REVIEW

Stem cell therapies in post-prostatectomy erectile dysfunction: a critical review

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Introduction: *Erectile dysfunction (ED) is still a common complication of radical prostatectomy. Current treatments of ED are mainly symptomatic. Mesenchymal stem cells (MSCs) have been widely investigated as a potential curative treatment. Although MSC therapy consistently improved erectile functions in the pre-clinical studies the initial expectations seem to be unmet. The aim of this study is to critically review the existing studies on use of stem cells in post-prostatectomy ED and understand factors that preclude clinical translation of the available evidence.*

Materials and methods: *A literature search for all pre-clinical and clinical studies investigating MSCs in the treatment of post-prostatectomy ED published between January 2009 and March 2016 was performed using the PubMed database.*

Results: *A total of 24 pre-clinical studies investigated MSC based treatments in cavernous nerve injury (CNI) rodent models. In the majority of these studies intracavernous injection of MSCs at the time injury improved erectile functions. There is less data on the efficacy of MSCs when applied in a chronic disease state. Allogeneic or xenogeneic MSCs were similarly effective with limited data on immunologic response. There is a lack of conclusive data on in vivo fate of MSCs and the best route of MSC administration.*

Conclusion: *MSC therapy consistently improved erectile functions after CNI. There seems to be a consensus on the disease model used and outcome evaluation however further studies focusing on immunologic response to MSCs, their mechanism of action and in vivo fate are needed before their widespread use in clinic.*

Key Words: radical prostatectomy, cavernous nerve injury, mesenchymal stem cells, erectile dysfunction

Introduction

Prostate cancer is the most common cancer in males with an estimated 1.1 million cases diagnosed worldwide in 2012.[1] After the prostate-specific antigen (PSA) screening era the median age at diagnosis decreased and the proportion of men with localized disease increased to nearly 80%.[2] Radical prostatectomy (RP) is the recommended treatment in low intermediate risk localized prostate cancer patients with a life expectancy > 10 years.[3] The aim of radical prostatectomy in men with localized prostate cancer is eradication of the disease while minimizing complications such as incontinence and erectile dysfunction (ED).

ED occurs in men undergoing RP irrespective of the surgical technique used and have major impacts on quality-of-life of men and their partners. The incidence of postoperative ED after RP changes between 25%

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and 90% depending on the population being studied, methods of data acquisition, ED evaluation and definition of the ED.[4] In a recent prospective series up to 75% of men reported ED 1 year after RP with minimal difference between robotic and open surgery groups.[5] Although there are reports to suggest lower rates of ED after robotic RP, this has not been definitely translated into better functional outcomes with longer follow up.[6] The sparing of cavernous nerves uni or bilaterally, age of the patient and experience of the surgeon are reported to be the main factors effecting postoperative erectile function outcomes.[7]

The mainstay of management in men with post-prostatectomy ED is pharmacotherapy with phosphodiesterase type 5 inhibitors (PDE5-Is).[8] In cases where PDE5-Is fail to improve erectile functions intracavernous injections and penile implants are the other available options. Another more historical modality can be vacuum device therapy. All these options are symptomatic rather than curative. The need to find a curative treatment option for ED has long been recognized and increasing amounts of research has been published within the last few years.[9] Therapeutic strategies such as stem cell and gene therapies, low intensity extracorporeal shock wave therapy (LI-SWT) and long term daily use of PDE5-Is are currently under investigation.[10,11]

Penile rehabilitation (PR) early after RP was first conceptualized in 1997.[12] The rational of penile rehabilitation relies on the fact that some degree of neuropraxia is unavoidable even if all principle steps in nerve sparing RP are mastered meticulously. This is due to the close proximity of the cavernous nerves to the prostate and their rather widespread distribution around the gland. The aim of PR is to prevent irreversible structural damage to corpora cavernosa (smooth muscle and endothelial cell apoptosis and formation of fibrosis) until recovery of the neuropraxia caused by poor oxygenization. Corpora cavernosa are oxygenated during erection. Therefore, the mainstay of PR is provocation of early erections after RP by pharmacological or physical interventions. Current means of penile rehabilitation is PED5-Is, vacuum devices and intracavernousal injections.

