Stem Cell Therapy for Erectile Dysfunction

Ethan L. Matz, MD,[1,2] Ryan Terlecki, MD,[2] Yuanyuan Zhang, MD, PhD,[1] John Jackson, PhD,[1] and Anthony Atala, MD[1,2]

ABSTRACT

Introduction: The prevalence of erectile dysfunction (ED) is substantial and continues to rise. Current therapeutics for ED consist of oral medications, intracavernosal injections, vacuum erection devices, and penile implants. While such options may manage the disease state, none of these modalities, however, restore function. Stem cell therapy has been evaluated for erectile restoration in animal models. These cells have been derived from multiple tissues, have varied potential, and may function via local engraftment or paracrine signaling. Bone marrow-derived stem cells (BMSC) and adipose-derived stem cells (ASC) have both been used in these models with noteworthy effects.

Aim: Herein, we will review the pathophysiology of ED, animal models, current and novel stem-cell based therapeutics, clinical trials and areas for future research.

Methods: The relevant literature and contemporary data using keywords, "stem cells and erectile dysfunction" was reviewed.

Main outcome measure: Examination of evidence supporting the association between erectile dysfunction and adipose derived stem cells, bone marrow derived stem cells, placental stem cells, urine stem cells and stem cell therapy respectively.

Results: Placental-derived stem cells and urine-derived stem cells possess many similar properties as BMSC and ASC, but the methods of acquisition are favorable. Human clinical trials have already demonstrated successful use of stem cells for improvement of erectile function.

Conclusion: The future of stem cell research is constantly being evaluated, although, the evidence suggests a place for stem cells in erectile dysfunction therapeutics. **Matz EL, Terlecki R, Zhang Y, et al. Stem Cell Therapy for Erectile Dysfunction. Sex Med Rev 2019;7:321–328.**

Key Words: Impotence; Regenerative Medicine; Stem Cell Therapy; Erectile Dysfunction

EPIDEMIOLOGY AND PATHOPHYSIOLOGY

Erectile dysfunction (ED) is defined by the NIH as the inability to maintain a penile erection sufficient for successful penetration.[1] The etiology may be psychogenic, organic, or mixed. Organic ED may be of a neurogenic, vasculogenic, hormonal, intra-cavernosal (ie, structural), or drug-induced origin (Table 1).[16] Despite technical advances in pelvic surgery (eg, radical prostatectomy) and in the management of pelvic trauma, patients experiencing either phenomena are still at considerable risk for consequent ED.[17] Excluding trauma-related cases, 10%–19% of ED is presumed neurogenic.[18]

Data from the late 1990s reported the prevalence of organic ED as 7% in men aged 18–29, 9% for those aged 30–39, 11% for those aged 40–49, and 18% for those aged 50–59 years.[18] In a longitudinal study by Johannes et al,[19] it was estimated that the incidence of ED was 25.9 cases per 1,000 man-years with an age-adjusted higher risk for men with lower education, pelvic injuries, diabetes mellitus (DM), coronary artery disease, and hypertension.[19–22] Increased physical activity is inversely associated with ED, while obesity is correlated with a higher risk. Men without chronic medical conditions, and who engage in healthy behavior have the lowest prevalence of ED.[23] Psychological disorders, such as anxiety and depression, can also threaten erectile function through psychogenic and medication-related pathways.[24]

Physiologically, an erection is achieved through the release of nitric oxide (NO) from parasympathetic nerve terminals. NO causes relaxation of cavernosal smooth muscle leading to increased blood flow.[25,26] Thus, conditions capable of

Received October 14, 2017. Accepted December 3, 2017.

[1]Wake Forest Institute for Regenerative Medicine, Winston Salem, NC, USA;
[2]Department of Urology, Wake Forest Baptist Medical Center, Winston Salem, NC, USA

https://doi.org/10.1016/j.sxmr.2017.12.008

Table 1. Rodent erectile dysfunction models

Clinical condition(s)	Model type	Basic description	References
Pelvic surgery (radical prostatectomy), trauma	Neurogenic or neuro-vascular injuries	CN crush, IPB ligation	2,3
Diabetes mellitus type 2	Diabetogenic	High-fat diet with streptozotocin injection	4,5
Atherosclerosis	Vascular	Bilateral internal iliac artery ligation	6–10
Hypogonadism, low testosterone	Hormonal	Orchiectomy, LH agonist	11,12
Advanced age	Aging	Allowing aging of animals	13–15

CN = cavernous nerve; IPB = internal pudendal bundle; LH = luteinizing hormone.

reducing NO release and/or restricting penile blood flow can result in ED.

