

Is there a role for stem cell therapy in erectile dysfunction secondary to cavernous nerve injury? Network meta-analysis from animal studies and human trials

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Abstract

Introduction: We carried out systematic review and network meta-analysis to investigate the role of stem cell therapy (SCT) in the management of erectile dysfunction (ED) secondary to cavernous nerve injury in rats and post-radical prostatectomy (RP) in humans.

Patients and Methods: The protocol was registered with PROSPERO database. We searched studies analyzing the efficacy of SCT for ED due to bilateral cavernous nerve injury (BCNI) in rats using Healthcare Databases Advanced Search (HDAS) Export software (MEDLINE, EMBASE, Scopus) from inception to September 2020. The outcome measurements, for 29 animal studies, were intracavernosal pressure (ICP), ICP/MAP (mean arterial pressure) ratio, and histological/molecular changes. All three available human trials evaluating SCT in post-RP ED were assessed for International Index for Erectile Function (IIEF) Score and Erection Hardness Score (EHS).

Results: For ICP measurement, animal studies were divided into adipose-derived stem cells (ADSCs) subgroup and bone marrow-derived stem cells (BMSCs) subgroup. Pooled analysis of these studies showed a beneficial effect of SCT in improving erectile function in rats with BCNI using network meta-analysis (95% confidence interval, CI; $p < 0.001$). There was an increase in ICP/MAP ratio in stem cell groups (including co-intervention) compared with control BCNI group. Histological and molecular evaluation of penile tissue revealed an increase in neuronal nitric oxide synthase (nNOS), smooth muscle content, and anti-apoptotic activity. Human trials revealed improved IIEF (70–150% from baseline at 6 months) and EHS (80–200% from baseline).

Conclusion: Our results confirm that SCT does improve the erectile function in rats having cavernous nerve injury. Similarly, early human results have shown promising results.

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Keywords: Radical Prostatectomy, Erectile dysfunction, Stem cells

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Introduction

Prostate cancer (PCa) is the second most frequent cancer diagnosis made in men and the fifth leading cause of death worldwide.¹ Radical prostatectomy (RP) remains the most commonly employed procedure for localized PCa in patients with a life expectancy of at least 10 years, which represents 25% of patients with PCa.^{1,2} Erectile

dysfunction (ED) is a common complication after RP.³ It is estimated that 86% patients experience ED after RP.^{4,5} Several factors determine the development of ED after RP. The important ones include patient age, preoperative potency status (baseline Erectile function), comorbidities, performance of nerve-sparing RP (unilateral *versus* bilateral), type of surgery (intra- *versus* inter- *versus*

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extra-fascial), surgical technique (open *versus* laparoscopic *versus* robot-assisted RP), and level of surgical experience.^{4,6} It was thought that robotic techniques will reduce ED in RP, but a recent meta-analysis has not been able to establish the same.⁷

Post-RP ED substantially decreases quality of life (QoL) of the afflicted men and their sexual partners.⁸ The main stay of treatment is phosphodiesterase type-5 inhibitors (PDE5Is) with other strategies being intraurethral alprostadil, intracorporal injection therapy, vacuum erection devices, and surgical procedures like penile revascularization and penile prosthesis implantation.⁹ It is important to mention that majority of these strategies treat ED symptomatically and do not address the underlying cause of ED. Furthermore, limitations to their use exist, such as intolerance to side effects, cost limitations, and unsatisfactory outcomes.¹⁰ Newer modalities [such as stem cell therapy (SCT) and low intensity extracorporeal shock wave therapy (LI-SWT)] are being investigated to develop a curative treatment for post-RP ED with the aim to restore cavernous nerves and rehabilitate penile erectile tissue.^{10,11} Stem cells are hypothesized to address both of these goals and as such being looked into their potential role in curative management of post-RP ED.

SCTs have been used in various clinical conditions due to immunoregulatory, immunosuppressive, and regenerative properties. In recent years, it has been established that within penile tissue, stem cells can differentiate into endothelial, neuronal, or smooth muscle cells and are capable of restoring possible structural damage in the penile tissue. Based on these very properties, several animal and human trials have been performed to evaluate the role of mesenchymal stem cells (MSCs) in the treatment of post-RP ED. In ED research, three types of stem cells are commonly used, including adipose-derived stem cells (ADSCs), bone marrow-derived stem cells (BMSCs), and muscle-derived stem cells (MDSCs).¹²

In this meta-analysis, an attempt has been made to summarize and analyze animal studies having SCT for ED due to cavernous nerve injury with outcome measurements being intracavernosal pressure (ICP), ICP/MAP (mean arterial pressure) ratio, and histological as well as molecular changes in penile tissue. All three available human trials evaluating SCT in post-RP ED were assessed for International Index for Erectile

Function (IIEF) and Erection Hardness Score (EHS).

Methods

The study protocol was registered with PROSPERO (CRD42020201343).

Evidence acquisition

Criteria for considering studies for this review. The inclusion criteria for animal studies included the following:

1. Animals/population: Male rats, ED secondary to cavernous nerve injury
2. Intervention/exposure: SCT
3. Comparator/control: Sham *versus* bilateral cavernous nerve injury (BCNI)/vehicle/stem cell/co-intervention/combined

The inclusion criteria for human studies included the following:

1. Animals/population: Male patients, ED secondary to RP
2. Intervention/exposure: SCT

The exclusion criteria for animal studies included the following:

1. Animals/population: Use of stem cells in male rats in which ED was due to other causes like metabolic/neurological.
2. Intervention/exposure: If the interventions are ill defined or dose/frequency is not mentioned or/ structural methods are inadequate.
3. Comparator/control: Studies which lacked proper grouping into control, Sham, and intervention.
4. Study design: Studies which did not fulfill above criterion and lacked any defined outcomes.

The exclusion criteria for human trials included studies using stem cells in male patients having ED due to non-prostatectomy causes.

Outcome measures

Animal studies. Primary outcomes:

1. ICP measurements
2. ICP/MAP ratio

Secondary outcomes: (histological/molecular)

1. Increase in nitric oxide synthase (NOS) fibers
2. Increase in smooth muscle content
3. Miscellaneous immune response-related changes

Human studies

1. IIEF score
2. EHS
3. Change in penile length
4. Adverse effects

Search method for the identification of studies

Electronic searches. The systematic review was done in accordance with Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA). The search was limited to the English language. Healthcare Databases Advanced Search (HDAS) Export software was used for searching MEDLINE, EMBASE, and also searched other databases (inception to September 2020). Search terms included ‘Prostatectomy’, ‘Erectile dysfunction’, ‘Stem cells’, ‘Rats’, ‘Animal experiments’, and ‘Human trials’. Boolean operators (‘And’/‘Or’) were used.

Data extraction. Two reviewers (M.M.W., S.M.) independently screened all abstracts and full-text articles for eligibility according to the criteria for considering studies for this review. Any disagreements were resolved by mutual consensus. Two authors (M.M.W., S.M.) extracted data from individual studies.

For animal studies, the data were grouped into data sheets having columns for type/source of stem cells, any co-intervention, total rats, randomization of rats, mode of cavernosal nerve injury (crush/electric/cryo), route (intracavernosal, intravenous, local) and dose of stem cells (most common dose used is 1.0×10^6 cells), and assessment interval (variable, most studies have performed at 4 weeks post-intervention) (Table 1).

For three human trials using SCT for post-RP, the data were categorized into total patients, age in years, duration since procedure, cell type used (adipose *versus* bone marrow), dose (most common dose being 1×10^9 cells), mode of delivery (intracavernosal, intravenous, local), side effects, mode of assessment (using standard tools like IIEF), and follow-up (Table 2).

Quality assessment. SYRCLE’s RoB tool (Systematic Review Centre for Laboratory Animal Experimentation) which is an adapted version of the Cochrane RoB tool was used for animal studies (Figure 1).⁴⁵ All three human trials were evaluated in this study; no quality assessment was performed for them.

Statistical analysis. Traditional meta-analysis was performed where possible. As all studies were unlikely to evaluate all treatments, a second set of analyses used a network meta-analysis approach to make indirect, as well as direct, comparisons between studies. A frequentist approach was utilized.⁴⁶ Specifically, the general approach used a model for treatment contrasts (the ‘contrast-based’), which considers treatment effects as fixed effects, and heterogeneity between studies as random effects.⁴⁷ All analyses were performed using the DerSimonian-Laird random-effects method, regardless of the amount of heterogeneity between studies. Statistical heterogeneity was assessed using the I^2 statistics. Substantial statistical heterogeneity was assumed if the I^2 value was above 50%.

An inverse variance method was used for continuous data and expressed as the mean difference with 95% confidence interval (CI), and for dichotomous data, a Mantel-Haenszel method was used. A p value of <0.05 was considered significant. The preferred method of variation was the standard deviation. Where this was not available, the value was imputed. The standard deviation was assumed to be a quarter of the data range.

The studies were divided into one of three subgroups depending on the source of adult stem cells: ADSC, BMSC, and Mixed. Meta-analyses were performed for the ADSC and BMSC subtypes, but due to a small number of studies (only two), no analysis of the mixed subgroup of studies was performed.

Results

Description of studies

Literature search

Animal studies. In total, 189 articles matched initial search. After removing duplicates, 71 were screened, of which further 37 were excluded (conference abstracts, language other than English, abstract-only studies). Furthermore, SQR3 (Survey, Question, Read, Recite, and Review)

Table 1. Animal study: data extraction results.

