations concerning the preservation of sexual functioning following treatment. It is recognized that sexual dysfunction is an independent determinant of a reduced quality of life after treatment for prostate cancer [5], whereas maintenance of quality of life is the principle argument in treatment choice for over 45% of patients [6]. It logically follows that an increasing body of research has been focused on understanding the pathophysiology of postprostatectomy ED and the concept of instituting prophylactic measures for prevention and/or early recovery from ED [7,8].

ED following RP is the result of inadvertent injury to the cavernous nerves that run close to the prostate capsule and innervate the corpora cavernosa of the penis. As unassisted nerve regeneration is a slow process, denervation-induced damage, including cavernosal fibrosis and smooth muscle apoptosis in the erectile tissue, frequently develops while the injured cavernous nerve is regenerating [9]. Various research groups have been focusing on how this time frame of nerve regeneration can be shortened and have investigated the effects of neurotrophic factors, type 5 phosphodiesterase (PDE-5) inhibitors, and immunomodulatory molecules in animal models of cavernous nerve injury (CNI) [9]. More recently, cellular therapy has received increasing attention regarding its potential merit for the recovery of erectile function following CNI [10–16]. In this review, we discuss the effects of multipotent stromal cell (MSC) therapy on erectile function, focusing on ED following CNI. Furthermore, we discuss possible mechanisms of action of MSC based on peer-reviewed literature and available research reports.

Methods

An extensive search was conducted on published English language literature between 1966 and 2011 on MSC therapy for the management of ED. The search engines SciVerse-sciencedirect, SciVerse-scopus, Google Scholar, and Pubmed were used, with search terms including "ED," "stem cells," "MSCs," "adipose- (tissue-) derived stem cells," "bone marrow-derived stem cells," "animal model," and "CNI." We opted to focus on recent publications (within the last 10 years) as older publications tended to contain preliminary results that have been more thoroughly or clearly defined in more recent studies. Relevant articles were identified and obtained in full-text form. Abstracts from international meetings were considered for inclusion only if the data had not been published in manuscript form. Information was critically reviewed and synthesized. Reference lists for several of the manuscripts identified via the search were reviewed and additional relevant citations were obtained from these.

Penile Rehabilitation Therapy and Clinical Need for MSC Therapy

There are a variety of insults to the nerves during RP, including thermal damage, ischemic injury, nerve stretching, and the local inflammatory effects from surgical trauma. Hence, the exact recovery time for return of erectile function after RP is somewhat unpredictable and complete erectile recovery is generally not witnessed until a mean of 18–24 months following surgery [17]. In effect, continued improvement in erectile function is seen beyond 2 years postsurgery [18]. These observations have encouraged clinicians to employ penile rehabilitation practices during the long recovery period.

The clinical strategy of postoperative penile rehabilitation after RP arose from the concept that induced early sexual stimulation and augmented blood flow oxygenating erectile tissue would facilitate the return of unassisted erectile function [19]. An additional (psychological) benefit of early penile rehabilitation after RP is the resumption of sexual activity and improved quality of life [20]. Unfortunately, there is no consensus on the implementation of penile rehabilitation including initiation time, frequency of application, type of vasoactive agents, and dose regimen to be used. After the first report by Montorsi et al. employing intracavernosal alprostadil injections to improve the recovery of spontaneous erections [21], several authors have developed other erectogenic rehabilitation programs utilizing oral PDE-5 inhibitors and vacuum erection devices. A recent prospective study reported greater numbers of men with spontaneous erections and increased responsiveness to oral PDE-5 inhibitor therapy after a regimen consisting of both intracavernosal injections and PDE-5 inhibitors [22]. Based on the available data, either intracavernosal injections and/or vacuum erection device are recommended as first-line options early in the post-RP period, as the mechanism of action does not require intact neural transmission. Thereafter, conversion to PDE-5 inhibitor therapy alone may be an option for men who can achieve at least partial tumescence [19].

In addition to penile rehabilitation protocols, a growing number of studies have employed neuro-

protection or stimulation of neurogenesis to restore cavernous nerve function after RP. In CNI animal models, a variety of neurotrophic factors, such as nerve growth factor (NGF), basic-fibroblast growth factor (bFGF), neurturin (neuro-)immunophilin ligands (FK506 and FK1706, rapamycin), growth hormone, vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), erythropoietin, and growth differentiation factor 5, have been investigated and demonstrated improved erectile function [23–33]. Similarly, gene therapies using various viral vectors coding for neurotrophic protein for tissue engineering and reconstruction have recently shown promise in CNI animal models [34–36].

Although current penile rehabilitation programs employ medications, which may partially restore erectile function, there is still a clinical need for measures that ensure maintenance of erectile tissue integrity and increase the chances of full cavernous nerve recovery. Cell-based therapies are a relatively novel regenerative approach. In principle, they will stimulate neurogenesis and thus enhance recovery of erectile function following CNI by shortening the time interval of cavernosal denervation and hypoxia.

Pathophysiology of ED Following CNI

ED following RP is often the result of injury to the cavernous nerves, combined with long-term alterations in the cavernosal tissues secondary to denervation. In the rat, the cavernous nerve is an easily identifiable structure originating from the major pelvic ganglion (MPG) located at the lateral surface of the prostatic capsule where it runs toward the crus of the corpus cavernosum. Several CNI models in animals have been developed, including nerve crush, transection, and freezing of the nerve [37]. Nerve-crush injury is currently suggested as the best animal model to study erectile function after RP [37]. This model in theory mimics nerve damage occurring during nerve sparing RP, with damage to the axon without transecting the nerve. Some researchers have proposed injury to accessory internal pudendal arteries as an important pathophysiological feature of post-RP ED [38]. However, the role of the latter vessels remains somewhat vague and under studied and therefore is not further addressed in this review.

It is recognized that even if the cavernous nerves are preserved with RP, ED develops in a majority of patients for up to 18–24 months [39]. This time frame represents the time needed for cavernous nerves to fully regenerate following neuropraxia and subsequent neurodegeneration. In neuropraxia, the affected cavernous nerves are injured by a combination of stretching, crushing, electrocoagulation, and blunt trauma. Though a majority of axons remain intact, relatively minor insults initiate a neurodegenerative process termed Wallerian degeneration [40]. Wallerian degeneration is initiated within minutes to hours following nerve injury and starts with degradation of the axoplasm and axolemma accompanied by the development of axonal and myelin debris. This is subsequently phagocytosed not only by resident Schwann cells but also by invading macrophages as the result of a neuroinflammatory reaction, which is coordinated by resident Schwann and glial cells [41]. This also occurs at the corresponding ganglia, containing both supporting cells and cell bodies of the injured neurons. The inflammatory reaction initiates a cascade resulting in the expression of chemoattractant molecules both at the site of injury and at the cell body of the affected nerve fiber (in the human situation, the pelvic and dorsal root ganglia or in the rat, the MPG). This inflammatory reaction affects the whole nerve and thereby causes damage to initially uninjured axons as well. These observations have suggested the application of immunomodulatory therapies to prevent ED from occurring following CNI [31,32,42]. The release of chemoattractant molecules by the injured tissues is an important stimulus for the use of stem cell therapy (vide infra) [43].