CURRENT MANAGEMENT OPTIONS

Management of organic ED involves an arsenal of therapies including phosphodiesterase (PDE) 5 inhibitors (PDE5i), prostaglandin (alprostadil) urethral suppositories, vasoactive intracavernosal injections (ICI), vacuum erection devices, and penile implants.[16,17] Psychosexual therapy also has a valid role, especially in the setting of couples' health. All patients should be counseled toward valuable lifestyle modifications such as smoking cessation, decreased alcohol consumption, weight management, and physical exercise.[16]

Once reversible causes have been addressed, oral PDE5i (eg, sildenafil) are often first line in management. These agents are cyclic guanosine monophosphate analogs and bind to the catalytic site of PDE5, inhibiting hydrolytic activity. Consequently, cyclic guanosine monophosphate levels rise and increase penile blood flow and amplify the neurologic signal for erection.[27] Non-responders or patients deemed ineligible for PDE5i (eg, men taking nitrates) may be offered vasoactive intracavernosal injections (ICI). Alternatively, vacuum erection devices can be considered and involve negative pressure to engorge the penis, coupled with a constriction band to effectively trap blood for a period of minutes so as to allow rigidity perhaps sufficient for penetration.[28] Penile implants have traditionally been reserved for failure of medical therapy. A 3-piece inflatable penile prosthesis is the most widely used design within the United States.[16,29]

STEM CELL THERAPY

Interest in stem cell technology for erectile restoration is increasing. The exact mechanism of benefit, however, remains unclear.[30] Mesenchymal stem cells (MSC), an adult stem cell population, are capable of self-renewal and can differentiate into multiple lineages, including muscle, cartilage, bone, and fat.[31] Mesenchyme, an embryonic connective tissue, is derived from mesoderm. Thus, MSCs can be obtained from the tissues they form (Table 2). MSC-mediated regeneration involves multiple cellular mechanisms. Positive effects from use of these undifferentiated stem cells are not due to cell differentiation and direct integration within target tissues. Rather, immunomodulation is achieved through a paracrine effect whereby secretion of cytokines and growth factors decrease inflammation and promote healing.[48–50] Efficacy of stem cell therapy may potentially be related to acuity. In theory, if reductions in inflammation occur proximate to the inciting event, normal wound healing may be facilitated.

A variety of cell-labeling techniques have been investigated to track the fate of injected stem cells. Examples of tracers include green fluorescent protein; LacZ; 4′,6-diamidino-2-phenylindole; bromodeoxyuridine; fluorescent carbocyanine (fluorescent dye);

Table 2. Stem cells studied in erectile dysfunction rat models

Cell type	Sources	Benefits	Drawbacks	References
BMSC	Trochanteric bone marrow Bx	Autologous, low immunogenic	Small number of cells in Bx, painful	32–34
ASC	Adipose extract (liposuction)	Autologous or xenogenous	Small number of cells, procedural	35–39
PSC	Placental tissue, discarded	Waste source, numerous cells	Potentially immunogenic	33,40,41
USC	Urine samples	Waste source, easily obtained from human and large animals, no enzymatic digestion, cost advantage, sufficient cell clones	Difficult to obtain in rodents due to limited urine production	42–47

ASC = adipose-derived stem cells; BMSC = bone marrow–derived stem cell; Bx = biopsy; PSC = placental-derived stem cells; USC = urine-derived stem cells.

and 5-ethynyl-2-deoxyuridine (EdU).[51] Green fluorescent protein, derived from jellyfish, is a widely used marker but can be difficult to discern due to autofluorescence in mammalian tissue.[35] EdU has the advantage of not requiring tissue manipulation, allowing tissue preservation for histology.[52]

To determine the destination of cells delivered through intracavernosal injection, Fandel et al[53] labeled adipose-derived stem cells (ASC) with 5EdU prior to administration. The cells migrated to the major pelvic ganglion (MPG), notably the site of cavernous nerve (CN) injury. They postulated that CN injury up-regulates stromal cell-derived factor−1, attracting both endogenous stem cells and the injected ASC. This appears promising as ASC have neuro-regenerative effects that may result in recovery of erectile function.[53]

Importantly, MSC express low levels of major histocompatibility complex (MHC) class I and no MHC class II in their undifferentiated state, making them minimally immunogenic.[36,54] This permits the use of either allogeneic or autologous stem cells. Selection of a specific stem cell type is then dictated by matters of practicality, such as ease of retrieval and packaging. Harvest of autologous bone marrow−derived stem cells (BMSC) or ASC involve procedural risks from trochanteric bone marrow biopsy and liposuction, respectively.[55,56] Placental stem cells (PSC), however, are simply acquired given they are considered medical waste after human birth.[32]

Prior work has sought to characterize the immunogenicity of allografted PSC and the optimal growth media. Evaluation of amnion-derived progenitor cells grown in the absence of animal-derived serum found the cells expressed MHC type I, but neither MHC II nor co-stimulatory molecules B7-1 and B7-2 for activation of T cells. In the absence of pre-activated T cells, PSC had an immunomodulatory effect.[37] Work with PSCs treated with interferon gamma demonstrated up-regulation of HLA-DR, an MHC class II cell surface receptor, and moderate T-cell proliferation.[36] Interestingly, BMSC appear more immunomodulatory than PSCs, displaying a better effect against activated T cells.[40]