Reference	Stem cell	Type/source	Co-intervention	Total rats	Experiment randomization	Type of injury	Route (average time of treatment 2-4h after injury)	Dose	Assessment interval	ED function assessment	Histological/Molecular assessment
1. Zheng et al. ¹³ China	ADSC	Allogenic SD rat Groin	ICA II	42 SD	Divided into six groups a. Sham group=7 b. BCNI only=7 c. BCNI + PBS=7 d. BCNI + ADSC=7 e. BCNI + ICI II=7 f. BCNI + ICI II + ADSC=7	BCNI (transection injury)	ICI, ICA II intragastric	ADSCs (5 × 10 ⁶) ICA (4.5 mg/kg/day)	1 week	ICP value and ICP/ MAP ratio	Western blotting CCK 8 assay RNA immunoprecipitation NOS content
2. Yang et al. ¹⁴ China	ADSC	Allogenic SD rat inguinal fat pad	rAd expressing hBDNF	40 SD	Divided into four groups a. Sham group=10 b. BCNI only=10 c. ADSCs infected with lenti-GFP=10 d. ADSCs infected with lenti-GFP=10	BCNI (crush)	ICI	ADSCs infected with lenti-GFP (1 × 10 ⁶ in 20 μl) and lenti-rBDNF (1 × 10 ⁶ in 20 μl)	4 weeks	Maximal ICP/ MAP ratio	Western blot Masson trichrome staining NOS content
3. Yang et al. ¹⁵ China	ADSC	Allogenic Rat epididymis	Nil	36 SD	Divided into six groups a. Sham group=6 b. PBS=6 c. ADSC=6 d. VEGF (ADSC-V)=6 e. GDNF (ADSC-G)=6 f. VEGF and GDNF (ADSC-G&V)=6	BCNI (crush injury)	MPG	Cell-fibrin scaffolds (1.5 × 10 ⁶ cells, 100 μl per rat) were prepared	2 weeks	Mean ICP	Western blotting
4. Chen et al. ¹⁶ China	ADSC Pluripotent stem cells	Human adipose tissue	iMSC and human adMSC	Not available	Divided into four groups a. Sham operation (sham group) b. adMSC group (adipose-derived MSC group) c. Induced pluripotent stem cell-derived MSC (iMSC group). d. PBS Group (PBS only group)	BCNI (crush injury)	Intracavernosal (ICI)	1 × 10 ⁶ adMSC cells 1 × 10 ⁶ iMSC cells Both in 20 μl of PBS	4 weeks	ICP/ MAP ratio	Western blot Masson trichrome staining Immunoreaction detection
5. Ying et al. ¹⁷ China	ADSC NAS	Allogenic Rat inguinal fat	Nil	45 SD rats	Divided into three groups a. Sham group=15 b. BCNI with PBS=15 c. BCNI with NAS=15	BCNI (crush injury)	ICI	One million NAS	4 weeks	Maximal ICP/ MAP ratio	Masson s trichrome staining Toluidine staining NOS content
6. Wu et al. ¹⁸ Taiwan	BM-MSC	Allogenic Rat femur	SPC	24 SD rats	Divided into three groups a. Sham group=8 b. Vehicle group=8 c. SPC group=8	BCNI (crush injury)	ICI	NA	4 weeks	Maximal ICP/ MAP ratio	Histology NOS content Immunofluorescence
7. Matsuda et al. ¹⁹ Japan	BM-MSCs	Allogenic Rat bone marrow	Nil	18 SD rats	Divided into three groups a. Sham group=6 b. Vehicle derived MSC=6 c. BM-MSC=6	BCNI (electric coagulation)	Intravenous, right jugular vein	1.0 × 10 ⁶ cells	4 weeks	ICP/ MAP ratio, with ICP-AUC analysis	Masson s trichrome staining Fluorogold study Green fluorescence

(continued)

Table 1. (continued)

Reference	Stem cell	Type/source	Co-intervention	Total rats	Experiment randomization	Type of injury	Route (average time of treatment 2–4 h after injury)	Dose	Assessment interval	ED function assessment	Histological/Molecular assessment
8. Ouyang <i>et al.</i> ²⁰ China	MSCs and MSC exosomes	Allogenic Rat bone marrow	Nil	32 SD rats	Divided into four groups a. Sham=8 b. PBS=8 c. MSC=8 d. MSC exosomes=8	BCNI (crush injury)	ICI	MSC cells: 1.5×10^6 MSC exosomes: 100 µg	4 weeks	Maximal ICP increase	Western blotting TUNEL staining
9. Li <i>et al.</i> ²¹ China	ADSCs and BMSCs	NA	Exosomes derived from ADSC-Exo and BMSC (BMSC-Exo)	48 SD rats	Divided into four groups a. Sham group=12 b. BCNI + vehicle MSC=12 c. BCNI + exosomes derived from ADSCs (ADSC-Exo group)=12 d. BCNI + BMSC-derived exosomes (BMSC-Exo)=12	BCNI (crush injury)	ICI	100 µg proteins of exosomes derived from ADSC/BMSC	3 weeks	Maximal ICP/MAP ratio	Masson's trichrome staining Imaging fluorescence
10. Zheng <i>et al.</i> ²² China	ADSCs	Allogenic SD rats	ICA II promoted the differentiation of ADSCs to SCs	36 SD rats	Divided into six groups a. Sham (n=6) b. BCNI (n=6) c. PBS (n=6) d. ADSCs (n=6) e. ICA II (n=6) f. ICA II + ADSCs (n=6)	BCNI (crush injury)	ICI and intragastric for ICA II	ADSC, 1×10^6 cells, ICA II (4.5 mg/kg/day)	4 weeks	ICP and MAP	Western blotting qRT-PCR
11. Wu <i>et al.</i> ²³ China	ADSCs	Allogenic SD rats	Nanotechnology-assisted (Nano-ADSC)-injected group with an external magnetic field	40 SD rats	Divided into four groups: a. Sham (n=10) b. BCNI (n=10) c. ADSCs (n=10) d. Nano-ADSC (n=10)	BCNI (crush injury)	ICI	2×10^6 cells	4 weeks	ICP/MAP ratio	Western blotting Immunohistochemistry <i>In Vivo</i> ADSC tracing
12. Chen <i>et al.</i> ²⁴ China	ADSCs	Allogenic SD rats	Nil	40 SD rats	Divided into four groups a. Sham=32 b. BCNI=32 c. PBS=8 d. ADSC=8 (four rats died in Sham and BCNI groups)	BCNI (crush injury)	Intracavernosal (IC)	2×10^6 cells	4 weeks	ICP/MAP ratio	Masson's trichrome staining Fluorescence Staining
13. Fang <i>et al.</i> ²⁵ China	BM-MS (r-MS)	Allogenic Rat bone marrow	Neural d-MSCs Using trans-retinoic acid for differentiation	50 SD rats	Divided into five groups (10 in each) a. sham operation group=10 b. BCNI + ICI of r-BM-MSCs (IC group)=10 c. PPI of r-BM-MSCs (IP group)=10 d. PPI of d-MSCs (IP-d group)=10 e. PPI of PBS (PBS group)=10	BCNI (crush injury)	Intracavernosal (ICI) and PPI	1×10^6 cells	2 weeks	ICP, MAP, and AUC	Western blotting Immunofluorescence staining Immunohistochemical staining

(continued)

Table 1. (continued)

Reference	Stem cell	Type/source	Co-intervention	Total rats	Experiment randomization	Type of injury	Route (average time of treatment 2-4h after injury)	Dose	Assessment interval	ED function assessment	Histological/Molecular assessment
14. Jeon et al. ²⁶ Korea	ADSC	Human fat	Low energy SWT	50 SD rats	Divided into five groups a. Sham group=10 b. BCNI group=10 c. BCNI + ADSC group=10 d. BCNI + SWT group=10 e. BCNI + SWT + ADSC group=10	BCNI (crush injury)	Around injured cavernous injury	1 × 10 ⁶ cells	4 weeks	ICP, ICP/ MAP	Immunostaining, Western blotting, and a cyclic guanosine monophosphate assay
15. Song et al. ²⁷ China	Umbilical cord-MSCs	hUCB mesenchymal stem cells (hUCB-MSCs)	BDNF	42 SD rats	Divided into four groups Group A: sham operation sham + PBS, n=6 Group B: BCNI + PBS, 12 Group C: BCNI + hUCB-MSCs, n=12 Group D: BCNI + BDNF-hUCB-MSCs n=12	BCNI (electric coagulation)	Intracavernosal (ICI)	2 × 10 ⁶ cells	4 weeks	ICP/ MAP	Western blotting
16. Yang et al. ²⁸ China	ADSC	Allogenic rats	Nil	39 SD rats	Divided into three groups a. Sham group=13 b. Cryo group=13 c. ADSC group=13	BCNI (cryoinjury)	ICI	1 × 10 ⁶ cells	4 weeks	ICP/ MAP	Western blotting Immunofluorescence staining Immunohistochemical staining
17. Kim et al. ²⁹ South Korea	BM-MSCs	Human (iliac crest)	SPION-MSC	30 SD rats	Divided into three groups a. Sham group=10 b. BCNI group=10 c. SPION-MSC group=10	BCNI (crush injury)	ICI	1 × 10 ⁶ cells	2 and 4 weeks	ICP/ MAP	MRI Histological evaluation
18. Lee et al. ³⁰ South Korea	ADSCs	Human	BDNF	75 SD rats	Divided into five groups a. Normal (N) group=15 b. Saline application=15 c. Fibroblast growth factor-hydrogel injection (bFGF)=15 d. ADSC application covered with BDNF membrane after BCNI (ADSC/BDNF)=15 e. The co-administration of bFGF-hydrogel injection and BDNF-membrane with ADSC after BDNF (bFGF + ADSC/BDNF)=15	BCNI (crush injury)	ICI	1 × 10 ⁶ cells	4 weeks	ICP/ MAP	Masson's trichrome staining Western blotting NOS expression
19. Kim et al. ³¹ South Korea	ADSCs	Human	NGF-hydrogel	25 SD rats	Divided into five groups a. Sham=5 b. BCNI + Saline application=5 c. BCNI + ADSCs=5 d. BCNI + NGF=5 e. BCNI + ADSC + NGF=5	BCNI (crush injury)	MPG	1 × 10 ⁶ cells	4 weeks	ICP/ MAP ratio	Immunofluorescence staining Western Blot for eNOS and nNOS