The process of Wallerian degeneration causes axonal interruption and denervation of the erectile tissues. Denervation of the erectile tissue over time results in loss of smooth muscle content and increased collagen deposition, leading to fibrotic changes in the extracellular matrix of the penis. In addition to ED, these changes in penile tissue architecture are thought to lead to penile shortening and an increased incidence of Peyronie's disease after RP [9]. It is hypothesized that in addition to the direct effect of denervation, indirect effects of penile hypoxia cause production of pro-inflammatory cytokines, which in turn stimulate collagen deposition and initiate apoptosis of smooth muscle cells and the endothelium [44]. CNIs also disrupt the sonic hedgehog homolog (SHH) cascade and corpora cavernosal homeostasis after which morphological changes in sinusoidal structure ensue, resulting in further deterioration of erectile function. Researchers have illustrated that neural activity and a trophic

factor from the MPG or cavernous nerves are necessary to regulate SHH protein and smooth muscle volume in the penis [45,46]. The resulting loss of smooth muscle and increase in collagen deposition in the penis provoke mechanical alterations, which reduce the elasticity and compliance of the cavernosal tissue [47]. These alterations impair expandability of the sinusoids, which normally compress the emissary veins against the tunica albuginea, resulting in cavernosal insufficiency (venous leak, cavernous veno-occlusive dysfunction).

Summarizing, ED following CNI occurs as a result of (i) denervation, (ii) loss of smooth muscle cell content, and (iii) fibrosis. Initially, stem cell therapy in the treatment of ED was instituted to allow stem cells to engraft and replace lost smooth muscle or endothelial cells with (*trans*)differentiated functional stem cells. This was the reason why most studies employed intracavernous injection for delivery. However, it is becoming more apparent that stem cells influence the process of neuroregeneration, thereby diminishing secondary damage to the erectile tissue by enhancing the nerve supply to these tissues. This paradigm transition is further elucidated in the upcoming paragraph on mechanisms of action of stem cell therapy for CNI-induced ED.

Multipotent Mesenchymal Stromal Cells

Bone Marrow-Derived Mesenchymal Stromal Cells

MSCs were first described by Friedenstein and colleagues in 1968 as fibroblast-like cells derived from the bone marrow, which generated colonies when plated at low densities (Figure 1A, B) [48].

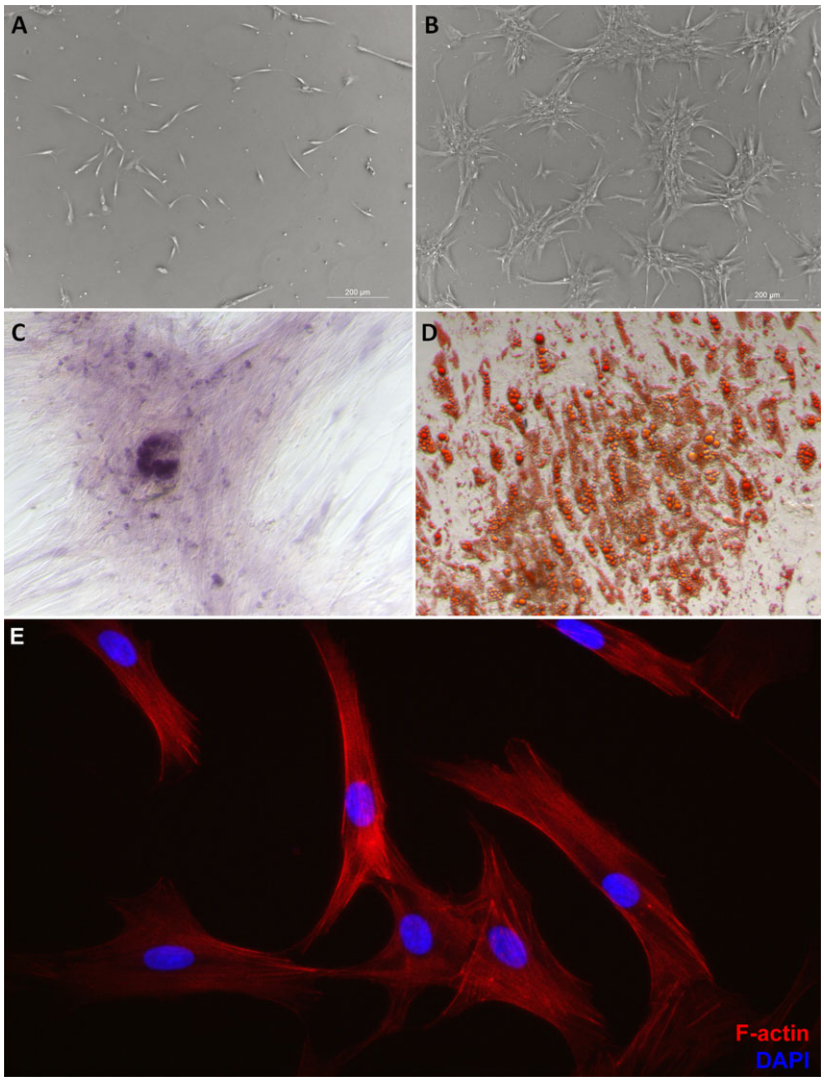


Figure 1 Multipotent stromal cells in culture. (A) Phase contrast microscopy image of passage 0 adipose tissue-derived stem cells (ADSCs) 2 days after plating. Note the individual spindle-shaped ADSC. (B) Phase contrast microscopy image of passage 0 ADSCs 7 days after plating: note the starting colony formation. (C) Alizarin red staining of osteogenic differentiated ADSC showing calcium depots (purple). (D) Oil red O staining of adipogenic differentiated ADSC showing fat droplets inside the cells (red). (E) Cytoskeletal organization of passage 0 ADSC in culture. Red: phalloidin (F-actin); blue: 4′,6-diamidino-2-phenylindole (DAPI) (nucleus).

Hence, they were originally referred to as fibroblastoid colony-forming units. Subsequently, they have been called mesenchymal stem cells and, most recently, multipotent mesenchymal stromal cells [49,50]. The International Society for Cellular Therapy has provided the following minimum criteria for defining multipotent human mesenchymal stromal cells [50]:

1. Plastic adherent when maintained in standard culture conditions.
2. MSC must express CD105, CD73, and CD90 and lack of expression of CD45, CD34, CD14, CD11b, CD79-α, CD19, and HLA-DR surface molecules.
3. MSC must differentiate into osteoblasts, adipocytes, and chondroblasts ex vivo (Figure 1C, D).

While it is estimated that MSCs represent only one in 10,000–1,000,000 of the total nucleated cells within bone marrow aspirates, they are easily cultured and expanded ex vivo under specific conditions [51,52]. They can be maintained for as many as 40 population doublings, resulting in cellular yields sufficient for most clinical cell therapy strategies in a time frame of 8–10 weeks [51]. As they are well characterized and have been extensively studied, bone marrow-derived MSCs (BM-MSCs) remain the principal source of MSCs for most preclinical and clinical studies. However, isolation of MSCs has been reported from several tissues, including adipose tissue, liver, muscle, amniotic fluid, placenta, umbilical cord blood, and dental pulp [51]. It is believed that MSCs represent a population of cells responsible for local growth and regeneration of the tissue in which they reside, and it is conceivable that there may be a distinct population of MSC-like cells endogenous to every tissue of the human body.