Mesenchymal stem cell (MSC) therapy emerges as a curative alternative to the current PR programs especially after the promising results obtained in animal studies within the last 10 years. However the apparent clinical benefits, although consistent, were mostly demonstrated as functional outcomes without clear consensus on several fundamental aspects such as the definition of evidence, *in vivo* distribution and the ultimate functions of MSCs which eventually led to a delay in translation of the basic science data to the clinic. This issue is increasingly being acknowledged in the literature as some authors recently noted that 'the beneficial effects given as a reason to move fast from insufficient science to translation or therapy are not clearly defined'.[13] Although this side of events may not be apparent to most clinicians it may be important to have a basic understanding of this newly developing area of medicine.

The aim of this review is to discuss the possible reasons underlying the delayed clinical translation of the pre-clinical MSC therapies in post-prostatectomy ED and to open a discussion on how to address them.

Pathophysiology of ED after RP

Penile erection is mainly mediated by nitric oxide (NO) secreted from non adrenergic non cholinergic nerve terminals of the cavernous nerve (CN) and the endothelial cells of cavernosal tissue. NO causes relaxation of the cavernosal smooth muscles through intracellular processes leading to cGMP mediated reduction in intracellular calcium. After relaxation of cavernosal smooth muscle, lacunar spaces are filled with blood which compresses subtunical venules resulting in erection (tumescence). Detumescence occurs when cGMP is degraded by PDE5.[14]

Cavernous injury is widely accepted to be responsible for post RP ED. Although the incidence of post RP ED decreased dramatically after implementation of nerve sparing RP technique was introduced in 1982,[15] potency rates after bilateral nerve sparing RP ranges between 31%-86%.[6] A recently developed prediction model gives a 35% chance to attain a functional erection suitable for intercourse 2 years after RP.[16] This is believed to be due to a temporary disruption of nerve transmission despite an anatomically intact nerve fiber, neuropraxia, which results in lack of or decreased number of erections that lead to poor oxygenation of the penile tissues.

The penile oxygen tension increases from pO2 levels of 35-40 mmHg at flaccid state to 75-100 mmHg at erect states.[17] The maintenance of healthy penile tissues in men requires some degree of regular erections such as those occurring in nocturnal penile tumescence.[18] A permanent deficiency of oxygen in the cavernousal tissue results in fibrogenic microenvironment with up regulation of cytokines such as TGF-beta and HIF-1alpha.[19,20] Also impaired penile oxygenation was shown to reduce the NO mediated relaxation of the cavernosal smooth muscle.[21] Thus a hypoxic state causes a variety of structural and functional changes in the corpora cavernosa.

Another potential factor to play a role in pathophysiology of post prostatectomy ED can be an intrinsic failure in the self-repair mechanisms of patients own tissues. Perivascular cells in many adult tissues contain MSCs which play a significant role in maintenance of normal tissue function and repair in case of an injury.[22] It has recently been suggested that penile endogenous stem/progenitor cells can be involved in the pathogenesis of ED and can be a therapeutic target.[23,24]

Mechanism of action of MSCs and rationale for using them in treatment of ED

MSCs, initially isolated from bone marrow, have later been isolated from many adult tissues such as adipose tissue, skeletal muscle, brain and skin. As their name suggests MSCs are defined by their ability of self-renewal and differentiation into various phenotypes (multipotency). There is a standard, widely applied definition of MSCs together with a lot of discussion about its limitations.[25] At the core of this discussion lies the fact that the multi differentiation capacity of MSCs has only been confirmed *in vivo* for bone marrow derived MSCs[13] and also the initial expectation that MSCs would replace a damaged tissue *in vivo* has not yet been met. In contrast the therapeutic effect of MSCs has consistently been demonstrated and these benefits are mostly attributed to their ability to produce an array of bioactive molecules. This is known as paracrine action of MSCs and it involves stimulation of angiogenesis and revascularization, modulation of immune and inflammatory responses, inhibition of apoptosis and trophic effects such as stimulation of mitosis, proliferation and differentiation of intrinsic stem/ progenitor cells.[26]