(continued)

Table 1. (continued)

Reference	Stem cell	Type/source	Co-intervention	Total rats	Experiment randomization	Type of injury	Route (average time of treatment 2–4 h after injury)	Dose	Assessment interval	ED function assessment	Histological/Molecular assessment
20. You <i>et al.</i> ³² South Korea	BM-MSC	Human (hBMSC)	Nil	40 SD rats	Divided into four groups a. Sham = 10 b. ICI of PBS + PPI of fibrin sealant = 10 c. hBMSCs (ICI group) = 10 d. hBMSCs (PPI + ICI group) = 10	BCNI (dissection)	MPG and ICI	1 × 10 ⁶ cells	4 weeks	ICP/MAP ratio	Western blotting Immunohistochemical analysis
21. You <i>et al.</i> ³³ South Korea	ADSCs	Human	Fibrin scaffolds for periprosthetic injection of ADSCs	60 SD rats	Divided into six groups a. Sham = 10 b. BCNI = 10 c. ICI of PBS + PPI of fibrin sealant = 10 d. PPI of ADSC-seeded fibrin sealant = 10 e. ICI of ADSC = 10 f. PPI of ADSC-seeded fibrin sealant and ICI of ADSCs = 10	BCNI (dissection)	MPG and ICI	1 × 10 ⁶ cells	4 weeks	ICP/MAP ratio with AUC	Western blotting Immunohistochemical analysis
22. Jeong <i>et al.</i> ³⁴ South Korea	ADSC	Human	BDNF, oral Udenafil in desired group	30 SD rats	Divided into five groups a. Sham group = 6 b. BCNI = 6 c. BCNI + Oral Udenafil = 6 d. BCNI + ADSC + BDNF = 6 e. BCNI + ADSC + BDNF + Oral Udenafil = 6	BCNI (crush injury)	Local application to injured nerve	1 × 10 ⁶ cells	4 weeks	ICP/MAP ratio	Immunohistochemical analysis Masson's trichrome cGMP eNOS
23. Qiu <i>et al.</i> ³⁵ USA	ADSC	Autologous rat	Adipose-derived SVF (immediate group received SVF after BCNI, delayed group received SVF after 4 weeks)	89 SD rats	Divided into four groups a. Sham group = 26 b. BCNI + Saline = 23 c. BCNI + Immediate SVF = 17 d. BCNI + Delayed SVF = 23	BCNI (crush injury)	ICI	2 × 10 ⁶ cells	12 weeks	ICP	Masson's trichrome
24. Kim <i>et al.</i> ³⁶ South Korea	BM-MSCs	Allogenic rats (femur/tibia)	Matrixen used (it is a collagen-based biocompatible polymer and it has been used widely as scaffolds in medical field)	50 SD rats	Divided into five groups a. Sham group = 5 b. BCNI = 10 c. BCNI + MSCs = 10 d. BCNI + Matrixen = 10 e. BCNI + MSC + Matrixen = 10	BCNI (crush injury)	MPG	1 × 10 ⁶ cells Matrixen 20 µl	4 weeks	ICP/MAP ratio	PKH-26/NGF co-staining into cavernous nerve, histological and immunohistochemical analysis
25. Kim <i>et al.</i> ³⁷ South Korea	BM-MSCs	Allogenic rats (femur/tibia)	BDNF (rAd/hBDNF represents MSCs infected with rAd expressing human BDNF)	40 SD rats	Divided into four groups a. Sham group = 10 b. BCNI = 10 c. BCNI + MSC = 10 d. BCNI + MSC infected with rAd/hBDNF = 10	BCNI (crush injury)	MPG	1 × 10 ⁶ cells	4 weeks	ICP/MAP ratio	Masson's trichrome staining eNOS and nNOS protein expression by Western blotting

(continued)

Table 1. (continued)

Reference	Stem cell	Type/source	Co-intervention	Total rats	Experiment randomization	Type of injury	Route (average time of treatment 2-4 h after injury)	Dose	Assessment interval	ED function assessment	Histological/Molecular assessment
26. Woo <i>et al.</i> ³⁸ South Korea	Muscle derived-MSC (MD-MSC)	Allogenic white rats (derived from femoral muscles)	MD-MSC was labeled with PKH-26 fluorescent cell linker	15 White rats	Divided rats into three groups a. Sham group = 5 b. BCNI = 5 c. BCNI + MD-MSC = 5	BCNI (transection injury)	ICI	1 × 10 ⁶ cells	4 weeks	ICP	Histology Measurement of cGMP
27. Albersen <i>et al.</i> ³⁹ USA	ADSC	Autologous (paratesticular pad of adipose tissue)	Nil	32 SD rats	Divided rats into four groups a. Sham group + PBS = 8 b. BCNI + PBS = 8 c. BCNI + ADSC Lysate = 8 d. BCNI + ADSC = 8	BCNI (crush injury)	ICI	1 × 10 ⁶ cells	4 weeks	ICP/MAP	Histology TUNEL staining
28. Kim <i>et al.</i> ⁴⁰ USA	MD-MSC and HBSS used (Hanks' Balanced Salt Solution)	Allogenic (MD-MSC from gastrocnemius muscle of female SD rat)	Rat MDC were transduced with retrovirus carrying the β-galactosidase reporter gene	30 SD rats	Divided rats into five groups a. Sham = 6 b. BCNI + MDSC + HBSS = 6 (2 weeks) c. BCNI + MDSC + HBSS = 6 (4 weeks) d. BCNI + HBSS = 6 (2 weeks) e. BCNI + HBSS = 6 (4 weeks)	BCNI (transection injury)	ICI	1 × 10 ⁶ cells	2/4 weeks	ICP	Histology
29. Bochinski <i>et al.</i> ⁴¹ USA	Embryonic stem cells Embryonal	Allogenic rats (from blastocyst)	NES (embryonic stem cells) were induced to differentiate into neural cells by transfecting them with BDNF	26 SD rats	Divided into four groups a. Sham group = 5 b. BCNI + culture medium into CC = 8 c. BCNI + NES to MPG = 4 d. BCNI + NES to CC = 9	BCNI (crush injury)	ICI and MPG	500 μl (10,000 cells/ml)	3 months	ICP	Histochemical analysis for NOS-containing fibers, tyrosine hydroxylase, and neurofilament staining

adMSC, adipose MSC; ADSC, adipose-derived stem cell; AUC, Area under curve; BCNI, bilateral cavernous nerve injury; BDNF, brain-derived neurotrophic factor; BM-MSCs, bone marrow-derived stem cells; CC, corpus cavernosum; CCK8, Cell Counting Kit-8; cGMP, cyclic guanosine monophosphate; d-MSCs, differentiated MSCs; ED, erectile dysfunction; bFGF, Basic fibroblast growth factor; eNOS, endothelial nitric oxide synthase; GDNF, glial cell-derived nerve growth factor; GFP, green fluorescent protein; hBDNF, human brain-derived neurotrophic factor; hBMSC, human bone marrow stem cells; HBSS, Hanks Balanced Salt solution; hUCB, human umbilical cord blood; ICA II, Icariside II; ICI, intracavernosal injection; ICP, intracavernosal pressure; iMSC, induced pluripotent stem cell-derived MSC; MAP, mean arterial pressure; MD-MSC, Muscle derived stem cells; MPG, major pelvic ganglion; MRI, magnetic resonance imaging; MSC, mesenchymal stem cell; NA, not available; NAS, neural-like cells from adipose-derived stem cells; NES, neural stem cells; NGF-hydrogel, nerve growth factor-incorporated hyaluronic acid-based hydrogel; nNOS, neuronal nitric oxide synthase; NOS, nitric oxide synthase; PBS, phosphate-buffered saline; PKH-26, red-fluorescent dye; PPI, periprostatic implantation; qRT-PCR, quantitative reverse transcription polymerase chain reaction; rAd, recombinant adenoviruses; SCs, Schwann cells; SD, Sprague-Dawley rats; SPC, smooth muscle progenitor; SPION-MSC, super-paramagnetic iron oxide nanoparticle labeling was performed for MSC; SVF, stromal vascular fraction; SWT, shock wave therapy; TUNEL, terminal deoxynucleotidyltransferase-mediated nick-end labeling; VEGF, vascular endothelial growth factor.

Table 2. Human study: data extraction results (post-radical prostatectomy).