To determine the best isolation and growth protocol for PSC, Yuan et al[41] isolated these cells using the time-gradient method. PSC were less immunogenic with neither HLA II nor HLA-D-related marker expression, compared to other MSCs. The time gradient method was such that non-adherent cells were re-plated 24 hours after the initial cell seeding. After 48 hours, the non-adherent cells were again seeded into another plate for 72 hours. The attached cells at the 3 time points were harvested and passaged for use.[41]

A current area of interest is the potential bioactivity of stem cell culture media. The so-called "secretome" is a collection of factors (growth factors, cytokines, and chemokines) secreted by cultured stem cells in vitro.[33] The biofactor-rich conditioned media (CM) could represent an effective therapeutic modality. This concept has been examined thoroughly in the context of wound healing. Lee et al[57] examined the secretory factors of endothelial precursor cells derived from human embryonic stem cells. They determined that the CM of these cells accelerated wound healing after both topical treatment and subcutaneous injection. Protein analysis showed that several key factors for tissue repair such as epidermal growth factor, fibroblast growth factor, granulocyte-macrophage colony-stimulating factor, interleukins, platelet-derived growth factor AA, and vascular endothelial growth factor were found in the CM.[57] Additionally, in 2007, Kim et al[38] established that CM from ASC promoted the proliferation and migration of human dermal fibroblasts, causing increased collagen type I secretion. This implied that CM from ASC may be useful in the treatment of wound healing.

Recently, exosomes have been investigated within both cancer research and stem cell−mediated tissue regeneration. Exosomes are phospholipid vesicles that bud from a cell and shuttle proteins, lipids, and nucleic acids to neighboring cells.[58] The paracrine mechanism of stem cell−based therapy is believed to involve secretion of exosomes or trophic factors to recruit resident endogenous stem cells. Signals from stem cell exosomes include microRNA, messenger RNA, growth factors, cytokines, and chemokines, which activate endogenous stem cells to participate in tissue repair.[59] Exosomes generated from stem cells demonstrate mitogenic and reparative properties that decrease scarring, apoptosis, and inflammation, and promote angiogenesis.[2] In a recent study by Chen et al,[58] exosomes isolated from ASC had a positive effect in a diabetogenic ED model in a cell-free experiment.

ANIMAL MODELS

Numerous animal models for ED research have been developed, each according to a different pathophysiology. Most of these are rodent models, but may serve to provide valuable insight into the human condition. Table 1 outlines the variety of ED scenarios in the rat model.

Neurogenic Model

The pudendal nerve is a sensory, autonomic, and motor nerve, carrying signals to and from the genitalia, urethra, and perianal region. A neurogenic ED rat model induced by internal pudendal bundle (IPB) or dual pudendal/CN injury may mimic the clinical scenario of ED consequent to radical prostatectomy or pelvic fracture. The rodent model primarily used in neurogenic ED research was first described by Lue and colleagues[3] at the University of California, San Francisco. In brief, the rat undergoes a midline incision under isoflurane anesthesia. The MPG and CN are exposed on either side of the prostate. A locking needle driver is applied to the CN 5 mm distal to the origin of the CN. A subsequent modification included a combined neurovascular model to simulate major pelvic injury. In this model, the IPB is suture ligated by placing the rat in lithotomy position and making a horizontal perineal incision. The IPB is identified between the ischiocavernosus muscle and the bulbospongiosus

muscle. The IPB is then suture ligated.[60] For protocols involving evaluation of potential therapeutics, pressure is applied to the nerve for 2 minutes prior to intra-cavernosal stem cell injections. To facilitate injections, the penis is exposed and a tourniquet is applied to the base, at the level of the pubic symphysis. Cells are injected at midshaft and the tourniquet is released after 3 minutes.[61]

Post-injury evaluations of erectile function have been well described by Albersen et al.[61] In this model, the MPG and CN were re-exposed at various time points after the original surgery. A 23-gauge butterfly needle was inserted into one of the proximal corpus cavernosa, filled with 250 U/mL of heparin solution, and connected to a pressure transducer to measure intra-cavernosal pressure (ICP). A bipolar hook was attached to the CN and stimulated for 50 seconds to induce erections. Mean arterial pressure (MAP) was recorded using a 23-gauge butterfly needle inserted into the aorta at the level of the iliac bifurcation. Final ICP/MAP ratio was determined for each animal.[60,61] Following ICP/MAP measurements, the animals were sacrificed and the cavernosal tissues were examined by histology, Western blot, and cell cycle assays for presence of endothelial cells, smooth muscle cells, neurocytes, and protein levels of neuronal and endothelial NO synthase (NOS).[6,34,50,53,54,60–63]

Lue's group has proposed using low-energy shock wave (LESW) therapy to treat neurologic injury.[34,50,60] Following application of LESW in a rat neuro-vascular injury model, Li et al[60] described structural recovery of collapsed dorsal penile arteries in the presence of Schwann cell proliferation into the s-phase. The effect of LESW on ED has also been studied in the streptozotocin-induced diabetic model. Following differing durations of LESW, ICP was recorded. It was determined that LESW provided benefit according to a dose-dependent response, with 300 shocks resulting in the most profound effect.[7]

