Stem Cell and Gene-Based Therapy for Erectile Dysfunction

Current Status and Future Needs

Ethan L. Matz, MD, Ryan P. Terlecki, MD*

KEYWORDS

• Mesenchymal stem cells • Gene therapy • Erectile dysfunction • Nitric oxide

KEY POINTS

- Stem cell therapy for erectile dysfunction has been effective in improving intracorporal pressure in animal models; small human trials have pointed toward efficacy.
- Gene therapy for erectile dysfunction aims to increase high expression efficacy for upstream genes in the erectogenic pathway and has promising results in models.
- Stem cell and gene therapy for erectile dysfunction are currently appropriate only within the context of clinical trial and should be used with caution.

INTRODUCTION

The high prevalence of erectile dysfunction (ED) and the associated detriment to quality of life for affected men and their partners underscores the need for effective therapy for this troubling condition.[1] Contemporary algorithms for ED management rely on the on-demand use of self-administered medications or surgical placement of a prosthetic device to offset the disease process and allow for sexual activity.[2] Although the introduction of effective oral and injectable agents for ED were revolutionary breakthroughs, no contemporary treatment options have been shown to restore the natural erectile physiology. Additionally, medications may be contraindicated, intolerable, unaffordable, or ineffective in many instances. Mechanical replacement of function, either through external or internal devices, has proven effective for many men, but remains generally less satisfactory than native function and carries associated risk.[3]

Given the large number of factors deemed contributory to development of ED, behavioral modifications (eg, weight loss, exercise, tobacco cessation) and optimization of conditions such as diabetes, hypertension, hypogonadism, and vascular disease are indicated.[4] Even when patients are compliant and successful with such measures, improvements in erectile function tend to be modest and frequently insufficient to meet the needs of most patients.

The desire for restorative, rather than palliative, management strategies for ED has stimulated interest in gene- and cell-based therapies. Herein, we present a brief overview of the relevant physiology and pathophysiology for these targeted therapies, describe the existing data to support these novel therapeutics, and highlight areas for future research. Therapies such low intensity shock wave therapy and platelet-rich plasma (PRP) are also advertised as regenerative therapies for ED.[5–7] Readers interested in energy-based therapies for ED are Raghav Pai and colleagues' article, "Energy-based Therapies for Erectile Dysfunction: Current and Future Directions," in this issue. We do not address PRP in detail in this report because, to our knowledge at the time of this writing, there is a paucity of published peer-reviewed data on this technology for management of ED.

Department of Urology, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA
* Corresponding author.
E-mail address: rterlecki@wakehealth.edu

Urol Clin N Am ■ (2021) ■–■
https://doi.org/10.1016/j.ucl.2021.06.014

urologic.theclinics.com

INSIGHT FROM PHYSIOLOGY

This article is not designed to provide an exhaustive overview of the complex cascade involved in the male erection. However, several steps are relevant to past and current research aimed at identification and further development of restorative therapy. Because the etiology of ED (in the absence of pure psychogenic dysfunction) can involve hormonal, neuronal, or vascular pathology, the targets of investigative research vary.[8]

In the cavernous arteries and spongy tissues of the penile corpora cavernosa, neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS) produce nitric oxide, which leads to the production of cyclic guanosine monophosphate (cGMP). The downstream effects of cGMP are mediated by a number of cellular pathways, many of which involve calcium, and result in smooth muscle relaxation, corporal dilation, and subsequent venous occlusion, which ultimately produces an erection.[9] Penile flaccidity returns as cGMP is hydrolyzed by phosphodiesterase type 5 (PDE-5).