Reference	Patient No.	Age (years)	Duration since procedure	Cell type used	Dose	Mode of delivery	Side effects	Mode of assessment	Follow-up
1. Yiou <i>et al.</i> ⁴² (NCT01089387)	12	63.6 (45–70)	22.9 months (6 months–3 years)	BM-MNCs	Four different doses 2 × 10 ⁷ 2 × 10 ⁸ 1 × 10 ⁹ 2 × 10 ⁹	Intracavernous	Mild postoperative pain at the BM aspiration No evidence of prostate cancer reactivation	IIEF-15 EHS Penile length, Color Doppler	1 year initially, extended to 61 months in phase II for 9 patients
2. Yiou <i>et al.</i> ⁴³ (NCT01089387)	6	59.9	26.3 months	BM-MNCs	1 × 10 ⁹	Intracavernous	No serious adverse events were reported.	IIEF-15 EHS Penile length, Color Doppler	6 months
3. Haahr <i>et al.</i> ⁴⁴ (NCT02240823)	21 Continent = 14 Incontinent = 7	60.2 (46–69)	10.7 months (6–15)	ADRC	8.4–37.2 million ADRCs	Intracavernous	No serious adverse events were reported; eight men experienced transient redness and swelling at the injection sites, three reported reaction in the penile area; five men stated minor abdominal hematomas; one man had an abdominal hematoma which led to scrotal and penile hematomas; this patient had taken large doses of non-steroidal anti-inflammatory drugs for back pain in the days before the treatment; all reported hematomas resolved within 14 days without any sequel; finally, eight patients reported light abdominal discomfort after liposuction	IIEF-5 EHS	1 year

ADRC, adipose-derived regenerating cell; BM-MNCs, bone marrow mononuclear cells; EHS, Erection Hardness Score; IIEF, International Index for Erectile Function.

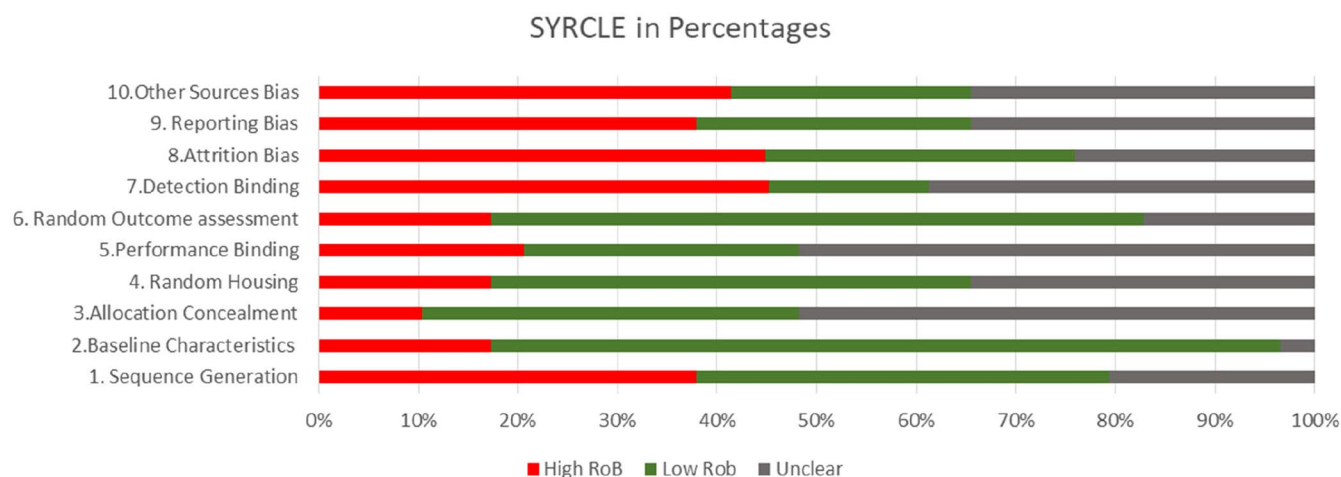


Figure 1. SYRCLE for animal studies.

technique was used and 34 articles were found relevant; however, 5 articles were excluded (nerve injury by radiation, studies involving oral therapy or use of cells other than stem cells, studies evaluating role in apoptosis); 29 studies were included for qualitative and 22 studies in quantitative analysis (Table 1). PRISMA flowchart is attached.

Human studies. Five articles matched initial search. After screening, two articles were excluded and three were included in qualitative research. PRISMA flowchart is attached.

Included studies

Animal studies. In 29 animal studies, a similar experimental protocol was followed. It involved dividing rats randomly in different groups – Sham, BCNI, Vehicle (in some), stem cell group, co-intervention group, and combined group (Stem cell and Co-intervention). Flowchart depicts stepwise approach carried out in animal experiments (Figure 2). Stem cells used included – ADSC (16 studies), BMSC (8 studies), and others (5 studies). Most studies used Sprague-Dawley (SD) rats, total of about 1,017, while only one study used white rats (15). Details are provided in Table 1.

Cavernous injury technique. Bilateral crush injury was most common form of cavernous nerve injury and used in 21 studies.^{14–18,20–26,30,31,34–39,41} Usually, this involved using a non-serrated hemostat away from major pelvic ganglion (MPG). In five studies, cavernous nerve injury was caused by transection injury,^{13,32,33,38,40} in two studies

electric coagulation,^{19,27} and in one study Cryo-injury²⁸ was used.

Co-Interventions and modifications. Some studies used certain co-interventions or modifications to enhance the effect of stem cell intervention. Co-interventions included brain-derived neurotrophic factor (BDNF) in six studies;^{14,27,30,34,37,41} oral Icariside II (ICA II) in two studies;^{13,22} and low shock wave therapy and oral Udenafil were used in one study each.^{26,34} Modifications included use of fibrin scaffolds, Matrixen (bio-compatible polymer), exosomes, nanotechnology, and super-paramagnetic iron oxide nanoparticles.

Route of administration. Most common route of administration of stem cells was intracavernosal route. It was used in 17 studies.^{13,14,16–18,20,21,23,24,27–30,35,38–40} The usual technique for IC injections (ICIs), included rolling up the prepuce to expose the penis and injecting to the lateral aspect of the penis. The needle was inserted around 3–4 mm. Before injection, drainage *via* the dorsal vein was halted by circumferential compression of the base of the penis using external compression (like an elastic band). The compression was released in around 1 min after injection of cells.

In four studies, stem cells were injected directly to MPG or cavernous nerves (periprostatic implantation, PPI).^{15,31,36,37} In five studies, ICI was used in combination with either MPG or PPI.^{22,25,32,33,41} For PPI of stem cells, different scaffolds like Fibrin or Matrixen were used. In most studies, mixtures were instilled into fissures between

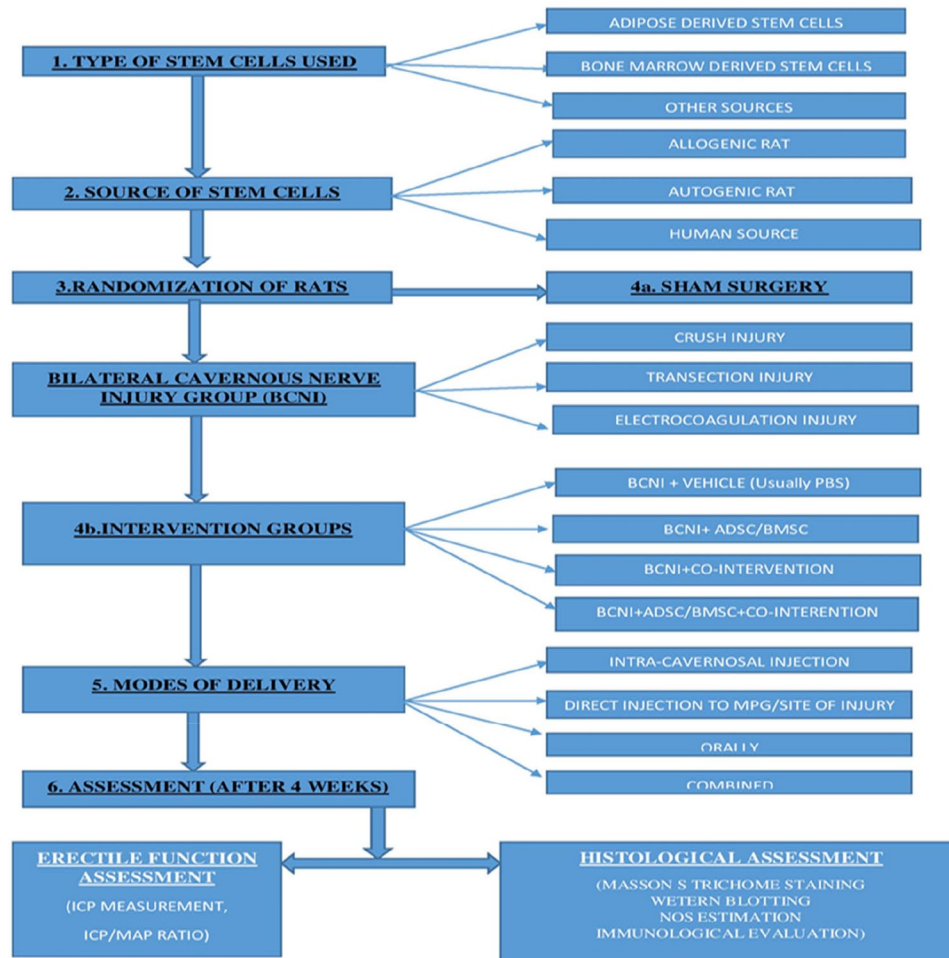


Figure 2. Flowchart depicting method of research in animal experiments.

vesico-prostatic junction and seminal vesicles. In two studies, stem cell infusion was done around injured nerve.^{26,34} In one study, stem cells were administered through intravenous route (right jugular vein).¹⁹ The most common dose of stem cells used is 1.0×10^6 cells.