Vascular Model and Atherosclerosis

Blood supply to the penis stems from the penile arteries, arising from the internal pudendal arteries, which are derived from the internal iliac arteries. Venous outflow from the penis is via cavernous venules to the deep and superficial dorsal veins. Vascular pathologies may be arteriogenic, secondary to impaired cavernosal flow, or veno-occlusive.[8] Atherosclerosis and endothelial dysfunction results in decreased NO biosynthesis and availability.[9]

Development of a vascular ED model is more challenging as the pathophysiology in human beings is typically gradual in onset. Vascular trauma has been shown to decrease ICP in the rat model. This involves a midline laparotomy in male rats, ligation of bilateral internal iliac arteries at their origin, and subsequent ICP/MAP measurements.[3] In a subsequent modification, Lee et al[10] followed ICP while progressively ligating vessels through a perineal approach until pressures were minimal or non-existent.[64]

Diabetogenic Model

ED secondary to DM is multi-factorial. Although it involves endothelial dysfunction, it is more complex than that of pure vasculogenic ED as it also incorporates neuropathic damage and reduced availability of NO. Chronic hyperglycemia results in glycation end products and reactive oxygen species that cause endothelial dysfunction and consequent impairment of erectile function.[4]

While multiple rodent models are established for type 1 DM, a type 2 model may be more reflective of the general ED population. Chiou et al[5] developed such a model using male Sprague-Dawley rats and dietary manipulation. 1 Group of rats were fed high-fat diets (58% fat, 25% protein, and 17% carbohydrate) for 2 weeks. Next, the rats were injected with low-dose streptozotocin and maintained their diet. Subsequently, rats with non-fasting plasma glucose of greater than 300 mg/dL were considered diabetic.[5] Albersen et al[11] later undertook a similar study where they fed rats a chow containing 2% cholesterol and 10% lard. After 2 weeks, they received 2 intra-peritoneal (IP) injections of streptozotocin 3 days apart. IP insulin challenge tests were done at 6 and 12 weeks to assess for DM.[11]

Castration Model

The hormonal model can be accomplished medically and/or surgically. In a prior study of surgically castrated rats, 1 subset was subsequently given testosterone and finasteride (a 5-alpha reductase inhibitor), and assessments were made in regard to erectile response, as well as neuronal and endothelial NOS activity.[12] In a rabbit study, Traish et al[65] compared surgically castrated rabbits to those receiving leuprolide acetate (a luteinizing hormone agonist) for 2, 4, and 8 weeks, respectively. ICP, NOS activity, and histologic evaluations were performed.

Aging Model

As life expectancy for men increases, the number desiring restoration of sexual function is likely to increase. The prevalence of ED increases with age from 30.7% in men aged 57–64 years, to 43.3% in those aged 75–85 years, with a linear increase in severity.[13,66]

For the aging model, Lin et al[14] obtained rats younger than 6 weeks, fed them a normal diet, and observed them over time. The rats were sacrificed in equal numbers at young (20 weeks), intermediate (40 weeks), and old (80 weeks) age.[14,15,67] Studies performed at different time points allowed assessment of veno-occlusive disease and the relationship to cavernosal function.

PRE-CLINICAL STUDIES

Numerous animal studies involving administration of various stem cell types (eg, ASC, BMSC) for ED have reported improvement in erectile response. Further attempts have been made to determine which cell type performs best. In 2010, using a rat model, Kendirci et al[39] performed ICI of multi-potent

BMSC activated by antibodies against p75 nerve growth factor receptor. They determined that both the activated and inactivated cells restored erectile function. However, the activated cells produced a more profound effect. It was determined that the p75-activated rat stem cell expressed a higher concentration of ß-fibroblast growth factor. Another rat study involved intracavernosal or IP injection of BMSC after bilateral CN crush injury.[6] Both injection types restored cavernous endothelial and smooth muscle content, penile NOS, and neuro-filament content. Additionally, while both IC and IP injections provided some recovery, IC was significantly (90%−100% of sham-control values) superior to IP.

In 2014, Xu et al[42] compared intra-cavernosal injection of plain (free) ASC to those that had been cultured for 3 days in a hanging drop method (spheroid micro-tissue). They noted significantly greater cell retention from administration of the aggregated ASC clusters. Additionally, injection of the ASC micro-tissues resulted in significantly improved erectile function based on the ratio of ICP/MAP. Recently Lin et al[43] created adipose deprived stem cells (ADSC) magnetized nanoparticles to help maintain the cells within the corpora after injection. They injected these nanoparticles into the corpora of rats and noted retention of the cells in the corpora at 3 days and improved erectile function of the nanoparticles as compared to injected ADSCs.