Adipose Tissue-Derived Stem Cells

Of the various tissues in which MSCs have been isolated, the fat tissue has been of particular interest for ED researchers investigating cellular therapy. Adipose tissue-derived stem cells (ADSCs) are a distinct population of MSCs residing at the perivascular niche in adipose tissue [53,54]. To obtain these cells, resected fat or lipoaspirate is minced and incubated with collagenase in order to dissociate the extracellular matrix. By centrifugation, floating mature adipocytes are then separated from the pelleted stromal vascular fraction (SVF). As the SVF comprises a heterogeneous cell population, the final isolation step consists of plating these cells in order to select the adherent population by successive washings [55]. They share many characteristics with BM-MSCs pertaining to morphology, phenotype, and ex vivo differentiation ability, and they have a highly similar gene expression profile [56]. However, the frequency of these cells is 100- to 500-fold higher in adipose tissue compared with the frequency of MSCs in bone marrow [55]. Furthermore, while bone marrow is obtainable in the gram range by a painful marrow aspiration, hundreds of grams of adipose tissue can be obtained with minimally invasive procedures under local anesthesia. The possibility of harvesting tissue in the hundreds of gram range allows for direct reinjection of cells (SVF) in the same surgical procedures during which they were harvested. Although SVF cells are not ADSCs per se, human clinical trials have recently been initiated to employ injection of uncultured SVF during breast reconstruction surgery and to treat myocardial infarction, traumatic calvaria defects, lipodystrophy, and type I and II diabetes [55]. As there is no need for expanding cells in culture to obtain sufficient numbers for cellular therapy, the risks of contamination or dedifferentiation of cells are minimized. Furthermore, MSCs present in SVF that have not been expanded in culture are more likely to retain their migratory and engraftment potential (see below). Therefore, it is reasonable to expect that ADSCs will become a valuable choice of MSCs for future clinical applications. Although the use of SVF may be clinically beneficial, especially during surgical procedures in which procurement and application can be combined within a period of 1–2 hours, cultured ADSCs are starting to make their way into clinical trials as well. Healing of Crohn's cryptoglandular fistula and maxillary reconstruction have been aided by application of autologous cultured ADSCs [55].

As BM-MSCs and ADSCs are the two stem cell sources most likely to make their way to clinical practice in the treatment of ED, this review will thus focus on efficacy and possible mechanisms of action of these specific cellular products for the reversal of ED, specifically following CNI.

MSC Therapy for Non-CNI-Induced ED

Reports on the efficacy of MSCs therapy for ED are, besides anecdotal clinical reports, to date limited to preclinical research. Besides ED following CNI, cellular therapy has been used in other conditions causing ED, such as diabetes and aging (summarized in Table 1).

Table 1 ED not related to cavernous nerve injury

First author, year [ref]	Type of MSC	Animal model (*age*)	Route of administration	Time from administration to evaluation	Benefit of MSC therapy?	Suggested* mechanism of action
Song, 2007 [57]	Human fetal immortalized BM-MSC (xenograft)	Healthy rat (*10 weeks*)	Intracavernous injection	2 weeks	N/A	Engraftment and differentiation
Bivalacqua, 2007 [58]	$_{lacZ}$BM-MSC (allograft)	Aged rat (*25 months*)	Intracavernous injection	1 and 3 weeks	Function: + Histology: +	Engraftment and differentiation
	$_{eNOS}$BM-MSC (allograft)	Aged rat (*25 months*)	Intracavernous injection	1 and 3 weeks	Function: + Histology: +	Engraftment and differentiation
Abdel Aziz, 2010 [59]	$_{GFP}$BM-MSC (allograft)	Aged rat (*24–30 months*)	Intracavernous injection	3 weeks, 4 weeks, 3 months, and 4 months	Function: + Histology: +	Engraftment
Garcia, 2010 [60]	$_{BrdU}$ADSC (autograft)	DM2, metS: Zucker diabetic fatty rats (*23 weeks*)	Intracavernous injection	3 weeks	Function: + Histology: +	Paracrine
Huang, 2010 [61]	$_{EdU}$ADSC (autograft)	Hyperlipidemic rat (*8 months*)	Intracavernous injection	4 weeks	Function: + Histology: +	Paracrine
Kendirci, 2006 [62]	$_{lacZ}$BM-MSC (allograft)	DM1: STZ injected rat (N/A)	Intracavernous injection	2 days	Function: –	Engraftment. Differentiation N/A
	$_{eNOS}$BM-MSC (allograft)	DM1: STZ injected rat (N/A)	Intracavernous injection	2 days	Function + (compared with both diabetic and nontransfected cells)	Engraftment. Differentiation N/A
Qiu, 2011 [63]	$_{CM\text{-}DiI}$BM-MSC (allograft)	DM1: STZ injected rat (*18 weeks*)	Intracavernous injection	4 weeks	Function: + Histology: +	Engraftment. Differentiation N/A
Qiu, 2011 [64]	$_{CM\text{-}DiI/VEGF}$BM-MSC (allograft)	DM1: STZ injected rat (*18 weeks*)	Intracavernous injection	4 weeks	Function: + Histology: +	Engraftment. Differentiation N/A

*Proposed by the author; see text for comments
Subscript depicts label/transfected gene (or both)
ADSC = adipose tissue-derived stem cell; BrdU = bromodeoxyuridine (*thymidine analogue, label*); CM-DiI = lipophilic fluorochrome (*label*); DM1 = diabetes mellitus 1; DM2 = diabetes mellitus 2; ED = erectile dysfunction; EdU = 5-ethynyl-2′-deoxyuridine (*thymidine analogue, label*); eNOS = endothelial nitric oxide synthase; GFP = green fluorescent protein (*transfected label*); lacZ = gene coding for β-galactosidase enzyme (*transfected label*); BM-MSC = bone marrow-derived multipotent stromal cell; N/A = not applicable; STZ = streptozotocin; VEGF = vascular endothelial growth factor

Healthy Rat

Song et al. transplanted immortalized human fetal BM-MSCs into the healthy corpus cavernosum of male rats under immunosupression and evaluated whether these cells were able to differentiate into endothelial and smooth muscle cells [57]. They conducted immunofluorescence microscopy to confirm in vivo differentiation and observed colocalization of human nuclear antibody with endothelial and smooth muscle markers in different cells. There were limitations in this study, namely, nuclear, membranous, and cytoplasmic antigen labeling appeared to overlap, suggesting cross-reactivity of the antihuman antibody with the host tissue. Overall, immortalized cells from human fetuses do not replicate easily in applications that will be used in future clinical practice.

Aging

Bivalacqua and associates investigated whether intracavernous injection of BM-MSCs alone or endothelial nitric oxide synthase (eNOS)-modified BM-MSCs could be used for the treatment of age-associated ED [58]. They observed improvement of erectile function as quick as 7–21 days after injection of eNOS-modified BM-MSCs. Improved erectile function was associated with increased eNOS protein, NOS activity, and cyclic guanosine monophosphate (cGMP) levels. Unmodified BM-MSCs were able to increase erectile function 21 days after injection, and injected cells were traced back to the corpus cavernosum where they expressed endothelial and smooth muscle markers. The authors concluded that injected cells were incorporated and differentiated into host tissue cells as a mechanism of action. Of note, BM-MSCs expanded in culture stained positive for smooth muscle actin and Von Willebrand factor. The exact location where the incorporated cells were observed was inconclusive. Hence, the assumption that differentiation into functional cells (e.g., endothelium-like cells located in the lining of sinusoids) at 7–21 days should be guarded at this time.