ICP measurement. Initially, the number of studies providing data in a suitable format for analysis was evaluated, along with the number of animals. A total of 22 studies provided data suitable for meta-analysis. This consisted of 13 studies in the ADSC subgroup, 7 in the BMSC subgroup, and 2 in the Mixed subgroup. Data from a total of 909 animals were collected. Information on the number of studies on which data in each treatment group were collected, along with the pattern of the treatment combinations, was analyzed using a network map.

ADSCs. The first set of analyses considered the ADSC studies only. Both direct compar-

isons only and a network meta-analysis were performed. The results of both sets of analyses are shown in Table 3. Both analyses show the mean difference between each pair of treatments, in addition to corresponding CIs and the significance of the group differences. On comparing, stem cell group performed significantly better than BCNI group as traditional meta-analysis revealed difference of 27 (95% CI, P value <0.001) and network meta-analysis revealed difference of 28 (95% CI, P value <0.001). Overall, the traditional meta-analysis and network meta-analysis gave fairly similar results. Typically, the same pairs of groups varied from each other (or not) in both sets of analyses. As expected, all groups were found to have significantly lower values than the sham group.

The BCNI group had significantly lower ICP values than all other groups, with the exception of the vehicle group, where no difference was observed. The traditional meta-analyses suggested a very

Table 3. Summary of meta-analysis results.

	Group 1	Group 2	Direct comparisons			Network meta-analysis	
			Difference (95% CI) ^a	P value	I ²	Difference (95% CI) ^a	P value
ADSC studies	Sham	BCNI	-71 [-84, -58]	<0.001	99%	-73 [-86, -59]	<0.001
		Vehicle	-68 [-85, -52]	<0.001	99%	-69 [-85, -53]	<0.001
		Stem cell	-44 [-54, -34]	<0.001	99%	-44 [-57, -32]	<0.001
		Co-intervention	-42 [-55, -28]	<0.001	99%	-42 [-57, 28]	<0.001
		Combined	-21 [-33, -9]	0.001	99%	-23 [-39, -7]	0.006
	BCNI	Vehicle	17 [-4, 38]	0.12	99%	3 [-15, 21]	0.73
		Stem cell	27 [15, 39]	<0.001	99%	28 [15, 42]	<0.001
		Co-intervention	31 [20, 50]	<0.001	99%	30 [15, 45]	<0.001
		Combined	48 [32, 65]	<0.001	99%	49 [33, 66]	<0.001
	Vehicle	Stem cell	30 [13, 48]	0.001	99%	24 [7, 40]	0.005
		Co-intervention	10 [-5, 24]	0.21	97%	25 [6, 44]	0.008
		Combined	15 [2, 27]	0.03	97%	44 [25, 65]	<0.001
Stem cell	Co-intervention	5 [-4, 13]	0.28	98%	2 [-13, 16]	0.83	
	Combined	22 [7, 36]	0.003	99%	21 [5, 37]	0.01	
Co-intervention	Combined	25 [11, 39]	<0.001	99%	20 [3, 37]	0.02	
BMSC studies	Sham	BCNI	-42 [-45, -39]	<0.001	(+)	-48 [-67, -31]	<0.001
		Vehicle	-72 [-92, -51]	<0.001	99%	-71 [-82, -60]	<0.001
		Stem cell	-27 [-39, -16]	<0.001	98%	-27 [-38, -16]	<0.001
		Co-intervention	-32 [-37, -27]	<0.001	83%	-36 [-48, -24]	<0.001
		Combined	-7 [-16, 1]	0.10	96%	-16 [-32, 0]	0.05
	BCNI	Vehicle	(#)	-	-	-22 [-41, -4]	0.02
		Stem cell	23 [11, 36]	<0.001	98%	21 [5, 38]	0.01
		Co-intervention	8 [6, 10]	<0.001	(+)	13 [-5, 31]	0.17
		Combined	30 [28, 32]	<0.001	(+)	32 [12, 53]	0.002
	Vehicle	Stem cell	43 [33, 52]	<0.001	96%	44 [32, 55]	<0.001
		Co-intervention	30 [12, 48]	0.001	98%	35 [22, 48]	<0.001
		Combined	37 [35, 39]	<0.001	(+)	54 [38, 71]	<0.001
	Stem cell	Co-intervention	-7 [-18, 4]	0.23	98%	-9 [-21, 4]	0.17
		Combined	10 [4, 16]	0.001	94%	10 [-5, 26]	0.18
	Co-intervention	Combined	22 [20, 24]	<0.001	0%	19 [3, 36]	0.02

ADSC, adipose-derived stem cell; BCNI, bilateral cavernous nerve injury; BMSC, bone marrow-derived stem cell; CI, confidence interval.

^aDifferences reported as Group 2 minus Group 1.

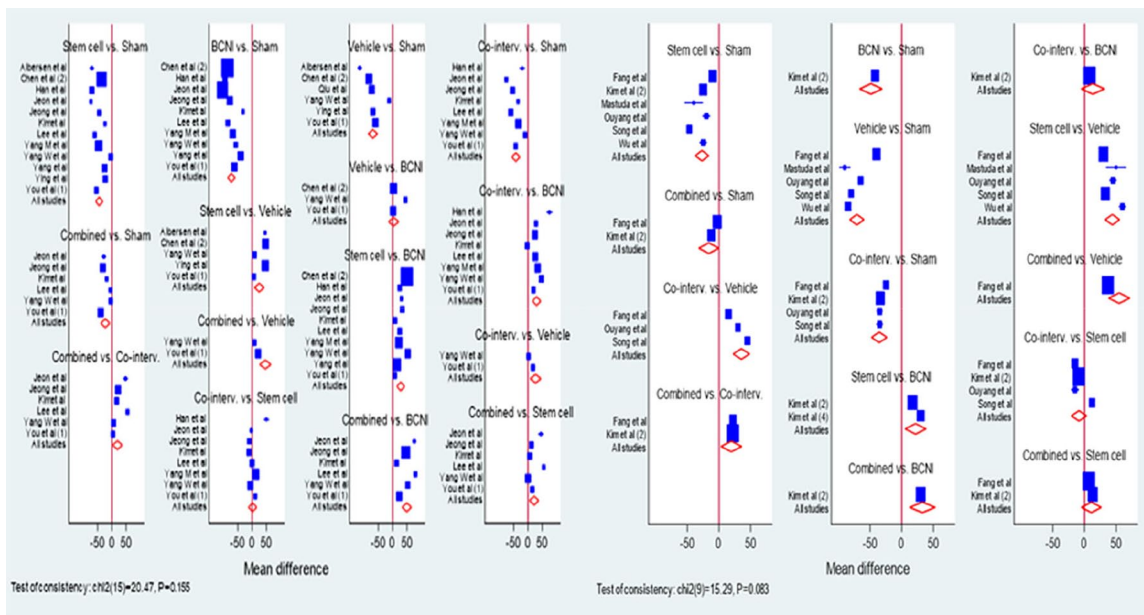


Figure 3. Forest plots for direct comparisons (ADSC studies – left side, BMSC studies – right side).

high degree of heterogeneity between the study results for all comparisons. A graphical illustration of these direct comparisons between groups is shown in a Forest plot in Figure 3.

BMSCs. A similar set of analyses was performed for the BMSC studies. The meta-analysis results, using both approaches, are summarized in Table 3. The results suggested that again the sham group tended to have the highest values. On comparing, again stem cell group performed significantly better than BCNI group as traditional meta-analysis revealed difference of 23 (95% CI, P value <0.001) and network meta-analysis revealed difference of 21 (95% CI, P value <0.001). For the traditional meta-analyses, the P values indicated heterogeneity between studies. For these studies, the vehicle group performed worst, with the network meta-analyses suggested significantly lower ICP values than all other groups. A graphical illustration of the results for the direct comparisons is shown in a Forest plot in Figure 3.

ICP/MAP ratio. ICP and MAP ratio was evaluated from 26 studies;^{13–37,39} 3 studies did not provide ICP/MAP ratio values.^{38,40,41} We found mean ICP/MAP ratio was higher in stem cell group (0.51) compared with BCNI group (0.25). Further for combined intervention group, ICP/MAP ratio was observed to be even

higher than stem cell group at 0.65 suggesting that the co-interventions further enhanced the benefit (Figure 4).

Histological/molecular evaluation. All 29 studies evaluated penile tissue as well as MPG (studies which involved periprostatic intervention); 21 studies reported an increase in level of neuronal nitric oxide synthase (nNOS), and 26 studies reported either increase in smooth muscle content or increase in ratio of smooth muscle to collagen ratio. Other changes reported included anti-apoptotic role played by stem cells. The summarized results are given in Table 4.

Human studies. The three phase I/II trials included in this study included two open label clinical trials and one open label extension study.^{42–44} The trials included patients who had ED post-RP and failed to recover using conventional therapy. Open-label trials can be used to compare treatments or gather additional information about the long-term effects in the intended patient population. Since all three trials included patients who had failed to recover on conventional therapy, these three trials were non-comparative and looked at long-term effect of stem cells on post-RP ED. One of the trials was open label extension.⁴³ A total of 35 patients were included in three trials. The results are summarized in Table 5.