Penile innervation is principally supplied by the cavernous nerve originating from the pelvic ganglia and containing both sympathetic and parasympathetic fibers.[10] During pelvic surgery (eg, radical prostatectomy), mechanical or thermal nerve injury may produce changes at the peripheral nerve cell body and target organ, resulting in ED.[11] Fortunately, the peripheral nervous system has a higher recovery potential relative to the central nervous system, and neurotrophins have been shown to result in erectile functional recovery in animal models.[12]

A patient's hormonal balance is also relevant to erectile function. In addition to the physical development of the penis and influence on libido, androgens are also essential to maintaining erectile physiology with an influence on the endothelium as well as the smooth muscle and fibroelastic properties of the corpora cavernosa.[13] Animal studies have demonstrated that decreases in testosterone levels result in changes to adrenergic and nonadrenergic receptor density and responsiveness, as well as alterations in nNOS activity.[14,15] Clinical studies have shown the association between testosterone replacement and improved frequency and rigidity of erections.[16] The conversion of testosterone to estradiol also has complex ramifications to erectile function; research on human tissue has shown that estrogen receptors are abundantly located throughout the corpora and around the neurovascular bundle.[17] Negative feedback mediated by estradiol receptors at the level of the brain and pituitary can result in indirect effects by lowering testosterone. However, direct effects are also possible, because exogenous estrogen has also been shown to lower smooth muscle within the corpora, with a corresponding increase in connective tissue.[18] Of note, estradiol levels were notably higher among men with ED attributed to venous leak, with authors speculating that this process was mediated by an influence on venous tone.[19]

The ultimate goal of any restorative ED therapy is to recreate the neurovascular milieu that permits spontaneous erectile responses. Development of a one-size-fits-all solution is complicated by the variety of etiologies. Thus, it is advantageous to study restorative therapies across a variety of models as vascular end-organ damage is distinct from what occurs after neurogenic injury (eg, after prostatectomy).

Numerous small animal (mostly rodent) models have been developed for studying pathophysiology and treatment response in ED. An early model described by Lue and colleagues[20] was based on ligation of the bilateral internal iliac arteries and could be considered a proxy for severe vascular disease. A cavernous nerve crush injury has been used within a rodent model of ED related to prior pelvic surgery; this approach may be used with or without concomitant internal pudendal bundle ligation.[21] Leuprolide acetate and orchiectomy have both been used to model the human condition of ED related to low serum testosterone levels.[22] A rodent model of ED related to diabetes may be achieved by using either streptozocin injection (models type 1 diabetes) or a high-fat diet in rats with or without propensity to develop abnormalities of glucose metabolism (models type 2 diabetes).[23,24] Another simple model for ED involves the use of aged rats that are otherwise healthy.[25,26]

MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSC) are a pluripotent adult stem cell population capable of both self-renewal and differentiation into multiple cell lines.[27] There are a variety of sources of MSC. Bone marrow–derived stem cells (BMSC), adipose-derived stem cells (ASC), amniotic fluid stem cells (AFSC), placental stem cells (PSC), and urine-derived stem cells have all been used in animal and human trials for management of ED and/or impaired penile hemodynamics.

The minimally immunogenic state of MSCs makes them an attractive therapeutic option. MSCs express low amounts of MHC class 1 and no MHC class 2 in their undifferentiated state.[28,29] Thus, autologous or allogenic MSC preparations

seem practical. Banas and colleagues examined the immunogenic characteristics of amnion-derived multipotent progenitor cells.[30] A low level of MHC 1 were seen and no MHC 2 antigens were detected. Additionally, these cells lacked costimulatory molecules B7-1 and B7-2. Cultured peripheral blood mononuclear cells did not react to these AMPs and actually had an immunomodulatory effect when prompted. Of note, MSC cell types seem to differ slightly in their immunogenic profiles. For example, BMSC seem to be more immunomodulatory than PSC.[31]

The mechanism(s) of MSC-induced functional recovery is/are not well-elucidated. Traditionally, 2 schools of thought existed regarding the therapeutic ability of these cells. These schools include cellular differentiation and direct integration into the target tissue and paracrine effects of the delivered MSC producing a more favorable cellular milieu. The total paracrine effect of the MSCs is not well-known, although the composition of the media produced by the cell has been reported.[32] It is likely that MSC functionality is related to paracrine immunomodulation rather than the direct action of the cells or integration of the cells into the existing matrix.[33,34] Cytokine and growth factor release seems to be the most likely fundamental therapeutic mechanism of stem cells in tissue.