Abdel Aziz et al. reported on the effects of intracavernous injection of BM-MSCs transfected with the green fluorescent protein (GFP) gene in aged rats [59]. They confirmed the findings of Bivalacqua et al. in terms of recovery of erectile function, both in short- (3–4 weeks) and long-term (3–4 months) follow-ups. Underlying changes included an increase of cGMP levels and alterations in the extracellular matrix, leading to markedly dilated sinusoidal vascular spaces. Fluorescence microscopy revealed dense autofluorescence in transplanted penile tissues. The morphology of transplanted cells and colocalization with smooth muscle or endothelial markers was not shown.

Diabetes and Metabolic Syndrome

Garcia and colleagues in Tom Lue's laboratory at University of California, San Francisco (UCSF) were the first to evaluate the effects of MSC therapy on erectile function in type II diabetic rats [60]. They used intracavernous injection of autologous undifferentiated ADSC labeled with the thymidine analogue bromodeoxyuridine (BrdU) to treat diabetes-induced ED. The observed increase in posttreatment intracavernous pressure (ICP) during cavernous nerve electrostimulation, measured 21 days after injection, was significantly higher in the treatment group compared with controls. In contrast with previous publications, only a small number of BrdU-labeled ADSC were detected within the corporal tissue of the treatment group. Whereas there was no significant incorporation of cells, a significant increase in neuronal NOS (nNOS) in the penile dorsal nerve and in the number of endothelial cells in the corpora cavernosa was observed in the treatment group. As the number of labeled ADSC retained in the corpus cavernosum was very small, the authors postulated that a paracrine function, not differentiation to smooth muscle or endothelial cells, was responsible for the observed improvement in penile function.

In agreement with these findings, Lue's lab further studied the effects of intracavernous application of autologous ADSCs in an animal model for metabolic syndrome (hyperlipidemia) and again found improvement in erectile function resulting from enhanced endothelial and neural function [61]. They labeled the ADSCs with another thymidine analogue, 5-ethynyl-2′-deoxyuridine (EdU), which is chemically detected and more sensitive than use of BrdU. This study also was not able to locate a significant number of stem cells that engrafted in the penis, in spite of the observed functional and structural improvements.

Kendirci et al. studied the feasibility of treatment with ex vivo expanded MSCs genetically modified with eNOS on improving erectile function in an animal model of streptozotocin-induced diabetes [62]. After isolating bone marrow from rat and expanding ex vivo, they transduced MSCs with genes coding for lacZ (for tracking purposes) and eNOS, and transplanted these cells into diabetic

rat corpora cavernosa. They observed significantly increased levels of eNOS protein and activity from cultured cells. Two days after intracavernous injection, lacZ-positive cells were detected in the corpus cavernosum. In diabetic animals injected with eNOS transfected MSCs, cavernous nerve electrostimulation caused ICP to be significantly higher than in either diabetic controls or in animals injected with lacZ-transfected MSCs. The authors demonstrated that intracavernous transfer of MSCs genetically enhanced to express eNOS improved diminished erectile activities in type I diabetic rats.

In two subsequent studies, Qiu et al. confirmed the benefits of MSC therapy to treat ED resulting from type I diabetes [63,64]. In the first study, uncommitted BM-MSCs were intracavernously injected, resulting in an increased ICP compared with untreated diabetic controls. The authors claimed that this observed change was the result of increased content of endothelium and smooth muscle in the corpus cavernosum. They further showed that CM-Dil-labeled cells persisted in the erectile tissue until 4 weeks following injection [63]. In an additional study, they showed that both functional and structural effects (smooth muscle and endothelium content) were enhanced after transfection of BM-MSCs with an adenoviral vector coding for VEGF [64].

MSC Therapy for ED Following CNI

Studies involving MSC therapy to treat CNI are summarized in Table 2. The first attempt in restoring erectile function following CNI using stem cells was made by Bochinski and colleagues from the UCSF laboratory who injected GFP-labeled neural embryonic stem cells either into the corpus cavernosum or adjacent to the MPG [16]. The authors observed significant improvement in erectile function. In treated groups, neurofilament and nNOS staining showed increased neuroregeneration or nerve preservation compared with injured controls. Interestingly, in both treated groups there was no direct evidence of engrafted stem cells after harvesting the tissue. In the opinion of the authors, this suggested that transplanted stem cells do not require prolonged presence in tissue to exert function. Instead, their mechanism of action may have occurred through growth factor expression, inhibition of demyelination, or as an initial lattice of cellular substrate. While this study did not employ MSCs, these findings provide a relevant clue about the method stem cells or transplanted cells in general heal injured tissues.

Fall et al. were the first to test whether intracavernous transplantation of adult bone marrow cells could improve erectile function following bilateral CNI [15]. In this study, bilateral CNI was induced by resection of a 5-mm segment of the cavernous nerves bilaterally. Following corporeal denervation, diffused apoptosis of mesenchymal cells, smooth muscle cells, and endothelial cells was noted in erectile tissues. Intracoporeal injection of 10^7 bone marrow mononuclear cells decreased apoptotic cell numbers, accelerated the normalization of nNOS and eNOS levels, and partially restored erectile responses at 5 weeks posttreatment. The authors reported that injected cells were retained in the corpus cavernosum based on identification of a dye label, PKH-26, in the erectile tissue of treated rats. Dye-labeled cells did not colocalize with smooth muscle, endothelial, or neural markers, indicating a lack of differentiation to those cell types [65]. However, the use of PKH-26 is questionable as a marker for engraftment because it can be taken up by surrounding cells, after cell death, and membrane disintegration of labeled cells. It may falsely label macrophages after phagocytosis of cellular debris of injected labeled cells. More importantly, it is not clear that MSCs or stem/progenitor cells per se were responsible for the benefits observed after treatment because an allogeneic bone marrow mononuclear cell fraction was used to treat the rats. Of this fraction typically one in 10,000 to one in a million cells are BM-MSCs [52]. As the BM-MSCs were not expanded in culture and very few BM-MSCs were injected, it is difficult to be certain whether the observed changes were the result of the MSC population or other cells in this cellular suspension.