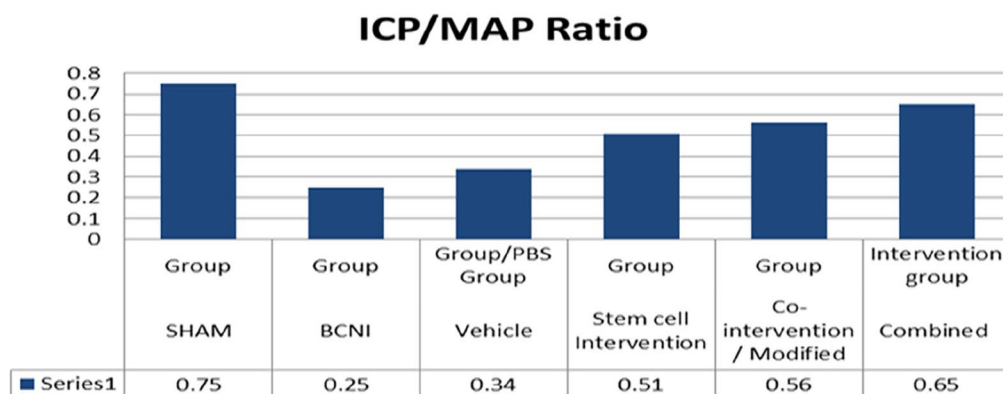


Figure 4. Mean ICP/MAP ratios.

Table 4. Results from histological/molecular evaluation.

Summarized results of tissue analysis				
Reference	NOS	Smooth muscle/ collagen ratio	Smooth muscle content	Other finding
1. Zheng <i>et al.</i> ¹³	Increased nNOS fibers			Suppression of miR-33 expression
2. Yang <i>et al.</i> ¹⁴	Increased nNOS fibers	Increased		
3. Yang <i>et al.</i> ¹⁵	Increased nNOS fibers	Increased		
4. Chen <i>et al.</i> ¹⁶	Increased nNOS expression		Increased	Anti-apoptotic Increased endothelial content
5. Ying <i>et al.</i> ¹⁷	Increased nNOS fibers		Increased	Increased myelinated axons
6. Wu <i>et al.</i> ¹⁸	No significant improvement		Increased	Anti-apoptotic
7. Matsuda <i>et al.</i> ¹⁹		Increased		
8. Ouyang <i>et al.</i> ²⁰	Increased nNOS expression	Increased	Increased	Anti-apoptotic
9. Li <i>et al.</i> ²¹	Increased nNOS expression			
10. Zheng <i>et al.</i> ²²				Suppression of miR-33 expression, promoting diffusion of ADSC to SC
11. Wu <i>et al.</i> ²³			Increased expression of α -SMA	Increased expression of β III tubulin and CD31
12. Chen <i>et al.</i> ²⁴	Increased nNOS fibers	Increased	Increased	
13. Fang <i>et al.</i> ²⁵	Increased nNOS fibers	Increased	Increased	
14. Jeon <i>et al.</i> ²⁶			Increased	Anti-apoptotic Increased VEGF expression
15. Song <i>et al.</i> ²⁷	Increased nNOS fibers	Increased	Increased expression of α -SMA	
16. Yang <i>et al.</i> ²⁸	Increased nNOS fibers			Elevation of neurotrophic factors (VEGF, NGF, and Neurturin)

(continued)

Table 4. (continued)

Summarized results of tissue analysis				
Reference	NOS	Smooth muscle/ collagen ratio	Smooth muscle content	Other finding
17. Kim <i>et al.</i> ²⁹			Increased	Increased
18. Lee <i>et al.</i> ³⁰	Increased nNOS fibers		Increased expression of α -SMA	
19. Kim <i>et al.</i> ³¹	Increased nNOS fibers		Decrease in smooth muscle atrophy	
20. You <i>et al.</i> ³²	Increased nNOS fibers	Increased		
21. You <i>et al.</i> ³³	Increased nNOS expression		Increased	
22. Jeong <i>et al.</i> ³⁴	Increased nNOS expression	Increased		Increase in cGMP level
23. Qiu <i>et al.</i> ³⁵	Increased nNOS fibers	Increased	Increased	
24. Kim <i>et al.</i> ³⁶	Increase in expressions of the eNOS and nNOS			Increase in neuronal cells
25. Kim <i>et al.</i> ³⁷	Increase in eNOS and nNOS	Increased		
26. Woo <i>et al.</i> ³⁸				Increase in cGMP level
27. Albersen <i>et al.</i> ³⁹	Increased nNOS fibers		Increased	Reduced apoptosis
28. Kim <i>et al.</i> ⁴⁰			Increased	Nerve fiber regeneration
29. Bochinski <i>et al.</i> ⁴¹				Differentiation of embryonic stem cells into neural cells leading to improved ED

ADSC, adipose-derived stem cells; cGMP, cyclic guanosine monophosphate; ED, erectile dysfunction; eNOS, endothelial nitric oxide synthase; miR-33, micro-RNA; NGF, nerve growth factor; nNOS, neuronal nitric oxide synthase; NOS, nitric oxide synthase; SC, Schwann cells; SMA, smooth muscle actin; VEGF, vascular endothelial growth factor.

1. *IIEF score (International Index For Erectile Dysfunction)*: Two trials used IIEF-15 score and found there was an increase in score; one study revealed an increase from baseline of 25.3 to 39.7 at 6 months,⁴² and the other study indicated an increase from 18.7 to 46.6 for same duration.⁴³ One trial used IIEF-5 scoring and found that in continent group of patients, it increased from 6 to 16 in a 6-month period; however, there was no improvement in incontinent group.
2. *EHS*: One trial reported an elevation in EHS from baseline 1.3 to 2.6 in 6 months,⁴² the second reported increase from 1.8 to 3.3 for same duration,⁴³ and third trial indicated an increase from 1 to 3 at 6 months in the continent group.⁴⁴ However, there was no change in incontinent group.
3. *Penile length*: In one trial, penile length was also considered. It increased from 12.4 to 13.3 cm by end of 3 months. However, by end of 6 months, it was around 12.9 cm.⁴²
4. *Adverse outcomes*: One trial reported mild postoperative pain at the bone marrow (BM) aspiration with no evidence of prostate cancer reactivation.⁴² Second trial reported 8 men developed transient redness and swelling at injection site. 5 men in this series also developed minor abdominal wall hematomas (post-liposuction).⁴⁴ The third trial has not reported any adverse outcome⁴³ (Table 2).

Table 5. Outcomes of SCT in human trials.

	Baseline	1 month	3 months	6 months	1 year
1. Yiou <i>et al.</i> ⁴²					
IIEF-15 score (Max 75)	25.3	28.3	39.7	43.5	44.4
EHS	1.3	1.7	2.3	2.6	3.0
Penile length (cm)	12.4	13.5	13.3	12.9	NA
2. Yiou <i>et al.</i> ⁴³					
IIEF-15 score (Max 75)	18.7	33.2	47.4	46.6	
EHS	1.8	2.2	2.7	3.3	
3. Haahr <i>et al.</i> ⁴⁴					
IIEF -5 (SHIM score)					
Continent group (11 pat)	6	6	11	16	
Incontinent group (6 pat)	5	5	5	5	
EHS					
Continent group (11 pat)	1	2	2	3	
Incontinent group (6 pat)	1	1	1	1	
EHS, Erection Hardness Score; IIEF, International Index for Erectile Function; NA, not available; SCT, stem cell therapy; SHIM, Sexual Health Inventory for men.					

Discussion

Cavernous injury is widely accepted to be responsible for post-RP ED due to the damage caused by incision, heat, and mechanical stress.⁴⁸ Although, nerve-sparing RP was introduced 30 years ago, ED still remains a challenge with RP surgery.^{49,50} A recent prospective series has shown that up to 75% of men reported ED, 1 year after RP with minimal difference between robotic and open surgery groups.⁵¹ Therefore, it is now commonly believed that, although leaving the cavernosal nerves (CNs) intact, nerve-sparing RP still causes subtle changes that are not obvious to the surgeons.⁵² These changes cause CNs to undergo Wallerian degeneration and eventually lose their connection to the corpora cavernosa.⁵³ Alternatively, the surgery-incurred insults may temporarily prevent the CNs from releasing nitric oxide (NO) into the corpus cavernosum (CC), and without NO-induced engorgement, the penile tissue becomes hypoxic and its cavernous musculature is replaced by collagens and fibrous scar tissue.^{52,53}

SCT is among different novel approaches being investigated to manage post-RP ED. Stem cells

are undifferentiated or partially differentiated cells and are classified as totipotent (e.g. zygote), pluripotent [e.g. embryonic stem cells (ESCs)], multipotent (e.g. hematopoietic and MSCs), and unipotent according to the number of cell lines in which they could be differentiated. Stem cells have been used for the treatment of cardiovascular,⁵⁴ neurological,⁵⁵ autoimmune,⁵⁶ and hematologic diseases.⁵⁷ In recent years, SCT has been proposed for the treatment of ED as stem cells can differentiate to endothelial, neuronal, or smooth muscle cells, and therefore restore possible structural damage in the penile tissue.⁵⁸ Most commonly used stem cells used in the treatment of ED are MSCs. MSCs are able to demonstrate therapeutic effects by their ability to produce an array of bioactive molecules including growth factors capable of inducing increased cell proliferation and immunomodulatory effects.⁵⁹ They lead to 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.⁶⁰ This is termed as paracrine action of

stem cells, because few stem cells can be detected after transplantation, and almost no direct evidence supports the theory that transplanted stem cells have differentiated into vascular endothelial cells, smooth muscle cells, or nerves.⁶¹