Evidence in support of this hypothesis stems from animal studies indicating that intracavernous injection of conditioned media from PSC induces erectile functional recovery in the form of increased intracorporal pressure/mean arterial pressure ratio at a level just below that of the cellular-based product.[32] Improvement from conditioned media (CM) produced from PSC (acellular) and directly from PSC administration (cellular) resulted in functional recovery to 57.1% and 66.6% of AMC groups, respectively.

Multiple cell-labeling experiments have attempted to track the fate of injected stem cells.[35] Fandel and colleagues labeled ASCs with 5-ethynyl-2-deoxyuridine before intracavernosal injection.[36] They produced ED in their model by crushing the cavernous nerve bilaterally. Histology demonstrated a higher concentration of cells in the major pelvic ganglion and cavernous nerve at the site of injury. Under a dual labeling system described by Dou and colleagues,[37] real-time in vivo imaging demonstrated cell migration to the area of injury at the pelvic crush sites and near immediate washout from the corpora. The proposed mechanism for this migration is still debated; however, Fandel and colleagues[36] postulated that stromal cell-derived factor 1 attracted the ASCs to the site of cavernous nerve injury.

Bone marrow was the first reported source of MSC.[38] In 2010, Kendirci and associates[39] performed intracavernosal injections of BMSC activated by antibodies against p75 nerve growth factor receptor and noted improved erectile function in a rat model. Although both activated and unactivated cells seemed to produce a restorative effect, this effect was more pronounced with activated cells. Another study with BMSCs evaluated the outcomes of injecting cells both intracavernosally and intraperitoneally.[40] Both approaches resulted in some degree of erectile functional recovery. Intracavernosal injection, however, was far superior to intraperitoneal injection with return of function equal to 90% to 100% of sham controls. Although efficacious, bone marrow aspiration to procure stem cells is invasive and tends to provide a low cellular yield.[40,41]

Surgically extracted fat may be used to procure ASC. Adipose tissue is ubiquitous and morally acceptable; although its procurement is not without risk, lipoaspiration is generally better tolerated than other means of MSC procurement.[42] Xu and colleagues[43] used ASC to produce microtissues that were injected into rat corpora, with a comparison arm involving injection of free ASC. Both the microtissues and free ASC resulted in erectile functional recovery, with the microtissues producing a significantly better response. Fandel and colleagues[36] described an animal model where ASC were injected intracavernosally in animals undergoing sham surgery and in another arm with bilateral cavernous nerve injury. Significantly more labeled ASC were seen at the major pelvic ganglion and animals with intracavernosal injection of ASC saw a significantly better recovery of erectile function as measured by intracorporal pressure at 7 and 28 days.

MSC from amniotic and placental sources have also shown promise.[31,44] Extraction of MSC from the mature placenta has become the method of choice as amniocentesis has fallen out of favor. Amniotic fluid cells, not from mature placenta, have been shown to produce erectile functional recovery versus control (saline injected animals), as recorded by maximum intracorporal pressure/mean arterial pressure (0.52, 0.26; $P<.05$) in an animal pelvic neurovascular injury model.[45] Gu and colleagues[46] subsequently evaluated PSC, extracted from mature placenta, also demonstrating functional recovery significantly better than controls (68% improved at the 12 week time point as compared with AMC) as demonstrated by intracorporal pressure/mean arterial pressure. Improved penile hemodynamic response in both of these studies was observed at 12 weeks after injection, implying a durable effect.