Albersen and colleagues at UCSF tested the applicability of intracavernosal ADSC treatment for ED following crush injury of the cavernous nerves as a model for neuropraxia [14]. Based on absence of incorporation of transplanted cells, in previous studies, the question arises whether ADSC-derived lysate would have beneficial effects on erectile function. Lysate treatment exposes injured tissues to soluble factors contained in ADSCs, without allowing live cells to directly act on the host tissue [66]. In this study, both ADSCs and ADSC lysate improved peak-ICP levels 4 weeks after injection, and both therapies partially restored smooth muscle content in the penis, decreased corpus cavernosum fibrosis, and, more importantly, restored nNOS expression in the

Table 2 ED following cavernous nerve injury

First author, year [ref]	Type of MSC	Animal model (*age*)	Route of administration	Time from administration to evaluation	Benefit of MSC therapy?	Suggested* mechanism of action
Fall, 2009 [15]	$_{PKH\text{-}26}$BM mononuclear cells (allograft)	Cavernous nerve resection (5-mm. gap), rat (*N/A*)	Intracavernous injection	3 and 5 weeks	Function: + Histology: +	Engraftment* differentiation N/A
Albersen, 2010 [14]	$_{EdU}$ADSC (autograft)	Cavernous nerve crush, rat (*12 weeks*)	Intracavernous injection	4 weeks	Function: + Histology: +	Paracrine
	ADSC-lysate (autograft)	Cavernous nerve crush, rat (*12 weeks*)	Intracavernous injection	4 weeks	Function: + Histology: +	Paracrine
Fandel, 2011 [71]	$_{BrdU}$ADSC (autograft)	Cavernous nerve crush, aged rat (*18 months*)	Intracavernous injection	4 weeks	Function: + Histology: +	Paracrine
	Adipose SVF (autograft)	Cavernous nerve crush, aged rat (*18 months*)	Intracavernous injection	4 weeks	Function: + Histology: +	Paracrine
Kendirci & Spees, 2011 [13]	$_{GFP}$BM-MSC (allograft)	Cavernous nerve crush, rat (*N/A*)	Intracavernous injection	4 weeks	Function: ± Histology: +	Paracrine
	$_{GFP\text{-}\ p75NGFR}$BM-MSC (allograft)	Cavernous nerve crush, rat (*N/A*)	Intracavernous injection	4 weeks	Function: + (compared with unselected BM-MSC) Histology: +	Paracrine
Fandel & Albersen, 2010 [43]	$_{EdU}$ADSC (autograft)	Cavernous nerve crush, rat (*12 weeks*)	Intracavernous injection	1 day, 3 days, 7 days, and 4 weeks	Function: + Histology: +	Paracrine and homing of ADSC toward MPG
	$_{EdU}$ADSC (autograft)	Cavernous nerve crush, rat (*12 weeks*)	Perineural injection (dorsal penile nerve)	1 day, 3 days, 7 days, and 4 weeks	Function: – Histology: –	No functional benefit

*Proposed by the author; see text for comments
Subscript depicts label/transfected gene (or both)
PKH-26 = (*label*); GFP = green fluorescent protein (*transfected label*); BrdU = bromodeoxyuridine (*thymidine analogue, label*); EdU = 5-ethynyl-2′-deoxyuridine (*thymidine analogue, label*); SVF = stromal vascular fraction; p75NGFR = p75 low affinity nerve growth factor receptor; ADSC = adipose tissue-derived stem cell; BM-MSC = bone marrow-derived multipotent stromal cell; N/A = not applicable; MPG = major pelvic ganglion

dorsal penile nerves. A limitation of this study was that the contents of the lysate were not characterized to identify potential regenerative substances. Furthermore, it was not elucidated whether the observed effects were a result of local or systemic distribution and whether retrograde axonal transport of lysate molecules may have played a role in conferring benefit. In accordance with previous studies, few engrafted ADSCs were found in the corpus cavernosum after 4 weeks. The lack of engraftment provided evidence for paracrine interactions between ADSCs and host tissue. The possibility for paracrine effects was further examined in culture studies at the Lue lab. First, it was shown that coculture of rat MPG tissue with ADSCs significantly improved neurite sprouting as compared with MPG fragments that were cocultured with smooth muscle cells [67]. The evidence for paracrine interaction was further supported by an experiment in which the MPG was cultured in conditioned medium of either ADSCs or smooth muscle cells [12]. Results from the latter experiment indicated that ADSCs might secrete substances that induce nerve regeneration and axonal sprouting. Zhang et al. identified LPS-induced CXC chemokine (LIX) (CXCL5) as a possible candidate for the neurotrophic effects of ADSCs. Blocking LIX reduced the effects of ADSC-conditioned medium and LIX was shown to activate the Janus kinase/signal (JAK/STAT) transducers and activators of transcription pathway in Schwann cells. This pathway is activated by other neurotrophins, such as BDNF, and has been documented to be important in mediating neuroregenerative effects in the MPG after CNI [12,68–70].

The USCF lab further investigated translational aspects of ADSC therapy in a study by Fandel and colleagues [71]. They assessed whether autologous adipose tissue-derived SVF therapy could replicate the effects of autologous ADSC treatment to improve on erectile recovery following CNI. They performed a study in aged rats (18 months old), testing the hypothesis that autologous cellular therapy with cells derived from aged animals could induce neuroregeneration. Besides demonstrating a significant improvement in erectile function in both treated groups compared with vehicle-treated rats, they showed an increase in nitrergic nerve regeneration and a restoration of smooth muscle that was otherwise lost in the corpus cavernosum. This finding raises hopes for clinical application of SVF. The use of SVF would provide an advantage over cultured cell therapy, as it can be harvested, prepared, and reinjected during a radical pelvic surgery such as RP.

Kendirci et al. isolated BM-MSCs from transgenic GFP rats and selected a subpopulation for p75 low affinity NGF receptor (p75NGFR) [13]. These cells were injected into the corpus cavernosum immediately after bilateral CNI (crush), followed after 4 weeks by ICP measurement in response to cavernous nerve electrostimulation. Intracavernous injection of p75NGFR-MSCs resulted in a significantly higher ICP measurements compared with all other groups except the sham-operated group. Rats injected with typical unselected MSCs had partial erectile function rescue compared with animals that received p75NGFR-MSCs. Fibroblast (cell control) and phosphate buffered saline (vehicle control) injection did not improve erectile function. Rare surviving engrafted MSCs and p75NGFR-MSCs had a fibroblastic rather than a neuronal morphology. No GFP positive cells were detected in the penile tissue of rats receiving fibroblasts. The rare long-term engraftment of MSCs and p75NGFR-MSCs and their morphology in vivo indicated that the beneficial effects of stem/progenitor cell administration were not likely the result of cell replacement by engraftment and differentiation, e.g., as neurons. In studies with cultured cells, the authors examined the secretion of bFGF, NGF, BDNF, VEGF, and IGF-1 by p75NGFR-MSCs, which are growth factors previously reported to decrease ED in rats with CNI [72–74]. Of all secreted proteins, bFGF was the growth factor secreted most by p75NGFR-MSCs compared with the other cell types. This growth factor is a promising candidate to further investigate, as it is well known to provide neuroprotection in the central and peripheral nervous systems [75], and bFGF was identified as a principal neurotrophic factor in the penis [76].

Considerations on the Mode of Action of MSC Therapy After CNI

While most of the above studies have shown that local (penile) cellular incorporation and differentiation are not the major mechanisms of MSC therapy following CNI, the question remains what happen to the cells after injection. To resolve this issue, a UCSF study in which labeled ADSCs were tracked in the penis and MPG at various time points following intracavernous injection was performed [43]. ADSCs migrate out of the injection site (corpus cavernosum) in a time-dependent manner and there is significant homing of ADSCs to the MPG in crush-injured animals. This

occurred after 1, 3, and 7 days but was transient. At 28 days, few ADSCs were traced back to the MPG. This observation was completely absent in sham animals. Elevated chemokine secretion (stromal cell-derived factor-1 [SDF-1]) at the MPG after crush injury appeared to be responsible for this observation. As no colocalization of labeled ADSCs with neuronal or Schwann cell markers was observed, increased numbers of ADSCs at the MPG after crush injury support nerve regeneration by releasing trophic factors to the neurons. Based on this and previous observations, a proposed mechanism of action for MSC therapy in CNI is illustrated in Figure 2. The difference in observed mechanisms of action between CNI and other disease models may be explained by the fact that after acute injury in CNI, there is short-term and localized release of homing factors (chemokines). In contrast, other disease models may invoke different signaling systems toward stem cells and thus different stem cell behaviors in vivo.