Homing of MSCs

MSCs are known to have a tendency to migrate to sites of tissue damage caused by ischemia, inflammation, trauma or tumor invasion when delivered systemically. This trafficking is called MSC homing and it involves migration within the blood stream (chemotaxis), cell attachment and rolling in vessel lumen and finally transmigration of MSCs across the endothelium and invasion into the tissue stroma. The leukocyte adhesion cascade which is studied extensively, can serve as a useful template to understand MSC migration and homing although the latter is not yet fully understood.[27]

Chemotaxis is migration of MSCs in response to chemical signals accumulated in the sites of tissue injury. This process most probably involves chemokines and their receptors. The chemokine receptors are classified as G-protein coupled receptors for CXC, CC, C and CX3C chemokines.[28] MSCs are known to express CCR1-10, CXCR1-2, CXCR4-6 and CX3CR1 receptors however there is significant variability among the studies depending on tissue of isolation, passage number and different isolation/ cultivation protocols.[29] The most commonly studied chemokine-receptor interaction both *in vivo* and in vitro is CXCL12 (or stromal cell derived factor [SDF]-1)-CXCR4.[30,31] The expression of CXCR4 by MSCs were shown to increase upon stimulation by cytokines such as IGF-1,[32] TNF-α[33] and cytokine cocktails[34] in vitro. These modulations have also been shown to improve the therapeutic efficacy of MSCs.[35]

The attachment and transmigration of MSCs through the vascular endothelium occurs in several coordinated steps: attachment of MSCs to the endothelium and rolling mediated by selectins and their ligands, firm adhesion after activation of integrins by chemokines, diapedesis across the endothelial tight junctions and basement membrane and finally migration through extracellular matrix. The adhesion molecule P selectin and the VCAM-1 (vascular cell adhesion molecule)-VLA-4 (very late antigen) has been shown to play a role in firm adhesion of MSCs to the activated endothelial cells.[36] After adhesion transendothelial migration occurs in a process mediated by junctional adhesion molecules (JAMs), cadherins, and platelet-endothelial cell adhesion molecule-1 (PECAM-1/CD31). Here cells adhere to ECM components via integrins, CD44, and other cell adhesion molecules. Afterwards ECM degrading enzymes, matrix metalloproteases 1 and 2 (MMPs), facilitate MSC invasion into the target tissues.

Paracrine effects of MSCs

Immunomodulation

Immunoregulatory activities of MSCs influence both innate and adaptive immune responses to develop either a pro-inflammatory or an anti-inflammatory phenotype depending on the microenvironment they are located. In the presence of an inflammatory environment where TNF-alpha and IFN-gamma levels are high MSCs adopt an immune-suppressive phenotype whereas low levels of these cytokines induce MSCs to adopt a pro-inflammatory phenotype.[37] Toll like receptors (TLR) on the surface of MSCs are also thought to contribute to this process as MSCs stimulated through TLR-3 and TLR-4 exert anti-inflammatory (MSC 2) and pro-inflammatory (MSC 1) phenotypes, respectively.[38] This process is called the MSC polarization, in analogy with macrophage polarization, and interactions with other cells of the innate immune system such as monocytes are also

reported to contribute to this process. MSCs also interact with the cells of adaptive immune response. Conclusively, MSCs plays a regulatory role in several phases of immune response through diverse mechanisms of actions and on various cell types.

Angiogenesis

Angiogenesis is the sprouting of capillaries from pre-existing blood vessels *in vivo*. This process involves a complex interaction between endothelial and non-endothelial cells as well as many enzymes, chemokines, growth factors, matrix metalloproteinases and adhesion molecules. A defective angiogenesis is implicated in many disease states such as ischemic heart disease, peripheral vascular disease and all defective wound healing processes. MSCs have demonstrated to secrete a wide variety of pro-angiogenic factors such as vascular endothelial growth factor, fibroblast growth factor 2, interleukin-6 that are shown to act in each step of the angiogenesis (endothelial cell proliferation, migration and tube formation).[39] Furthermore the secretion of pro-angiogenic factors by MSCs has been shown to be increased significantly by exposing the cultured MSCs to hypoxia (hypoxic pre conditioning)[40] as well as resulting in better functional results *in vivo*.[41]