Urine-derived stem cells, procured from voided urine samples, are attractive given the ease of procurement, ability to forego enzymatic digestion, and a lower cost profile.[47–49] Animal data suggest that urine-derived stem cells may afford some functional recovery in vitro, but in vivo results from animal model studies are lacking.[50]

A diverse mixture of secreted growth factors, cytokines, and chemokines is present within the CM across MSC subtypes.[41] This "secretome" is of interest as a potential therapeutic modality. Lee and colleagues[51] found that the application of CM from human embryonic stem cells accelerated wound healing. Likewise, Kim and colleagues[52] saw similar changes with increased collagen type 1 deposition using CM from ASC. CM from PSC were examined in vitro and in an animal model to determine cytokine make-up and changes in erectile function.[32] Intracavernosal injection of PSC, CM of PSC, serum-free media, and phosphate-buffered saline, with serum-free media and phosphate-buffered saline acting as controls, was performed in an animal model of neurovascular ED and the intracorporal pressure/mean arterial pressure was recorded at 6 weeks. There was an improvement in the intracorporal pressure/mean arterial pressure by 57.1% and 66.6% in CM of PSC and PSC, respectively.

Despite promising data from animal models, human studies of MSC for the management of ED are scant. The vast majority of published studies are fraught with experimental flaws, low numbers of participants, and absence of control groups. Bahk and colleagues[53] injected umbilical MSC intracavernosally across 7 had patients with ED and diabetes. All 7 patients absence of erections, despite medications, for at least 6 months and received intracavernosal injections of 1.5×10^7 human umbilical cord blood stem cells. When coupled with PDE-5 inhibitors, there was rigidity sufficient for penetration in 2 patients. There were improvements in reported outcomes (International Index of Erectile Function [IIEF-5], Sexual encounter profile, Global Assessment Questionnaire, and erection diary) for up to 11 months in 1 patient.

Yiou and colleagues[54] performed intracavernosal injection of BMSC in 12 men with medication-refractory ED after radical prostatectomy at increasing doses (2×10^7, 2×10^8, 1×10^9, and 2×10^9). Erectile function was assessed with IIEF-15, erection hardness scale, and color duplex Doppler ultrasound examination. At 6 months, there were significant improvement in intercourse satisfaction and erectile function components of the IIEF and improvements of the erection hardness scale (2.6 ± 1.1 and 1.3 ± 0.8, respectively; $P = .008$). Outcomes seemed to be somewhat dose dependent.

Haahr and colleagues[55] performed a single intracavernosal injection of ASC into 17 men after radical prostatectomy to examine safety. The amount of ASC was proportional to the amount of lipoaspirant with a mean of 1.4×10^5 per gram of fat, correlating with 8.4 to 37.2 million ASC injected. Five patients reported adverse events related to the lipoaspiration or injection that were all minor and included pain at the procedural sites or penile hematoma. Eight of the 17 men treated recovered their erectile function to a level that permitted penetrative sex.

Finally, Levy and coworkers[56] recruited men with ED for at least 6 months with an IIEF of less than 21. All participants refrained from using PDE-5 inhibitors at the time of study and underwent a 4-week washout if used previously. All 8 men previously required injectable medication for erection. One milliliter of PSC diluted in 2 mL of isotonic saline was prepared, and a single injection of 1.5 mL was injected into the base of each corpus cavernosum. The exact number of cells was unknown owing to the proprietary formulation. Evaluation was performed at 6 weeks, 3 months, and 6 months. At follow-up, 3 men were able to achieve erections without additional medications, 4 required low-dose PDE-5 inhibitors, and 1 required additional injectable therapy.

GENE THERAPY

Gene therapy aims to transform the function of existing cell populations. Despite initial interest, the field has been slow to develop secondary to safety concerns related to gene therapy-related deaths in the late 1990s and early 2000s.[57] Unlike approaches to malignancies and systemic inflammatory diseases, where the goal of genetic therapy is high expression efficiency leading to a robust systemic impact, strategies targeting ED aim for minimal cellular perturbation with minimal systemic effects. Gene therapy may be administered with vectors or without (sometimes known as naked DNA).[57] The vector is the package in which the gene is placed, and these vectors are usually viral.[58] The optimal technique for gene transfer, particularly to the penis, remains unclear; however, the benign nature of ED and the local smooth muscle targets allow for plasmid-free, naked DNA, transfer.