The exact interactions between recruited MSC and the host neural tissue remain unclear in the CNI rat model. It has been proposed that the paracrine actions of these stem cells include the rescue of neurons from apoptosis, modulation of the immune response following CNI, and induction of switching of microglia phenotype [77]. Another mechanism that has been observed in the central nervous system is the activation of endogenous neurogenesis by stimulation of tissue-residing neural progenitor cells [77,78]. However, while the presence of resident neural progenitor cells has recently been confirmed in the peripheral nervous system, it is unclear whether activation of these cells takes place after stem cell treatment of peripheral nerve injury [79].

Mesenchymal Stromal Cell Migration and Recruitment

In view of the therapeutic potential for MSCs to treat a wide variety of diseases and tissue injuries, there is great interest in mechanisms that control MSC migration and recruitment [80]. This is the case for cultured progenitor cells as well as for noncultured endogenous MSC-like cells that may be amenable to directed mobilization in vivo. MSCs are relatively simple to isolate from bone marrow and adipose tissue and can be rapidly expanded into clinically relevant numbers of cells for cell therapy [81]. However, culture expansion of MSCs can alter their expression and/or presentation of cell surface proteins such as chemokine receptors, altering their homing and migratory characteristics (Figures 2 and 3) [82,83]. In addition, MSCs express a wide variety of integrin subunits that are important for adhesion to endothelium. Notably, cell surface expression of integrins can also change with MSC culture conditions [84], and these integrins are likely to affect MSC adhesion and extravasation following intravenous or intra-arterial infusion.

Improved understanding of cell surface protein expression and function on both cultured and noncultured MSCs will help to identify culture conditions that increase the ability of expanded cells to home and engraft diseased or injured tissue after transplantation. For example, similar to many types of immune cells, MSCs express C-X-C chemokine receptor (CXCR4), which is the receptor for SDF-1. However, only a few percentages of cultured MSCs exhibit this cell surface receptor. In contrast, C-C chemokine receptor (CCR) type 4, which is a receptor that binds monocyte chemotatactic protein 1, is functionally and structurally present on the cell surface and in the intracellular compartment of ADSC after prolonged culturing (Figure 3, unpublished data). Activation of this receptor leads to conformational changes in the cell cytoskeleton that prepares the cell for migration, endothelial adhesion, and transendothelial migration.

Means of Improving MSC Recruitment

Lee et al. demonstrated that a subset of rapidly self-renewing human bone marrow MSCs was preferentially engrafted in mice following intravenous infusion compared with larger MSCs that were dividing at a slower rate [85]. Migration assays indicated that CXCR4 and CX3CR1 (fractalkine receptor) were differentially expressed on rapidly dividing MSCs, suggesting that these receptors contribute to enhanced engraftment of this cellular subset in vivo. Shi et al. reported increased homing of fetal liver kinase 1-positive human MSCs to the marrow compartment of irradiated nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice following treatment of MSCs with a cytokine cocktail that caused intracellular pools of CXCR4 to be expressed on the cell surface [86]. Cultured human MSCs were also reported to express the chemokine receptors CXCR5 and CXCR6, as well as CCR type 1 (CCR1), CCR type 2, CCR type 7 (CCR7), and CCR type 9 [83,87,88]. Human MSCs that migrated into cultured pancreatic islets were re-isolated and shown to express CXCR4, CXCR6, CCR1, and CCR7, suggesting that this complement of receptors may in part mediate MSC migration to injured tissues [88].

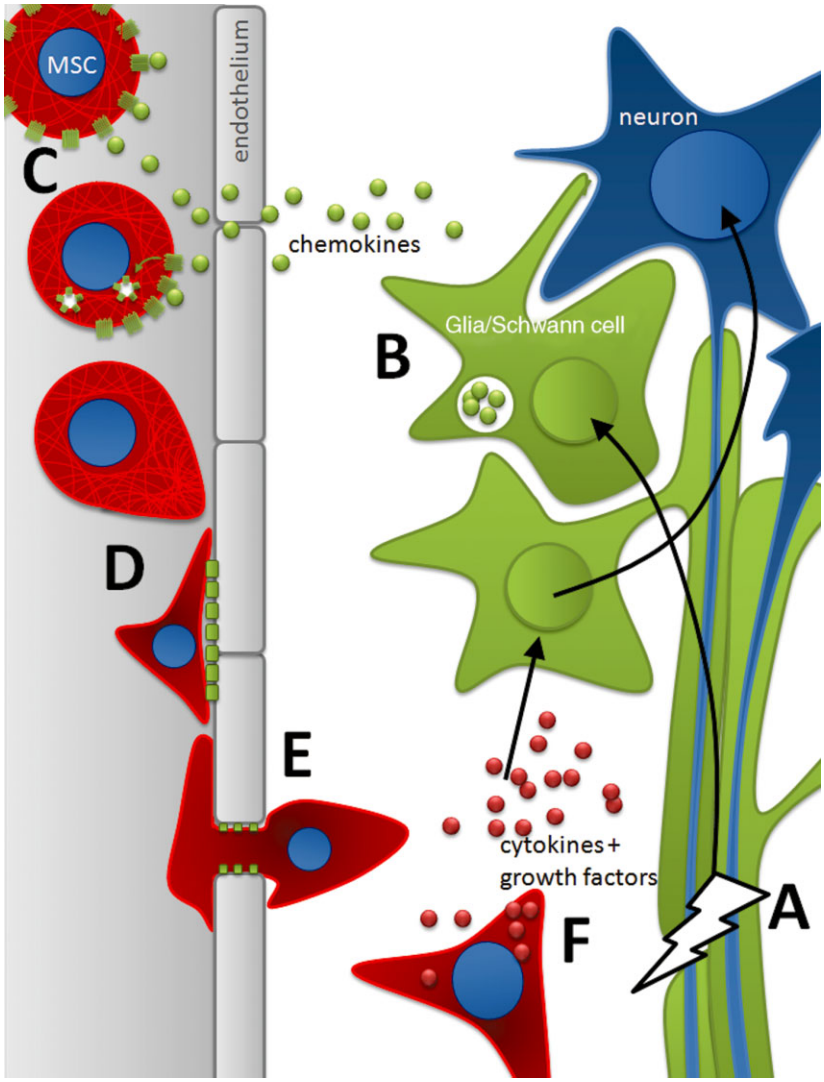


Figure 2 Schematic drawing of processes in the major pelvic ganglion (MPG) of a cavernous nerve injured rat treated with multipotent stromal cells (MSCs). Proposed sequence of events in MSC therapy for cavernous nerve injury: (A) axonal stretch, tear, crush, cut, freeze, or coagulation causes neuropraxia resulting in (B) neuroinflammation accompanied by production of pro-inflammatory proteins that activate neuroglia and Schwann cells in the MPG. This activation results in the production and release of a mixture of chemokines, among which stromal cell-derived factor-1. (C) Chemokines activate their respective receptor on MSC coursing through the capillaries of the MPG. Activation of these receptors results in receptor-ligand complex internalization in endosomes and submembranous polymerization of G-actin to filamentous (F)-actin, as the cell prepares to attach to the endothelium. (D) MSC attaches to the endothelium by binding of integrins to endothelial adhesion molecules. (E) MSC migrates through the endothelium into the interstitium of the MPG, a process coordinated by CD31, junctional adhesion molecules, and endothelial adhesion molecules. (F) The activated MSC releases neurotrophic growth factors and cytokines, among which are vascular endothelial growth factor, basic fibroblast growth factor, and LPS-induced CXC chemokine (LIX). These neurotrophic substances stimulate neuroregeneration through, among others, activation of the Janus kinase/signal transducers and activators of transcription pathway in neuroglia and Schwann cells.