A population of stem cells can be easily isolated from voided human urine. A small subpopulation of cells isolated from urine samples expresses qualities of multi-potent progenitor cells and has potential for use in tissue engineering.[44–47,68] Urine-derived stem cells (USC) secrete proangiogenic factors that can induce differentiation into endothelial cells. This could prove useful in diabetogenic ED. In a study involving intra-cavernosal injection of USC, there was a significant increase in ICP/MAP ratio although few cells were seen, suggesting mediation via paracrine effect.[69] Some data suggest USC have a higher affinity toward ectodermal cells as compared to mesodermal or endodermal cells.[70]

Stem Cell Clinical Trials

4 Clinical trials with limited participants have been described. Bahk et al[71] injected 1.5×10^7 umbilical MSC into the corpora of 7 ED patients with DM and noted improvement when coupled with oral PDE5i. There were no reported adverse effects of the injections. The International Index of Erectile Function (IIEF)-5, global assessment questionnaire, erection diary, blood glucose diary, and medication dosage were monitored for 9 months. 3 Participants regained morning erections in 1 month, 2 participants achieved erection successful for penetration in conjunction with PDE5i for 6 months, and all but 1 reported increased desire.

Yiou et al[56] administered BMSC in men with ED after radical prostatectomy. 4 Equal groups of patients were given escalating doses of BMSC (2×10^7, 2×10^8, 1×10^9, and 2×10^9, respectively). IIEF-15, erection hardness scale, penile duplex, and penile NO release tests were all used to assess erectile function. Significant improvement was noted in 9 of 12 patients treated in combination with an oral PDE5i. Increased dosages were associated with greater incidence of spontaneous erections. This study reported no serious adverse events.

Haahr et al[55] injected ASC into 17 men with a history of prostatectomy to determine safety and efficacy. 5 Patients had minor adverse events related to liposuction, 2 men has redness or swelling at the injection site, and 1 patient developed a scrotal and penile hematoma. They used IIEF-5 to evaluate erectile function and found 8 of 17 men able to achieve an erection for successful intercourse with no mention of use of oral medications. Post hoc analysis showed an improved response for those men who had regained continence after prostatectomy.

In a study of 8 men with ED, Levy et al[72] injected adult placental-matrix-derived stem cells (unknown cell number) and assessed peak systolic velocity, end-diastolic velocity, stretched penile length, penile width, and erectile function via IIEF-5. 2 Patients at 2 months, and 3 additional patients at 3 months achieved erections for successful intercourse with use of PDE5i. The only measure significantly improved was peak systolic velocity. There were no reported adverse events in this study.

Future of Stem Cells for ED

Many questions remain regarding stem cell therapy for ED. Uncertainties regarding immunogenicity and whether allogeneic or autologous cells should be preferred beckon further clarity. While autologous stem cells would mitigate immunogenic concerns, acquisition is more intensive than banked allogeneic cells. It is possible that some cell types may produce untoward effects. The optimal passage number of the cells may be different based on stem cell type.[73] The optimal cell concentration for injection remains to be determined. It is unknown if dosing should be based on patient weight or a standard concentration relative to the target tissue.[74] Proper dosing schedules have not been established and it will be valuable to determine whether single injections will be adequate, or if multiple injections at distinct time points would prove advantageous. Furthermore, determining the detailed stem-cell-mediated mechanism of therapeutic effect is essential. Determination of whether stem cells may help treat other co-morbid conditions such as Peyronie disease may help produce widespread use.[75]

Clinical efforts will need to be standardized. Prior to implementation of large-scale clinical trials, agreed upon validated measures must be selected. These may involve instruments such as the IIEF or erection hardness scale, as well as quantitative measures such as penile duplex. In addition, access to these therapeutics may be difficult. Kymriah (Novartis, Basel, Switzerland), tisagenlecleucel, which was approved as a cell therapy for acute lymphoblastic leukemia in August 2017 uses the patient's own T cells has a cost of $475,000.[76] Barriers like this may also become evident in the stem cell realm.

Despite the level of evidence and scientific explanations regarding the benefits of stem cell therapy for the variety of maladies stemming from DM and other conditions, the future of stem cell research remains in jeopardy. Ethical concerns have stirred nationwide and global debate that threatens therapeutic applications. Additionally, attempts by some to market therapies in unsound and unscientific ways threatens this field of research. However, PSC and USC may represent valuable alternatives to other cell types. Their abundance, accessibility, and the ability to harvest them in the absence of human harm has led to a consensus regarding safety of use. Future research with PSC, USC, and the CM derived from them may open the door to a multitude of novel therapies. Commercial banking and storage of both cells and CM may provide a reliable source for research and clinical efforts. Further scientific inquiry is necessary to determine the long-term utility of stem cells for ED.

Corresponding Author: Anthony Atala, MD, 391 Technology Way NE, Winston-Salem, NC 27101, USA. Tel: 336-716-5876; Fax: 336-713-7290; E-mail: aatala@wakehealth.edu

Conflict of Interest: The authors report no conflicts of interest.

Funding: None.