The most prominent gene therapy candidate for treatment of ED is the calcium sensitive potassium channels (Maxi-K), a regulator of intracellular calcium.[59] Results from the first human trial of gene transfer of Maxi-K channels were published in

2006.[60] In this study, a single dose of *h*Maxi-K (human) was injected into the corpora of a small group of men who were monitored for 24 weeks. Three men were treated at each of 3 dosages of 500, 1000, and 5000 μg, and 2 men treated at 7500 μg. No adverse events were reported and no plasmids (evidence of residual genetic material) were detected in the semen of the treated men. There was a trend toward improved erectile function on the IIEF that was maintained at 24 weeks at the 2 higher dosages (5000 μg and 7500 μg). Despite apparent safety and possible efficacy, no subsequent studies have been published on this treatment modality.

The investigation of gene therapy to stimulate alternative molecular signaling pathways has included study of nitric oxide synthase (NOS), which is known to be germane to erectile function in men.[9] After intracorporal injection of an adenoviral vector containing the gene for eNOS, aged rats demonstrated increased levels of eNOS, cGMP, and a higher intracorporal pressure as compared with vehicle alone.[61] Another study placed adenoviral vectors containing the eNOS gene into MSC before also performing intracavernosal injection among aged rats.[62] Treated animals manifested increased levels of eNOS protein, NOS activity, and cGMP. Viral vector-based gene therapy specific to nNOS and inducible NOS may improve erectile function in animal models.[63,64] Penile nNOS was transfected into 5-month and 24-month aged rats. intracorporal pressure was increased in aged rats compared with controls when electroporated penile nNOS was transfected into these animals.[63]

Growth factors such as vascular endothelial growth factor (VEGF), neurotrophin-3, glial cell-derived neurotrophic factor (GDNF), and brain-derived neurotrophic factor have been postulated to enhance erectile function. In a diabetic rat model, Bennett and colleagues[65] examined herpes simplex virus (HSV)–mediated neurotrophin-3 transmission. Injection of HSV-NT3 was compared with injection of the β-galactosidase (Lac-z) gene, acting as a control, inserted into HSV. The researchers noted significantly higher density of NOS-stained neurons and higher intracorporal pressure levels in the neurotrophin-3 group as compared with the Lac-z group.

GDNF is part of the transforming growth factor-β family and has a role in the maintenance of autonomic axons.[66] These neurons are found within the penis and GDNF has a role in their survival and regeneration.[67] Two studies by Kato and colleagues[68,69] examined HSV delivery of GDNF and neurturin (NTN) in an animal model of cavernous nerve injury. intracorporal pressure and histology were evaluated at 2 and 4 weeks after the injection of HSV-GDNF and HSV-NTN around the site of injury. At 4 weeks, the intracorporal pressure was significantly improved (approximately twice as high as control) in groups treated with GDNF and NTN when compared with HSV transfected with green fluorescent protein and lacZ. The HSV-NTN groups were shown to have significantly higher levels of fluorogold positive neurons in the major pelvic ganglion than the control groups.

Studies involving animal models have shown that administration of VEGF after arterial injury better facilitates recovery of erectile function, with increased levels of neuronal nitric oxide among treated participants.[70] Animals treated with VEGF, bovine serum albumin, and phosphate-buffered saline at concentrations of 2 and 4 μg were examined at different times. At 6 weeks, there was a significant improvement in erectile function as noted by increased intracorporal pressure/mean arterial pressure.

Choe and colleagues[71] examined sonic hedgehog (SHH), a known regulator of penile smooth muscle. Their laboratory designed a nanofiber hydrogel capable of injection into the cavernosa aimed at extended release after cavernous nerve injury in an animal model. Rats were injected with SHH concentrations of 6.25 μg per rat (1×) or 2× (n = 5). SHH suppressed apoptosis and preserved smooth muscle by 48% while delivering the SHH to the cavernous nerve and cavernosa in combination preserved smooth muscle at 100%.