Ponte et al. determined that incubation of MSCs with the inflammatory cytokine tumor necrosis factor alpha resulted in enhanced migration toward chemokines, although they observed the strongest migration of MSCs in response to the growth factors platelet-derived growth factor (PDGF) and IGF-1 [87]. In a landmark article identifying conditions that differentially mobilized subsets of hematopoietic, endothelial, and stromal progenitor cells, Pitchford et al. showed that AMD3100, the CXCR4 antagonist, preferentially mobilized stromal cells from bone marrow when administered after 4 days of pretreatment with VEGF [89]. Therefore, in addition to CC and

CXC chemokines, tyrosine kinase receptors for growth factors also mediate the trafficking of stromal progenitor cells. Chronic hypoxia is reported to also mobilize MSCs into peripheral blood [90] and successful attempts have been made to increase the homing potential of MSC in response to SDF-1 and fractalkine by culturing the cells under hypoxic conditions [91]. Accordingly, characterization of the relative levels of circulating growth factors and chemokines in normoxic and hypoxic animals may provide further insight into mechanisms that control MSC homing.

Lessons Learned from Paracrine Activity of MSC Therapy

Data from injury models in multiple organ systems suggest that paracrine activity of adult stem/progenitor cells is an important component for many of the benefits conferred by cell therapy [92]. Because of low numbers of engrafted cells, many studies demonstrating rescue and/or repair of injured tissue after MSC injection suggest that a paracrine effect may be operative [93,94]. Supportive evidence for paracrine action also comes from experiments in which MSC-conditioned medium or MSC-derived lysate rather than live cells was administered to injured animals to provide benefit. Conditioned medium from MSCs has been shown to reduce infarct size and improve cardiac function after myocardial infarction when injected directly into left ventricular muscle [95], administered intra-arterially [96], or administered intravenously [97]. Adipose stromal cell-conditioned medium infused intravenously was shown to protect neonatal rats against hypoxia-ischemia-induced brain injury [98], and intra-arterial infusion of conditioned medium from a subpopulation of human BM-MSCs protected adult mice against stroke [99]. In a wound-healing model, MSC-conditioned medium increased re-epithelialization, cell infiltration, granulation formation, and angiogenesis [100].

Approaches to inhibit particular secreted factors by siRNA transfection of MSCs or viral transduction of MSCs to express shRNA have identified a few key factors involved in their ability to reduce injury through paracrine activity. Suppression of hepatocyte growth factor (HGF) production by adipose-derived MSCs transduced by lentivirus to express HGF-specific shRNA was shown to reduce the angiogenic effects of the MSCs in a model of limb ischemia [101]. MSC-secreted keratinocyte growth factor (KGF) was shown to improve alveolar epithelial fluid transport after *E. Coli*-induced acute lung injury through siRNA-based inhibition experiments [102]. TNF-stimulated gene 6 protein (TSG-6), which is a secreted anti-inflammatory protein, was shown to mediate, in part, the benefits of human MSC administration in a murine model of myocardial infarction [102].

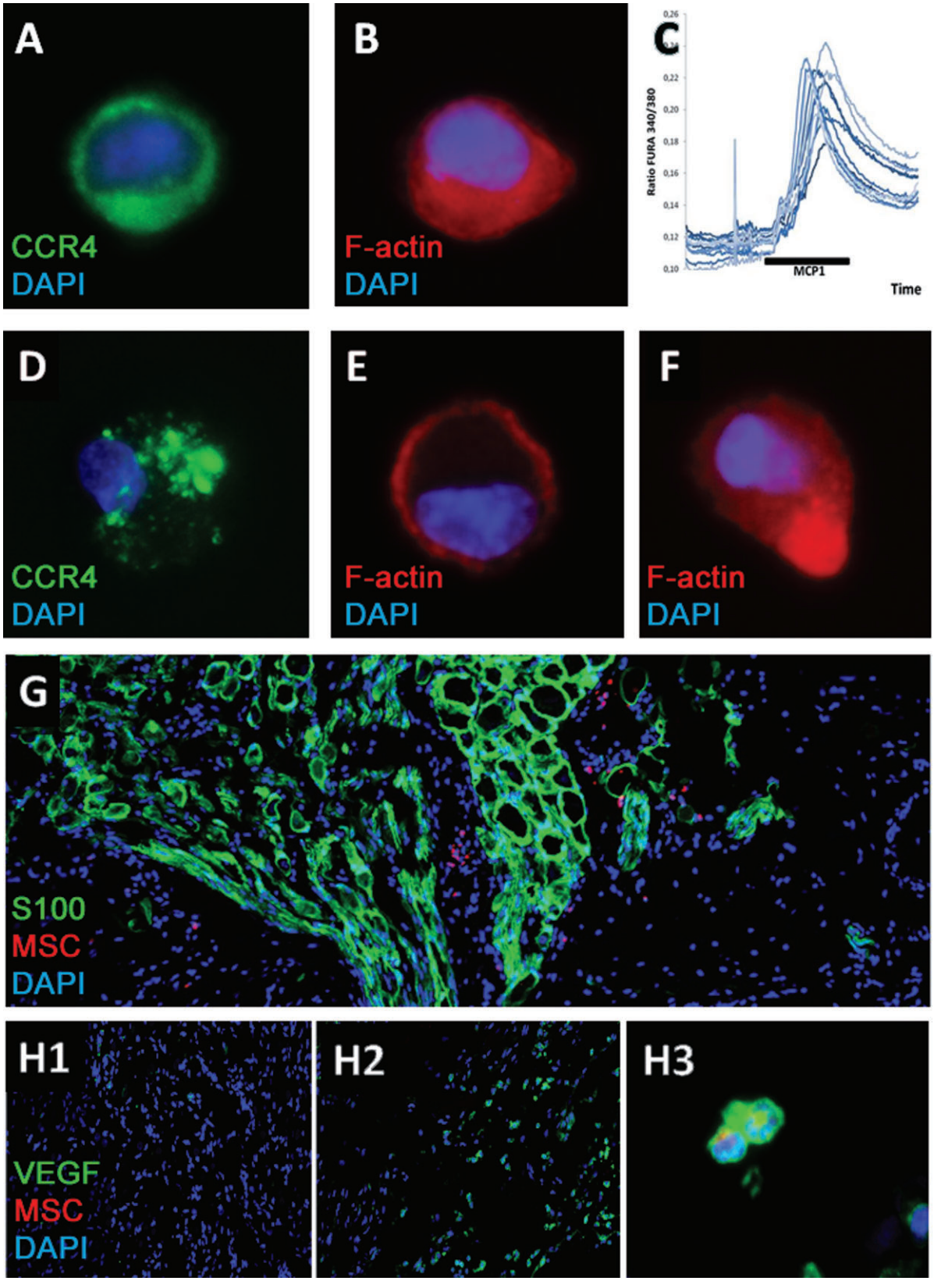
One aspect of adult stem/progenitor cell therapy that has received relatively little attention is that injected cells may actually alter their expression and secretion of many factors in response to changes in in vivo environments, exposure to inflammatory cytokines, etc. For example, human MSCs injected into the peri-infarct area of mice with stroke significantly increased their expression of SDF-1 in comparison with MSCs injected into the analogous cortical area in sham-operated mice [103]. Human MSCs delivered intravenously activated their production of TSG-6 upon embolization in the lung vascular bed [102]. In terms of using bone marrow- or adipose-derived MSCs to treat CNI, further work is needed to identify the key secreted factor or factors that protect erectile tissues. Experiments with MSC-conditioned medium may help to determine whether such molecules are constitutively expressed and secreted or are alternatively induced after transplantation of MSCs in vivo.