The current meta-analysis examined 29 preclinical studies of SCT in the treatment of ED secondary to BCNI in rats and also evaluated three human clinical trials. The animal studies were analyzed for ICP measurement, ICP/MAP ratio, and histological/molecular results. Our study focused mainly on ICP measurements as it is considered a reliable method for direct measure of erectile function. It allows for the acquisition of data on basal ICP, peak ICP, plateau ICP, time to erection and detumescence time, duration of response.⁶²

Meta-analysis of ICP values (post-SCT) including ADSC as well as BMSC stem cell groups revealed statistically significant improvement in ED in SCT intervention group as compared with BCNI group. There is minimal literature in the form of a systemic review or a meta-analysis available regarding ICP measurements in ED in BCNI rats to compare. We also evaluated ICP/MAP ratio for animal studies. We found that there is an increase in ICP/MAP ratio in stem cell intervention group as compared with BCNI group. A previous meta-analysis based on ICP/MAP ratio had also revealed that there is a significant difference of erectile functions between stem cell transplantation group and control group.⁶³ We noticed that for both ICP (meta-analysis) and ICP/MAP ratio, the efficacy of SCT was enhanced by the addition of co-interventions. This has been reported previously as well.⁶³

In case of animal studies, histology of penile tissue post-SCT was also analyzed. It revealed an increase in NOS, ratio of smooth muscle to collagen, and anti-apoptotic activity in intervention groups. In a previous systematic review, it has been established that SCT does lead to structural changes in CC resulting in increased endothelial and smooth muscle cell markers, increase in neural cell markers, decrease in apoptosis, as well as a decrease in collagen content.⁴⁸

The three human trials in post-RP patients did reflect improvement in SHIM and EHS scores after SCT. The trials have revealed that SCT in humans has a potential efficacy suggested by a

significant improvement in IIEF scores, erectile function with minimal adverse effects. However, in one trial, the SCT therapy results could not produce desired results in men having urinary incontinence.⁴⁴ This has been already established that urinary continence seems to be a prerequisite for having sexual intercourse post-RP.⁶⁴

Adult stem cells in regenerative medicine do have issues and concerns. The most important concern being development of recurrences as well as other tumors in patients receiving SCT due to conflicting evidence that adult stem cells can promote tumor genesis.⁶⁵ Several hypotheses have proposed that the events in either stem and/or differentiated cells, such as genomic instability, inflammatory microenvironment, cell fusion, and lateral gene transfer, should be considered as the possible origin of cancer stem cells (CSCs), are responsible for the sustained and uncontrolled growth of malignant tumors, and are proposed to play significant roles in metastasis and recurrence⁶⁶ but also the chemotaxis and subsequent migration of patient stem cells to the hypoxic tumor mass promoting angiogenesis; however, the specific response of the stem cell to the malignant mass may well be due to the malignancy type.⁵⁹ In the majority of animal trials, animal was sacrificed at around 4 weeks, so this aspect has not been addressed. The issues with human trials included in this study include very limited number of patients, SCT treatment started after a gap of few months to years, sometimes overlapping both SCT with other therapies, no long-term follow-up details, and also pre-screening for risk factors, and the presence of secondary malignancy may well be required to reduce risk factors.

In order to understand SCT in post-RP patients, a double-blind randomized controlled study is need of hour; however, it will not be an easy task, as a number of questions need to be addressed – When to start? Which type of stem cell to use? What quality control of stem cells to be used? What source? What is ideal dose? Which route? How to do follow-up?

Conclusion

Our results confirm that SCT does improve the erectile function in rats having cavernous nerve injury. Furthermore, co-interventions and specific modifications do improve efficacy of SCT. Similarly, early human results have shown

promising results. Thus, regenerative medicine approach to the treatment of ED appears to hold much promise.

Author contributions

Mudassir M. Wani: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Visualization; Writing – original draft; Writing – review & editing.

Bhavan P. Rai: Supervision; Validation; Writing – review & editing.

William Richard Webb: Project administration; Validation; Visualization.

Sanjeev Madaan: Conceptualization; Data curation; Formal analysis; Project administration; Resources; Software; Supervision; Validation; Writing – review & editing.

Conflict of interest statement

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Supplemental material

Supplemental material for this article is available online.

References

1. Rawla P. Epidemiology of prostate cancer. *World J Oncol* 2019; 10: 63–89.
2. Heidenreich A, Bellmunt J, Bolla M, *et al.* EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and treatment of clinically localised disease. *Eur Urol* 2011; 59: 61–71.
3. Montorsi F, Adaihan G, Becher E, *et al.* Summary of the recommendations on sexual dysfunctions in men. *J Sex Med* 2010; 7: 3572–3588.
4. Salonia A, Burnett AL, Graefen M, *et al.* Prevention and management of postprostatectomy sexual dysfunctions. Part 1: choosing the right patient at the right time for the right surgery. *Eur Urol* 2012; 62: 261–272.
5. Weyne E and Albersen M. Post-RP erectile dysfunction – therapies for the next decade. *Nat Rev Urol* 2014; 11: 616–618.
6. Rabbani F, Stapleton AM, Kattan MW, *et al.* Factors predicting recovery of erections after radical prostatectomy. *J Urol* 2000; 164: 1929–1934.
7. Fode M, Ohl DA, Ralph D, *et al.* Penile rehabilitation after radical prostatectomy: what the evidence really says. *BjU Int* 2013; 112: 998–1008.
8. Litwin MS, Nied RJ and Dhanani N. Health-related quality of life in men with erectile dysfunction. *J Gen Intern Med* 1998; 13: 159–166.
9. Lue Tom F. Erectile dysfunction. *N Engl J Med* 2000; 342: 1802–1813.
10. Alwaal A, Hussein AA, Lin CS, *et al.* Prospects of stem cell treatment in benign urological diseases. *Korean J Urol* 2015; 56: 257–265.
11. Vardi Y, Appel B, Kilchevsky A, *et al.* Does low intensity extracorporeal shock wave therapy have a physiological effect on erectile function? Short-term results of a randomized, double-blind, sham controlled study. *J Urol* 2012; 187: 1769–1775.
12. Yiou R. Stem-cell therapy for erectile dysfunction. *Biomed Mater Eng* 2017; 28(s1): S81–S85.
13. Zheng T, Zhang T, Zhang W, *et al.* Icariside II facilitates the differentiation of ADSCs to Schwann cells and restores erectile dysfunction through regulation of miR-33/GDNF axis. *Biomed Pharmacother* 2020; 125: 109888.
14. Yang M, Sun JY, Ying CC, *et al.* Adipose-derived stem cells modified by BDNF gene rescue erectile dysfunction after cavernous nerve injury. *Neural Regen Res* 2020; 15: 120–127.
15. Yang W, Chen Z, Ma X, *et al.* Co-overexpression of VEGF and GDNF in adipose-derived stem cells optimizes therapeutic effect in neurogenic erectile dysfunction model. *Cell Prolif* 2020; 53: e12756.
16. Chen Z, Han X, Ouyang X, *et al.* Transplantation of induced pluripotent stem cell-derived mesenchymal stem cells improved erectile dysfunction induced by cavernous nerve injury. *Theranostics* 2019; 9: 6354–6368.