STATEMENT OF AUTHORSHIP

Category 1

(a) Conception and Design
Ethan L. Matz; Ryan Terlecki; Yuanyuan Zhang; John Jackson; Anthony Atala

(b) Acquisition of Data
Ethan L. Matz

(c) Analysis and Interpretation of Data
Ethan L. Matz

Category 2

(a) Drafting the Article
Ethan L. Matz; Ryan Terlecki; Yuanyuan Zhang; John Jackson; Anthony Atala

(b) Revising It for Intellectual Content
Ethan L. Matz; Ryan Terlecki; Yuanyuan Zhang; John Jackson; Anthony Atala

Category 3

(a) Final Approval of the Completed Article
Ethan L. Matz; Ryan Terlecki; Yuanyuan Zhang; John Jackson; Anthony Atala

REFERENCES

1. NIH National Cancer Institute. Consensus development conference statement. Impotence. **Int J Impot Res 1992;5:181-284.**
2. Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. **Exp Mol Med 2013;45:e54.**
3. El-Sakka A, Yen TS, Lin CS, Lue TF. Traumatic arteriogenic erectile dysfunction: a rat model. **Int J Impot Res 2001;13:162-171.**
4. Castela Â, Costa C. Molecular mechanisms associated with diabetic endothelial-erectile dysfunction. **Nat Rev Urol 2016;13:266-274.**
5. Chiou W-F, Liu H-K, Juan C-W. Abnormal protein expression in the corpus cavernosum impairs erectile function in type 2 diabetes. **BJU Int 2010;105:674-680.**
6. Ryu JK, Kim DH, Song KM, Yi T, Suh JK, Song SU. Intracavernous delivery of clonal mesenchymal stem cells restores erectile function in a mouse model of cavernous nerve injury. **J Sex Med 2014;11:411-423.**
7. Liu J, Zhou F, Li G-Y, et al. Evaluation of the effect of different doses of low energy shock wave therapy on the erectile function of streptozotocin (STZ)-induced diabetic rats. **Int J Mol Sci 2013;14:10661-10673.**
8. Baumann F, Hehli D, Makaloski V, Schumacher M, Schönhofen H, Diehm N. Erectile dysfunction—overview from a cardiovascular perspective. **Vasa 2017;46:347-353.**
9. Goksu C, Deveer M, Sivrioglu AK, et al. Peripheral atherosclerosis in patients with arterial erectile dysfunction. **Int J Impot Res 2013;26:55-60.**
10. Lee M-C, El-Sakka AI, Graziottin TM, Ho H-C, Lin C-S, Lue TF. The effect of vascular endothelial growth factor on a rat model of traumatic arteriogenic erectile dysfunction. **J Urol 2002;167:761-767.**
11. Albersen M, Lin G, Fandel TM, et al. Functional, metabolic, and morphologic characteristics of a novel rat model of type 2 diabetes-associated erectile dysfunction. **Urology 2011;78:476.e1-476.e8.**
12. Lugg JA, Rajfer J, Gonzalez-Cadavid NF. Dihydrotestosterone is the active androgen in the maintenance of nitric oxide-mediated penile erection in the rat. **Endocrinology 1995;136:1495-1501.**
13. Albersen M, Orabi H, Lue TF. Evaluation and treatment of erectile dysfunction in the aging male: a mini-review. **Gerontology 2011;58:3-14.**
14. Lin JSN, Tsai YS, Lin YM, Lin CS, Chow NH. Age-associated changes in collagen content and its subtypes within rat corpora cavernosa with computerized histomorphometric analysis. **Urology 2001;57:837-842.**
15. Davila HH, Rajfer J, Gonzalez-Cadavid NF. Corporal veno-occlusive dysfunction in aging rats: evaluation by cavernosometry and cavernosography. **Urology 2004;64:1261-1266.**
16. Shamloul R, Ghanem H. Erectile dysfunction. **Lancet 2013;381:153-165.**
17. Mulhall JP, Bella AJ, Briganti A, McCullough A, Brock G. Erectile function rehabilitation in the radical prostatectomy patient. **J Sex Med 2010;7:1687-1698.**
18. Lue TF. Physiology of penile erection and pathophysiology of erectile dysfunction. In: Wein AJ, Kavoussi LR, Partin AW, Peters CA, eds. Campbell-Walsh urology. 26th ed. Philadelphia: Elsevier; 2016. p. 612-642.