Anti-apoptosis

MSCs prevent cell death through modifying the microenvironment in a pro-proliferative way, by producing some of the well-known anti-apoptotic proteins and by direct cellular interactions. Co- culture studies showed that MSCs improved survival of ischemic cardiac cells via direct cell-cell connections and intercellular nanotube formation.[42] Also in a pig model of cardiac ischemia-reperfusion injury MSC-conditioned medium decreased Caspase-3 activity and improved functional outcomes.[43] MSC conditioned media is demonstrated to contain pro-survival factors such as B-cel lymphoma 2 (Bcl- 2), Akt, VEGF, bFGF and Stromal derived growth factor-1.[44-46]

Tissue growth and regeneration

Another important property of MSCs is to secrete growth factors and other chemokines to induce cell proliferation and tissue regeneration in many organ systems including peripheral nerves. MSCs are shown to secrete NGF (nerve growth factor), BDNF (brain-derived neurotrophic factor) and GDNF (glial cell line-derived neurotrophic factor).[47,48] In a rat cavernous nerve injury model, MSCs were shown to improve functional outcomes equally as good as NGF releasing hydrogel[49] and BDNF immobilized synthetic membrane.[50]

Current pre-clinical evidence of MSC based therapies in post-prostatectomy ED

In the last 15 years a total of 24 pre-clinical studies investigated MSC based treatments in cavernous nerve injury animal models. Among these, 17 were cavernous nerve crush injuries where MSCs were used as a cellular therapy, Table 1, whereas 9 studies used an MSC based tissue engineering approach in crush injury or nerve resection models where MSCs are delivered to the site of tissue injury via cell carriers, Table 2. This section will provide an evaluation of these studies from a clinical translational point of view.

Animal model

A rat model of CN injury seems to be the standard animal model to study post-RP ED,[51] only two studies used a mouse model of CN injury.[52,53] The CN crush injury is mostly performed by a hemostatic clamp without disrupting the continuity of the nerve (neuropraxia model) whereas in a nerve resection a short or long segment resection of the CN nerve was performed.

In most of CN injury models, the MSCs are injected into the corpus cavernosum whereas the actual tissue injury is to the nerves exiting the MPG. A cell tracking study in this model demonstrated that ADSCs injected to the corpus cavernosum several days after nerve crush injury migrated preferentially to the bone marrow.[54] Subsequent work from the same group than revealed that intracavernously (IC) injected ADSCs exerted their effects by migrating to MPG days after CN injury.[55] The animal model in these studies represents an acute injury state where the physiological homing signals are intense to allow engraftment of MSCs to the site of injury, the MPG in this case. Taken together with the consistent improvement in erectile functions it is likely that the MSCs injected into the cavernosum will migrate to the cavernous nerves and aid in tissue repair.

Treatment with MSCs in acute versus chronic injury states can have an effect on their homing potential. There is only one pre-clinical study which compared immediate and delayed treatments with SVF which showed similar efficacy.[56] However it is not clear how the MSCs will exert their effects in a chronic injury state, where the homing signals from the host may be minimal or absent. It can be speculated that absence of regular penile erections will create a hypoxic microenvironment with increased cytokine secretion that can attract MSCs to the corpus cavernosum to improve tissue regeneration. In the first pilot phase I clinical studies that evaluated the safety of bone marrow derived mononuclear cells in patients with

TABLE 1. **Pre-clinical studies evaluating the effect of mesenchymal stem cell (MSC) based cellular therapies on improvement of erectile functions after cavernous nerve (CN) injury**