17. Ying CC, Yang M, Wang Y, *et al.* Neural-like cells from adipose-derived stem cells for cavernous nerve injury in rats. *Neural Regen Res* 2019; 14: 1085–1090.
18. Wu YN, Chen KC, Liao CH, *et al.* Smooth muscle progenitor cells preserve the erectile function by reducing corporal smooth muscle cell apoptosis after bilateral cavernous nerve crush injury in rats. *Biomed Res Int* 2019; 2019: 8520523.
19. Matsuda Y, Sasaki M, Kataoka-Sasaki Y, *et al.* Intravenous infusion of bone marrow-derived mesenchymal stem cells reduces erectile dysfunction following cavernous nerve injury in rats. *Sex Med* 2018; 6: 49–57.
20. Ouyang X, Han X, Chen Z, *et al.* MSC-derived exosomes ameliorate erectile dysfunction by alleviation of corpus cavernosum smooth muscle apoptosis in a rat model of cavernous nerve injury. *Stem Cell Res Ther* 2018; 9: 246.
21. Li M, Lei H, Xu Y, *et al.* Exosomes derived from mesenchymal stem cells exert therapeutic effect in a rat model of cavernous nerves injury. *Andrology* 2018; 6: 927–935.
22. Zheng T, Zhang TB, Wang CL, *et al.* Icariside II promotes the differentiation of adipose tissue-derived stem cells to Schwann cells to preserve erectile function after cavernous nerve injury. *Mol Cells* 2018; 41: 553–561.
23. Wu H, Tang WH, Zhao LM, *et al.* Nanotechnology-assisted adipose-derived stem cell (ADSC) therapy for erectile dysfunction of cavernous nerve injury: in vivo cell tracking, optimized injection dosage, and functional evaluation. *Asian J Androl* 2018; 20: 442–447.
24. Chen X, Yang Q, Zheng T, *et al.* Neurotrophic effect of adipose tissue-derived stem cells on erectile function recovery by pigment epithelium-derived factor secretion in a rat model of cavernous nerve injury. *Stem Cells Int* 2016; 2016: 5161248.
25. Fang JF, Jia CC, Zheng ZH, *et al.* Periprostatic implantation of neural differentiated mesenchymal stem cells restores cavernous nerve injury-mediated erectile dysfunction. *Am J Transl Res* 2016; 8: 2549–2561.
26. Jeon SH, Shrestha KR, Kim RY, *et al.* Combination therapy using human adipose-derived stem cells on the cavernous nerve and low-energy shockwaves on the corpus cavernosum in a rat model of post-prostatectomy erectile dysfunction. *Urology* 2016; 88: 226.e1–226.e9.
27. Song L, Zhu J, Zhang X, *et al.* BDNF-hypersecreting human umbilical cord blood mesenchymal stem cells promote erectile function in a rat model of cavernous nerve electrocautery injury. *Int Urol Nephrol* 2016; 48: 37–45.
28. Yang R, Fang F, Wang J, *et al.* Adipose-derived stem cells ameliorate erectile dysfunction after cavernous nerve cryoinjury. *Andrology* 2015; 3: 694–701.
29. Kim JH, Lee HJ, Doo SH, *et al.* Use of nanoparticles to monitor human mesenchymal stem cells transplanted into penile cavernosum of rats with erectile dysfunction. *Korean J Urol* 2015; 56: 280–287.
30. Lee SH, Kim IG, Jung AR, *et al.* Combined effects of brain-derived neurotrophic factor immobilized poly-lactic-co-glycolic acid membrane with human adipose-derived stem cells and basic fibroblast growth factor hydrogel on recovery of erectile dysfunction [published correction appears in *Tissue Eng Part A* 2016; 22: 194]. *Tissue Eng Part A* 2014; 20: 2446–2454.
31. Kim IG, Piao S, Lee JY, *et al.* Effect of an adipose-derived stem cell and nerve growth factor-incorporated hydrogel on recovery of erectile function in a rat model of cavernous nerve injury. *Tissue Eng Part A* 2013; 19: 14–23.
32. You D, Jang MJ, Lee J, *et al.* Periprostatic implantation of human bone marrow-derived mesenchymal stem cells potentiates recovery of erectile function by intracavernosal injection in a rat model of cavernous nerve injury. *Urology* 2013; 81: 104–110.
33. You D, Jang MJ, Lee J, *et al.* Comparative analysis of periprostatic implantation and intracavernosal injection of human adipose tissue-derived stem cells for erectile function recovery in a rat model of cavernous nerve injury. *Prostate* 2013; 73: 278–286.
34. Jeong HH, Piao S, Ha JN, *et al.* Combined therapeutic effect of udenafil and adipose-derived stem cell (ADSC)/brain-derived neurotrophic factor (BDNF)-membrane system in a rat model of cavernous nerve injury. *Urology* 2013; 81: 1108.e7–1108.e14.
35. Qiu X, Fandel TM, Ferretti L, *et al.* Both immediate and delayed intracavernous injection of autologous adipose-derived stromal vascular fraction enhances recovery of erectile function in a rat model of cavernous nerve injury. *Eur Urol* 2012; 62: 720–727.
36. Kim SJ, Park SH, Sung YC, *et al.* Effect of mesenchymal stem cells associated to matrixen on the erectile function in the rat model with bilateral cavernous nerve crushing injury. *Int Braz J Urol* 2012; 38: 833–841.
37. Kim SJ, Choi SW, Hur KJ, *et al.* Synergistic effect of mesenchymal stem cells infected with

- recombinant adenovirus expressing human BDNF on erectile function in a rat model of cavernous nerve injury. *Korean J Urol* 2012; 53: 726–732.
38. Woo JC, Bae WJ, Kim SJ, *et al.* Transplantation of muscle-derived stem cells into the corpus cavernosum restores erectile function in a rat model of cavernous nerve injury. *Korean J Urol* 2011; 52: 359–363.
 39. Albersen M, Fandel TM, Lin G, *et al.* Injections of adipose tissue-derived stem cells and stem cell lysate improve recovery of erectile function in a rat model of cavernous nerve injury. *J Sex Med* 2010; 7: 3331–3340.
 40. Kim Y, de Miguel F, Usiene I, *et al.* Injection of skeletal muscle-derived cells into the penis improves erectile function. *Int J Impot Res* 2006; 18: 329–334.
 41. Bochinski D, Lin GT, Nunes L, *et al.* The effect of neural embryonic stem cell therapy in a rat model of cavernosal nerve injury. *BJU Int* 2004; 94: 904–909.
 42. Yiou R, Hamidou L, Birebent B, *et al.* Safety of intracavernous bone marrow-mononuclear cells for postradical prostatectomy erectile dysfunction: an open dose-escalation pilot study. *Eur Urol* 2016; 69: 988–991.
 43. Yiou R, Hamidou L, Birebent B, *et al.* Intracavernous injections of bone marrow mononucleated cells for postradical prostatectomy erectile dysfunction: final results of the INSTIN clinical trial. *Eur Urol Focus* 2017; 3: 643–645.
 44. Haahr MK, Jensen CH, Toyserkani NM, *et al.* Safety and potential effect of a single intracavernous injection of autologous adipose-derived regenerative cells in patients with erectile dysfunction following radical prostatectomy: an open-label phase I clinical trial. *EBioMedicine* 2016; 5: 204–210.
 45. Hooijmans CR, Rovers MM, de Vries RB, *et al.* SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* 2014; 14: 43.
 46. Salanti G, Higgins JPT, Ades AE, *et al.* Evaluation of networks of randomized trials. *Stat Methods Med Res* 2008; 17: 279–301.
 47. White IR, Barrett JK, Jackson D, *et al.* Consistency and inconsistency in network meta-analysis: model estimation using multivariate metaregression. *Res Synth Methods* 2012; 3: 111–125.
 48. Mangır N and Türkeri L. Stem cell therapies in post-prostatectomy erectile dysfunction: a critical review. *Can J Urol* 2017; 24: 8609–8619.
 49. Ficarra V, Novara G, Ahlering TE, *et al.* Systematic review and meta-analysis of studies reporting potency rates after robot-assisted radical prostatectomy. *Eur Urol* 2012; 62: 418–430.
 50. Pardo Y, Guedea F, Aguiló F, *et al.* Quality-of-life impact of primary treatments for localized prostate cancer in patients without hormonal treatment [published correction appears in *J Clin Oncol* 2011; 29: 779]. *J Clin Oncol* 2010; 28: 4687–4696.
 51. Haglind E, Carlsson S, Stranne J, *et al.* Urinary incontinence and erectile dysfunction after robotic versus open radical prostatectomy: a prospective, controlled, nonrandomised trial. *Eur Urol* 2015; 68: 216–225.
 52. Fode M, Ohl DA, Ralph D, *et al.* Penile rehabilitation after radical prostatectomy: what the evidence really says. *BJU Int* 2013; 112: 998–1008.
 53. Albersen M, Kendirci M, Van der Aa F, *et al.* Multipotent stromal cell therapy for cavernous nerve injury-induced erectile dysfunction. *J Sex Med* 2012; 9: 385–403.
 54. Chen S, Liu Z, Tian N, *et al.* Intracoronary transplantation of autologous bone marrow mesenchymal stem cells for ischemic cardiomyopathy due to isolated chronic occluded left anterior descending artery. *J Invasive Cardiol* 2006; 18: 552–556.
 55. Mazzini L, Mareschi K, Ferrero I, *et al.* Mesenchymal stromal cell transplantation in amyotrophic lateral sclerosis: a long-term safety study. *Cytotherapy* 2012; 14: 56–60.
 56. M zes G and Sipoş F. Issues and opportunities of stem cell therapy in autoimmune diseases. *World J Stem Cells* 2019; 11: 212–221.
 57. Ball LM, Bernardo ME, Roelofs H, *et al.* Multiple infusions of mesenchymal stromal cells induce sustained remission in children with steroid-refractory, grade III-IV acute graft-versus-host disease. *Br J Haematol* 2013; 163: 501–509.
 58. Protogerou V, Chrysikos D, Karampelias V, *et al.* Erectile dysfunction treatment using stem cells: a review. *Medicines (Basel)* 2021; 8: 2.
 59. Kokai LE, Marra K and Rubin JP. Adipose stem cells: biology and clinical applications for tissue repair and regeneration. *Transl Res* 2014; 163: 399–408.
 60. Liang X, Ding Y, Zhang Y, *et al.* Paracrine mechanisms of mesenchymal stem cell-based therapy: current status and perspectives. *Cell Transplant* 2014; 23: 1045–1059.

61. Lin CS, Xin Z, Dai J, *et al.* Stem-cell therapy for erectile dysfunction. *Expert Opin Biol Ther* 2013; 13: 1585–1597.
62. Pan F, Zhang J, Liu Y, *et al.* Intracavernosal pressure recording to evaluate erectile function in rodents. *J Vis Exp* 2018; 136: 56798.
63. Shan H, Chen F, Zhang T, *et al.* Stem cell therapy for erectile dysfunction of cavernous nerve injury rats: a systematic review and meta-analysis. *PLoS ONE* 2015; 10: e0121428.
64. Koehler N, Holze S, Gansera L, *et al.* Erectile dysfunction after radical prostatectomy: the impact of nerve-sparing status and surgical approach. *Int J Impot Res* 2012; 24: 155–160.
65. Chu DT, Nguyen TT, Tien NLB, *et al.* Recent progress of stem cell therapy in cancer treatment: molecular mechanisms and potential applications. *Cells* 2020; 9: 563.
66. Afify SM and Seno M. Conversion of stem cells to cancer stem cells: undercurrent of cancer initiation. *Cancers (Basel)* 2019; 11: 345.

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