Future Directions and Translational Issues

Besides advancements in our knowledge pertaining to the modes of action of injected MSCs, there are a number of translational issues that need attention regarding the potential clinical use of cell-based therapies in ED following RP.

First, what type of stem cells would be the most appropriate candidate for the purpose of preserving and/or restoring erectile function? Although the answers are yet to be answered, both BM-MSCs and ADSCs seem to be convenient based on accumulating animal data. Furthermore, these cell types can be used in an autologous fashion and are not exposed to the ethical repercussions embryonic stem cells pose. Several other factors to determine the type of stem cells used include cost, ethical issues, ease of isolation and culturing, risks, effectiveness, and abundance from the source.

Second, it is currently unknown for what period stem cells survive in the target tissue after implantation or injection. Current data support that there is a time-dependent decline of stem cells after implantation into the target tissues [42]. Recent studies using quantitative analysis demonstrate that injected stem cells survive for a couple of days after implantation and disappeared soon after


A
CCR4
DAPI
B
F-actin
DAPI
C
Ratio FURA 340/380
0,26
0,22
0,20
0,18
0,16
0,14
0,12
0,10
MCP1
Time
D
CCR4
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E
F-actin
DAPI
F
F-actin
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G
S100
MSC
DAPI
H1
VEGF
MSC
DAPI
H2
H3

Figure 3 Overview of changes in multipotent stromal cells (MSCs) after activation by chemokines and interaction of MSC with the major pelvic ganglion (MPG). (A–F) Cells depicted are MSC, passage 2, derived from human subcutaneous adipose tissue of consenting donors. (A) Unstimulated adipose-derived MSC (adipose tissue-derived stem cell [ADSC]) in suspension shows even distribution of C-C chemokine receptor type 4 (CCR4) in cytoplasm and cell membrane. (B) Unstimulated ADSC in suspension shows even distribution of filamentous actin (F-actin) in the cell. (C) Calcium imaging: upon addition of monocyte chemotactic protein-1 (MCP1) to the culture medium of ADSC, an influx of calcium into the cell is observed, indicating presence of functional receptors (CCR4) for MCP1. (D) Stimulation of ADSC with MCP1 for 5 minutes causes receptor-ligand complex internalization in endosomes (correlates to Figure 1C). (E) Stimulation of ADSC with MCP1 for 30 seconds causes actin polymerization in the submembranous compartment (correlates to Figure 1C). (F) Stimulation of ADSC with MCP1 for 60 seconds causes formation of a so-called "leading edge" (correlates to Figure 1C). (G) 1–7 days following injection, 5-ethynyl-2′-deoxyuridine-labeled ADSC (MSC, red) is located in the MPG in cavernous nerve injured rats. S100 (green): neuroglia marker (adapted from reference 43 with permission). H1: MPG of a cavernous nerve injured rat. H2-3: MPG of a cavernous nerve injured rat treated with ADSC: an increase in local vascular endothelial growth factor (VEGF) staining is noted. H3: high magnification of MSC located in the MPG showing colocalization of EdU (red), with which the cells were labeled before injection, and VEGF (green). DAPI = 4′,6-diamidino-2-phenylindole.

[43,61]. This is not only the case in ED models but has also with other disease models, such as myocardial infarction [66]. If disappearance of cells follows implantation, then incorporation and differentiation of stem cells may not be the only modes of action that could restore erectile function. Recently, researchers have postulated that paracrine mechanisms are involved in restoration of erectile physiology [13,14]. A number of growth factors secreted from the implanted stem cells could be responsible for restoration of erectile function following CNI. Furthermore, injected MSCs targeting the injured tissues were recruited toward the MPG following CNI. Why MSCs target certain tissues following injury, and whether MSCs mobilize and home under steady-state conditions remains a topic of debate. Progress in this area has been stifled by the difficulties in identifying and isolating native MSCs; most studies utilize culture-expanded MSCs that do not express many of the cell-adhesion or chemokine receptors that are responsible for the homing of stem cells [80]. Elucidation of the mode of action of MSC is a major hurdle that needs to be delineated before advances and clinical application.

Third, unwanted side effects of stem cell transplantation in this specific setting need to be defined, specifically tumorogenicity and differentiation of injected cellular preparations in unwanted directions. Lin et al. proposed that ADSC may influence growth of prostate tumor cells in a nude mouse model [104]. This issue needs to be further elucidated before we can apply stem cell therapy in men who may have residual tumor cells at section margins or in circulation. Another issue is where these cells end up after disappearing from the MPG to determine what changes take place in these tissues.

Last but not least, stem cell use in humans is lacking. To the best of our knowledge, the only study that has used stem cells to treat ED in a clinical setting is by Bahk et al. from Korea. The researchers implanted umbilical cord stem cell into the penis of seven men with diabetes-related ED and reported that six regained morning erections within 6 months [105]. However, despite increased penile rigidity, none of the men were able to achieve vaginal penetration unless aided by PDE-5 inhibitors before sexual intercourse. In postprostatectomy patients, no clinical trials to date have been initiated.

Conclusion

From the many emerging studies utilizing MSCs for ED, a growing optimism by both basic scientists and clinicians exists. Advances have been made from in vitro and in vivo preclinical studies using MSCs. Although MSCs were originally believed to provide for tissue regeneration through engraftment and long-term survival in injured tissues via their presumed plasticity, recent findings suggest a plethora of additional mechanisms, including paracrine processes. MSC therapy has shown efficacy in animal models of CNI. Further research is warranted to overcome a number of translational hurdles on the path toward clinical application of MSC for the treatment of postprostatectomy ED.

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(b) Revising It for Intellectual Content
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Category 3

(a) Final Approval of the Completed Article
Maarten Albersen; Jeffrey L. Spees; Muammer Kendirci; Frank Van der Aa; Tom F. Lue; Wayne J.G. Hellstrom

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