Although not gene therapy, injection of MSC or platelet-based therapies have been promoted with hopes of increasing the presence of VEGF and possibly improving the density of healthy vascular channels, but washout remains a major concern and the evidence of benefit is lacking. There are minimal human data examining PRP for ED. One small study addressed the safety profile of using PRP for multiple genitourinary conditions.[72]

CONCERNS AND LIMITATIONS

Ethical concerns exist in regard to stem cell and gene therapies. The appealing nature of autologous restorative therapy across the spectrum of disease, coupled with the slow regulatory process, may tempt providers and patients to pursue or offer therapies with poorly understood benefits and risks.[73] Direct-to-consumer marketing in men's health is increasing, evidenced by the ability to obtain prescription medications without ever meeting a provider.[74] Predatory practices by clinics targeting men with ED have been investigated repeatedly and reported in popular news

outlets.[75] This multibillion dollar per year industry in the United States has led to unevidenced, expensive, and potentially dangerous interventions.[76] The Sexual Medicine Society of North America has warned against the inappropriate use of cell-based and gene therapies. Although the Sexual Medicine Society of North America recognizes the merits of research and "strongly supports the development of novel erectogenic therapies," they also assert that these novel therapeutics should be considered experimental and administration conducted only under research protocols in compliance with institutional review board approval."[77]

It is important to consider the placebo effect, the phenomenon of improvement in symptoms based on patient belief and desire for a positive outcome. The placebo effect is particularly strong with respect to issues in sexuality; participants in the placebo arm of studies on erectogenic therapy often have statistically meaningful improvements in their self-evaluated erectile function.[78] Younger patients, those with milder ED, and men with substantial psychological dimensions to their ED may be particularly susceptible to this and more hence more likely to fall prey to therapies of unclear verified benefit.[79,80] The possibility of a strong placebo effect must be considered in any study involving a novel therapeutic in a small subset of men with no placebo group (eg, as in most of the human studies of gene and stem cell therapy to date).

FUTURE DIRECTIONS

The future direction of cellular therapies must be large-scale human studies. There are very few, if any, large, randomized, placebo-controlled studies in the field of novel therapeutics for ED. Thus, coordinated multicenter studies of adequate power are required. Efforts must establish safety and efficacy, as well as optimal dosing and delivery. Despite the number of animal studies suggesting benefit, more translation to human trials is essential. Comparisons between cellular and cell-free (eg, CM) therapies should continue as the latter may obviate some cost and ethical concerns if deemed satisfactory.

SUMMARY

Stem cell and gene therapy represent a promising area of study relative to developing truly restorative options for erectile function. The data from animal studies are impressive, but larger human trials are necessary. Given existing fears, it seems important that the future of valuable research isn't jeopardized by opportunists. Both studies and clinical interventions should be conducted ethically, with appropriate patient counseling and fully informed consent.

CLINICS CARE POINTS

- Stem cell and gene therapy for Erectile dysfunction is only currently appropriate within the context of clinical trial.
- Patients asking about stem cell and gene therapy for ED should be informed of the current SMSNA position statement.
- Sexual health and urologic providers can consider participating in well run clinical trials if/when they become approved.

DISCLOSURE

R.P. Terlecki: Consultant/Advisory Board/Grant support for Boston Scientific; Grant support for Department of Defense. E.L. Matz: nothing to disclose.

REFERENCES

1. 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(9):1502–9.
2. Burnett A, Nehra A, Breau R, et al. Erectile dysfunction: AUA guideline. 2018.
3. Bettocchi C, Palumbo F, Spilotros M, et al. Patient and partner satisfaction after AMS inflatable penile prosthesis implant. J Sex Med 2010;7(1 PART 1):304–9.
4. Wing RR, Rosen RC, Fava JL, et al. Effects of weight loss intervention on erectile function in older men with type 2 diabetes in the Look AHEAD Trial. J Sex Med 2010;7(1 PART 1):156–65.
5. 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(1):22–32.
6. Man L, Li G. Low-intensity extracorporeal shock wave therapy for erectile dysfunction: a systematic review and meta-analysis. Urology 2018;119:97–103.
7. Qiu X, Lin G, Xin Z, et al. Effects of low-energy shockwave therapy on the erectile function and tissue of a diabetic rat models. J Sex Med 2009;6(SUPPL. 6):1–9.
8. Lue TF. Physiology of penile erection and pathophysiology of erectile dysfunction. In: Alan W Partin. Campbell- Philadelphia, PA:Elsevier; Walsh Urology. 26th edition. 2016. p. 612–42.