	Nerve injury	Follow up time (days)	Source of MSCs	MSC labelling	Route of MSC delivery	Comment
Bae[86]	crush	28	Xenogeneic (human) ADSC	None	ICI	ADSC were compared with a hydrogel and were similarly effective in improving erectile functions
Choi[75]	crush	28	Xenogeneic (human) Testicular SCs	CM-Dil	Periprostatic instillation	CD34/CD73 double positive (highly potent) cells compared with BMSCs
Kendirci[48]	crush	28	Rat BMSC	GFP	ICI	A special subpopulation of MSCs (p75dMSC) were used
Qiu[56]	crush	84	Autologous SVF	None	ICI	Immediate and delayed treatments were equally effective
Song[52]	crush	14	Allogeneic SVF	GFP	ICI	Suggests a mechanism for SVF action: induction of angiogenesis
Ying[88]	crush	90	Allogeneic ADSCs	None	ICI	Demonstrated improved nerve regeneration after CN injury
Zhu[59]	crush	28	Xenogeneic (human) umbilical cord MSCs	BrdU	ICI	Beneficial effect of MSCs on cavernous nerve regeneration were demonstrated on electron microscopic analysis
Albersen[79]	crush	28	Allogeneic ADSCs and ADSC lysate	EdU	ICI	Both ADSC and ADSC lysate improved erectile functions. Paracrine action of ADSCs demonstrated
Fandel[80]	crush	28	Autologous ADSCs	EdU	ICI & PI	PI of ADSCs did not improve erectile functions
Kim[84]	crush	28	Allogeneic BMSCs	None	Injection into the MPG	MSCs were infected with brain drived neurotrophic factor expressing adenoviruses
Kovanecz[85]	Nerve resection	45	Xenogeneic (mouse) muscle derived MSCs	None	ICI	Daily Tacrolimus was given to rats to prevent immune-rejection of xenogeneic MSCs
Ryu[53]	crush	14	Allogeneic BMSCs	PKH26	ICI & IPI	ICI and IPI equally effective for tissue repair. IC was better for erectile function recovery
Xu[89]	crush	28	Allogeneic ADSCs	EdU	IC injection	Scaffold free micro tissues were injected instead of cell suspensions
Mangir[64]	crush	28	Allogeneic & autologous ADSCs	None	IC injection	First direct comparison autologous and allogeneic cell sources in this model
You[90]	crush	28	Autologous SVF and ADSCs	PKH26	IC injection	SVF and ADSCs equally effective
Jeon[76]	crush	28	Xenogeneic (human) ADSCs	None	PI	ADSCs were used with low energy shock waves
Fall[66]	Nerve resection	12 & 35	Allogeneic (littermates) BM mononuclear cells	PKH26	IC injection	The mechanism of action of MSCs were suggested as inhibition of apoptosis

ADSC = adipose derived stem cells; BMSC = bone marrow stem cells; SVF = stromal vascular fraction; ICI = intracavernosal injection; IPI = intraperitoneal injection; MPG = major pelvic ganglion; PI = perineural injection

TABLE 2. **Pre-clinical studies using scaffold materials together with mesenchymal stem cells (MSC) to regenerate tissues after cavernous nerve (CN) injury**

	Nerve injury	Follow up time (days)	Source of MSCs	MSC labelling	Route of MSC delivery	Comment
Lin[55]	Nerve resection	90	Autologous ADSCs	Human adipose extracellular matrix	MSC seeded tissue matrix was applied as a nerve conduit	MSC viability and proliferation on the scaffold after transplantation was studied in detail
Ying[91]	Nerve resection	90	Allogeneic ADSCs	Autologus vein graft	Injection of ADSCs into grafted saphenous vein	MSCs were simply injected inside the grafted vein
You[92]	crush	28	Xenogeneic (human) BMSCs	Fibrin scaffold	Periprostatic implantation + ICI	ICI + periprostatic implantation is better than ICI alone
You[93]	crush	28	Xenogeneic (human) ADSCs	Fibrin scaffold	Periprostatic implantation + ICI	Direct comparison of ICI, PPI and the combination, all were equally effective
Lee[94]	crush	28	Xenogeneic (human) ADSCs	BDNF immobilized PLGA membrane	Perineural application	The PLGA membrane was applied on the cavernous nerve immediately after MSCs were applied to the area
Jeong[95]	crush	28	Xenogeneic (human) ADSCs	BDNF immobilized PLGA membrane	Perineural application (as above)	Combination of oral Udenafil was investigated MSCs were produced according to GMP
Piao[50]	crush	28	Xenogeneic (human) ADSCs	BDNF immobilized PLGA membrane	Perineural application (as above)	MSCs were produced according to GMP
Kim[77]	crush	28	Allogeneic BMSCs	Matrixen (collagen based cell carrier)	Application on the MPG	MSCs are more effective when introduced with a cell carrier
Miyamato[87]	Nerve resection	84	Xenogeneic (human) BMSCs	Alginate gel sponge	Perineural implantation of MSC containing gel sponge	A special subtype (CD133+) of bone marrow mononuclear cells were used