19. Johannes CB, Araujo AB, Feldman HA, Derby CA, Kleinman KENP, Kinlay JBMC. Incidence of erectile dysfunction in men 40 to 69 years old: longitudinal results from the Massachusetts Male Aging Study. **J Urol 2000; 163:460-463.**
20. Rosen RC, Wing R, Schneider S, Gendrano N. Epidemiology of erectile dysfunction: the role of medical comorbidities and lifestyle factors. **Urol Clin North Am 2005;32:403-417.**
21. Araujo A, Mohr B, McKinlay J. Changes in sexual function in middle-aged and older men: longitudinal data from the Massachusetts Male Aging Study. **J Am Geriatr Soc 2004; 52:1502-1509.**
22. Inman BA, Sauver JLS, Jacobson DJ, et al. A population-based, longitudinal study of erectile dysfunction and future coronary artery disease. **Mayo Clin Proc 2009;84:108-113.**
23. Bacon CG, Mittleman MA, Kawachi I, Giovannucci E, Glasser DB, Rimm EB. Sexual function in men older than 50 years of age: results from the health professionals follow-up study. **Ann Intern Med 2003;139:161-169.**
24. Quek KF, Sallam AA, Ng CH, Chua CB. Prevalence of sexual problems and its association with social, psychological and physical factors among men in a Malaysian population: a cross-sectional study. **J Sex Med 2008;5:70-76.**
25. Prieto D. Physiological regulation of penile arteries and veins. **Int J Impot Res 2008;20:17-29.**
26. Lue TF. Erectile dysfunction. **N Engl J Med 2000; 342:1802-1813.**
27. Brant WO, Bella AJ, Lue TF. Treatment options for erectile dysfunction. **Endocrinol Metab Clin North Am 2007; 36:465-479.**
28. Hatzimouratidis K, Hatzichristou DG. A comparative review of the options for treatment of erectile dysfunction: which treatment for which patient? **Drugs 2005;65:1621-1650.**
29. Bettocchi C, Palumbo F, Spilotros M, et al. Patient and partner satisfaction after AMS inflatable penile prosthesis implant. **J Sex Med 2010;7:304-309.**
30. Soebadi MA, Moris L, Castiglione F, Weyne E, Albersen M. Advances in stem cell research for the treatment of male sexual dysfunctions. **Curr Opin Urol 2016;26:129-139.**
31. Park JS, Suryaprakash S, Lao Y-H, Leong KW. Engineering mesenchymal stem cells for regenerative medicine and drug delivery. **Methods 2015;84:3-16.**
32. Oliveira MS, Barreto-Filho JB. Placental-derived stem cells: culture, differentiation and challenges. **World J Stem Cells 2015;7:769-775.**
33. Jayaraman P, Nathan P, Vasanthan P, Musa S, Govindasamy V. Stem cells conditioned medium: a new approach to skin wound healing management. **Cell Biol Int 2013;37:1122-1128.**
34. Qiu X, Villalta J, Ferretti L, et al. Effects of intravenous injection of adipose-derived stem cells in a rat model of radiation therapy-induced erectile dysfunction. **J Sex Med 2012; 9:1834-1841.**
35. Billinton N, Knight AW. Seeing the wood through the trees: a review of techniques for distinguishing green fluorescent protein from endogenous autofluorescence. **Anal Biochem 2001;291:175-197.**
36. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. **Exp Hematol 2003;31:890-896.**
37. Banas RA, Trumpower C, Bentlejewski C, Marshall V, Sing G, Zeevi A. Immunogenicity and immunomodulatory effects of amnion-derived multipotent progenitor cells. **Hum Immunol 2008;69:321-328.**
38. Kim W-S, Park B-S, Sung J-H, et al. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. **J Dermatol Sci 2007;48:15-24.**
39. Kendirci M, Trost L, Bakondi B, Whitney MJ, Hellstrom WJG, Spees JL. Transplantation of nonhematopoietic adult bone marrow stem/progenitor cells isolated by p75 nerve growth factor receptor into the penis rescues erectile function in a rat model of cavernous nerve injury. **J Urol 2010;184:1560-1566.**
40. Fazekasova H, Lechler R, Langford K, Lombardi G. Placenta-derived MSCs are partially immunogenic and less immunomodulatory than bone marrow-derived MSCs. **J Tissue Eng Regen Med 2011;5:684-692.**
41. Yuan W, Zong C, Huang Y, et al. Biological, immunological and regenerative characteristics of placenta-derived mesenchymal stem cell isolated using a time-gradient attachment method. **Stem Cell Res 2012;9:110-123.**
42. Xu Y, Guan R, Lei H, et al. Therapeutic potential of adipose-derived stem cells-based micro-tissues in a rat model of postprostatectomy erectile dysfunction. **J Sex Med 2014; 11:2439-2448.**
43. Lin H, Dhanani N, Tseng H, et al. Nanoparticle improved stem cell therapy for erectile dysfunction in a rat model of cavernous nerve injury. **J Urol 2016;195:788-795.**
44. Zhang Y, McNeill E, Tian H, et al. Urine derived cells are a potential source for urological tissue reconstruction. **J Urol 2008;180:2226-2233.**
45. Bodin A, Bharadwaj S, Wu S, Gatenholm P, Atala A, Zhang Y. Tissue-engineered conduit using urine-derived stem cells seeded bacterial cellulose polymer in urinary reconstruction and diversion. **Biomaterials 2010;31:8889-8901.**
46. Wu S, Wang Z, Bharadwaj S, Hodges SJ, Atala A, Zhang Y. Implantation of autologous urine derived stem cells expressing vascular endothelial growth factor for potential use in genitourinary reconstruction. **J Urol 2011;186:640-647.**
47. Bharadwaj S, Liu G, Shi Y, et al. Characterization of urine-derived stem cells obtained from upper urinary tract for use in cell-based urological tissue engineering. **Tissue Eng Part A 2011;17:2123-2132.**
48. Peak TC, Anaissie J, Hellstrom WJG. Current perspectives on stem cell therapy for erectile dysfunction. **Sex Med Rev 2016; 4:247-256.**
49. Lin C-S. Advances in stem cell therapy for the lower urinary tract. **World J Stem Cells 2010;2:1-4.**
50. Xin Z-C, Xu Y-D, Lin G, Lue T, Guo Y-L. Recruiting endogenous stem cells: a novel therapeutic approach for erectile dysfunction. **Asian J Androl 2016;18:10-15.**