ADSC = adipose derived stem cells; BMSC = bone marrow stem cells; ICI = intracavernosal injection; MPG = major pelvic ganglion; PLGA = poly-lactic-co-glycolic acid)

long term post prostatectomy ED (chronic disease), an IC injection method was used.[57,58]

In conclusion current pre-clinical evidence suggests that IC application of MSCs at the time of CN injury results in consistent improvement in erectile functions. There is less data on the efficacy of MSCs when applied in a chronic disease state. As this information will have a significant impact on treatment planning a better

understanding of the *in vivo* fate of MSCs in relation to their homing efficacy in acute and chronic injuries is needed.

Source of stem cells

The sources of stem cells used in these studies are highly variable. Most studies used adult stem cells, mostly bone marrow or adipose tissue derived, whereas only one study used umbilical cord stem cells.[59] The use of adult MSCs in animal models seems to be a more suitable approach to mimic the desired clinical application, compared to embryonic stem cells. However adult stem cells cannot be expected to be as pluripotent as embryonic stem cells unless they are reprogrammed to become induced pluripotent stem cells[60,61] which is a rather newly developing area. Also culture expanded autologous, allogeneic or xenogeneic MSCs were variably implicated, except two studies where a stromal vascular fraction (SVF) was used.[52,56] SVF has obvious advantages of being immediately available and omitting the contact of cells with serum in culture media. In one of the existing two clinical trials a SVF was used[62] however SVF is a heterogeneous population of cells.

MSCs express low levels of major histocompatibility complex (MHC) class 1 and do not express MHC class 2 molecules thus they are minimally (or none) immunogenic.[63] In a CN injury model, although only one pre-clinical study directly compared autologous and allogeneic ADSCs,[64] there appears to be random use of MSCs from different sources. Some researchers used xenogeneic donors while others preferred to use autologous cells[56,65] or cells from the littermates.[66] It might also be argued that genetic variability in laboratory rodents is not huge and transplantation between littermates could be considered 'autologous' however autologous, from a clinicians point of view, refers to a situation where each animal receives its' own cells. Although no clear difference between autologous, allogeneic or even xenogeneic cell sources in terms of efficacy has been demonstrated,[64] a recent ISCT working proposal recommended that data from xenorecipient animal models should be used with caution in clinical trial planning.[67] Thus a xenogeneic transplantation model in a pre-clinical study is unlikely to be immediately translated.

In conclusion, autologous transplantation of MSCs has a clear advantage in terms of safety since there is always a risk of re-expression of MHC after interaction with the microenvironment[63] and also there is always some risk of contamination by unknown pathogens.[68] On the other hand, allogeneic MSCs has the advantage of being acutely available in standardized, ready to use form. Also there is data to suggest decreased potency of MSCs with increasing age and certain comorbidities,[69,70] in which case allogeneic transplantation can be the best choice. So far both allogeneic and autologous MSCs have been used in pre-clinical and clinical studies both of which revealed promising efficacy and safety data.[71] More in depth discussions on many aspects of stem cell immunity is needed as the extend of immunological reactions might have an impact on efficacy of the treatment in long term.[72]

Cell application methods

Stem cells were applied mostly by means of injecting them into the corpus cavernosum as a means of cellular therapy. Also MSCs has directly been applied on to/around the damaged nerve via a cell carrier material such as a gel or a scaffold which is sometimes used in combination with several bioactive factors, Table 2.

The bioavailability and efficacy of any therapeutic agent is affected by its method of delivery. IC injection of MSCs seems to be implemented in a standard fashion in the vast majority of the pre-clinical and all of the clinical trials,[62,73] despite absence of comparative data to show the best way of MSC administration in terms of entrapment and functional outcomes. However it is likely to change the clinical outcomes because IV, intracoronary and endocardial injections when compared directly in a swine model of acute MI demonstrated to result in better engraftment of MSCs in intracoronary and endocardial injections compared to IV delivery.[74]

Existing pre-clinical studies have addressed this issue by comparing IC injection to intraperitoneal (IP) injection[53] and peri-neural injection/implantation[75] of MSCs which revealed conflicting results and no study up to now used an IV injection method. IP injection was equally effective to restore damaged tissue but was not as good in improving functional outcomes. In this study peri-neural injection when compared directly with IC injection did not improve functional outcomes however this observation was not universal and in studies by other groups peri-neural injection improved erectile functions and nerve regeneration.[76,77]

Cell labeling/quantification of engraftment

Another factor that delays clinical translation of MSCs can be related to their *in vivo* fate. It has been shown that after intravenous injection most of the MSCs are trapped in the lungs initially, however they eventually home to sites of tissue injury and finally 0.1% to 2.7% of MSCs can be found engrafted to the sites of injury after 2 weeks.[78] Most of the studies reviewed in this article used pre-labeled MSCs and mostly a fluorescent

nuclear dye was used[59,79,80] whereas others simply did not track the *in vivo* fate of the MSCs.

Current methods used to track the MSCs are mainly histologic detection of pre-labeled cells or certain proteins in target organs and non-invasive imaging technologies. Because detection of MSCs in harvested tissue samples had disadvantages of requiring numerous animals to be sacrificed at several time points and also the need of sampling several organs with histologic sectioning being performed only on some parts of excised organs dynamic imaging techniques started to develop. These non invasive dynamic imaging studies include MR imaging of superparamagnetic iron oxide (SPIO) labeled MSCs,[81] single photon emission computed tomography (SPECT) imaging of radioisotope (^{111}In oxine, ^{99m}Tc) MSCs[82] and quantum dot labeling.[83] Each of these non invasive imaging modalities have their own advantages and limitations however they are very likely to become an integral part of stem cell based therapies and further improvements in this area needed.

Outcome evaluation

The duration of follow up after treatment with stem cells was mostly 4 weeks, ranging between 2 weeks to 3 months. The functional outcomes were consistently reported as ICP/MAP measurement in all of the studies whereas histologic evaluation and quantification of tissue collagen deposition, endothelial and smooth muscle cell content in corpora cavernosa as well as quantification of expressed proteins in the tissue was included in others.

In all of the existing pre-clinical studies, the MSC treatment resulted in restoration of intracavernosal pressure up to 60%-80% in neuropraxia models[80,84] whereas a two fold increase compared to control was demonstrated in CN resection studies.[66,85] The structural changes in the corpus cavernosum in response to nerve injury were an increase in endothelial and smooth muscle cell markers and their cellular products,[52,84,86] an increase in neural cell markers,[56,59] a decrease in collagen content and a decrease in apoptosis.[79] An increase in angiogenesis in the corpus cavernosum after MSC injection was studied in detail in only one study.[87] Thus in cavernous nerve injury animal models MSC treatment consistently resulted in improved functional and structural outcomes.

Current clinical trials of MSC based therapies in post-prostatectomy ED

The purpose of pre-clinical studies is to provide rigorous safety and efficacy information together with a proof of principle for therapeutic effects. Above mentioned pre-clinical studies have consistently demonstrated a short term safety by universal characterization of stem cell population used, some information on bio-distribution and tumorigenicity. The efficacy was also demonstrated consistently in a relevant small animal model suggesting a possible mechanisms of action involving improved peripheral nerve regeneration via secreted nerve growth factors.[47,48] Also optimal conditions for stem cell applications such as route of administration and dosage were delineated. This data led to two clinical trials[57,58] evaluating the role of stem cells in the treatment of post-prostatectomy ED primary endpoints being patient safety and efficacy at 6 months follow up. Both studies recruited patients with chronic ED unresponsive to available treatments, both administered stem cells via intra cavernousal injection, both used patients own stem cells obtained by adipose tissue after liposuction or bone marrow after bone marrow aspiration avoiding any period of cell culture.

Conclusion

Mesenchymal stem cell based therapies have proved to be promising in the treatment of post- prostatectomy ED. Accumulated evidence from pre-clinical studies demonstrates efficacy of MSCs to improve erectile functions in addition to safety and tolerability data from two clinical studies. Further studies focusing on the *in vivo* fate and mechanism of action of MSCs are needed. Implementation of MSC tracking with non invasive imaging modalities can be critical to elucidate the best method of cell application. □

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