51. Lin C-S, Xin Z-C, Wang Z, et al. Stem cell therapy for erectile dysfunction: a critical review. **Stem Cells Dev 2012; 21:343-351.**
52. Salic A, Mitchison TJ. A chemical method for fast and sensitive detection of DNA synthesis in vivo. **Proc Natl Acad Sci U S A 2008;105:2415-2420.**
53. Fandel TM, Albersen M, Lin G, et al. Recruitment of intracavernously injected adipose-derived stem cells to the major pelvic ganglion improves erectile function in a rat model of cavernous nerve injury. **Eur Urol 2012;61:201-210.**
54. Mangir N, Akbal C, Tarcan T, Simsek F, Turkeri L. Mesenchymal stem cell therapy in treatment of erectile dysfunction: autologous or allogeneic cell sources? **Int J Urol 2014; 21:1280-1285.**
55. 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.**
56. 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.**
57. Lee MJ, Kim J, Chung H-M, et al. Enhancement of wound healing by secretory factors of endothelial precursor cells derived from human embryonic stem cells. **Cytotherapy 2011; 13:165-178.**
58. Chen F, Zhang H, Wang Z, et al. Adipose-derived stem cell-derived exosomes ameliorate erectile dysfunction in a rat model of type 2 diabetes. **J Sex Med 2017;14:1084-1094.**
59. Ratajczak MZ, Jadczyk T, Pedziwiatr D, Wojakowski W. New advances in stem cell research: practical implications for regenerative medicine. **Polish Arch Intern Med 2014;124:7-8.**
60. Li H, Matheu MP, Sun F, et al. Low-energy shock wave therapy ameliorates erectile dysfunction in a pelvic neurovascular injuries rat model. **J Sex Med 2016;13:22-32.**
61. 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.**
62. Qiu X, Lin G, Xin Z, et al. Effects of low-energy shockwave therapy on the erectile function and tissue of a diabetic rat model. **J Sex Med 2009;6(Suppl. 6):1-9.**
63. 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.**
64. De Young L, Bella A, Howard J, Brock G. Arteriogenic erectile dysfunction alters protein expression within the cavernosal tissue in an animal model. **J Sex Med 2005;2:199-206.**
65. Traish AM, Munarriz R, O'Connell L, et al. Effects of medical or surgical castration on erectile function in an animal model. **J Androl 2003;24:381-387.**
66. Laumann EO, Waite LJ, Das A. Sexual dysfunction among older adults: prevalence and risk factors from a nationally representative US probability sample of men and women 57–85 years of age. **J Sex Med 2008;5:2300-2311.**
67. Hannan JL, Blaser MC, Pang JJ, Adams SM, Pang SC, Adams MA. Impact of hypertension, aging, and antihypertensive treatment on the morphology of the pudendal artery. **J Sex Med 2011;8:1027-1038.**
68. Guan J, Niu X, Gong F, et al. Biological characteristics of human urine derived stem cells: potential for cell-based therapy in neurology. **Tissue Eng Part A 2014;20:1-36.**
69. Ouyang B, Sun X, Han D, et al. Human urine-derived stem cells alone or genetically-modified with FGF2 improve type 2 diabetic erectile dysfunction in a rat model. **PLoS One 2014; 9:e92825.**
70. Kang HS, Choi SH, Kim BS, et al. Advanced properties of urine derived stem cells compared to adipose tissue derived stem cells in terms of cell proliferation, immune modulation and multi differentiation. **J Korean Med Sci 2015;30:1764-1776.**
71. Bahk JY, Jung JH, Han H, Min SK, Lee YS. Treatment of diabetic impotence with umbilical cord blood stem cell intracavernosal transplant: preliminary report of 7 cases. **Exp Clin Transplant 2010;8:150-160.**
72. Levy J, Marchand M, Iorio L, Cassini W, Zahalsky M. Determining the feasibility of managing erectile dysfunction in humans with placental-derived stem cells. **J Am Osteopath Assoc 2016;116:e1-e5.**
73. Oliveira PH, Boura JS, Abecasis MM, Gimble JM, da Silva CL, Cabral JMS. Impact of hypoxia and long-term cultivation on the genomic stability and mitochondrial performance of ex vivo expanded human stem/stromal cells. **Stem Cell Res 2012;9:225-236.**
74. Liu S, Zhou J, Zhang X, et al. Strategies to optimize adult stem cell therapy for tissue regeneration. **Int J Mol Sci 2016; 17:1-16.**
75. Soebadi MA, Milenkovic U, Weyne E, Castiglione F, Albersen M. Stem cells in male sexual dysfunction: are we getting somewhere? **Sex Med Rev 2017;5:222-235.**
76. Bach PB, Giralt SA, Saltz LB. FDA approval of tisagenlecleucel: promise and complexities of a $475 000 cancer drug. **JAMA 2017;318:1861-1862.**