9. Lue TF. Erectile Dysfunction. N Engl J Med 2000; 342(24):1802–13.
10. Jung J, Jo HW, Kwon H, et al. Clinical neuroanatomy and neurotransmitter-mediated regulation of penile erection. Int Neurourol J 2014;18(2):58–62.
11. Burnett AL. Erectile function outcomes in the current era of anatomic nerve-sparing radical prostatectomy. Rev Urol 2006;8(2):47–53.
12. Chen KC, Minor TX, Rahman NU, et al. The additive erectile recovery effect of brain-derived neurotrophic factor combined with vascular endothelial growth factor in a rat model of neurogenic impotence. BJU Int 2005;95(7):1077–80.
13. Isidori AM, Buvat J, Corona G, et al. A critical analysis of the role of testosterone in erectile function: from pathophysiology to treatment - A systematic review. Eur Urol 2014;65(1):99–112.
14. Reilly C, Stopper V, Mills T. Androgens modulate the alpha-adrenergic responsiveness of vascular smooth muscle in the corpus cavernosum. J Androl 1997;18(1):26–31.
15. Lugg J, Ng C, Rajfer J, et al. Cavernosal nerve stimulation in the rat reverses castration-induced decrease in penile NOS activity. Am J Physiol Endocrinol Metab 1996;271(2 34–2).
16. Chiang HS, Cho SL, Lin YC, et al. Testosterone gel monotherapy improves sexual function of hypogonadal men mainly through restoring erection: evaluation by IIEF score. Urology 2009;73(4):762–6.
17. Dietrich W, Haitel A, Huber JC, et al. Expression of Estrogen Receptors in Human Corpus Cavernosum and Male Urethra. J Histochem Cytochem 2004; 52(3):355–60.
18. Srilatha B, Adaikan PG. Estrogen and phytoestrogen predispose to erectile dysfunction: do ER-α and ER-β in the cavernosum play a role? Urology 2004; 63(2):382–6.
19. Mancini A, Milardi D, Bianchi A, et al. Increased estradiol levels in venous occlusive disorder: a possible functional mechanism of venous leakage. Int J Impot Res 2005;17(3):239–42.
20. El-Sakka a, Yen TS, Lin CS, et al. Traumatic arteriogenic erectile dysfunction: a rat model. Int J Impot Res 2001;13(3):162–71.
21. 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(6):904–9.
22. 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(3):381–7.
23. Castela Â, Costa C. Molecular mechanisms associated with diabetic endothelial-erectile dysfunction. Nat Rev Urol 2016;13(5):266–74.
24. 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(5): 674–80.
25. Lin JSN, Tsai YS, Lin YM, et al. Age-associated changes in collagen content and its subtypes within rat corpora cavernosa with computerized histomorphometric analysis. Urology 2001;57(4):837–42.
26. Davila HH, Rajfer J, Gonzalez-Cadavid NF. Corporal veno-occlusive dysfunction in aging rats: evaluation by cavernosometry and cavernosography. Urology 2004;64(6):1261–6.
27. Park JS, Suryaprakash S, Lao Y-H, et al. Engineering mesenchymal stem cells for regenerative medicine and drug delivery. Methods 2015;84:3–16.
28. Le Blanc K, Tammik C, Rosendahl K, et al. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 2003;31(10):890–6.
29. Mangir N, Akbal C, Tarcan T, et al. Mesenchymal stem cell therapy in treatment of erectile dysfunction: autologous or allogeneic cell sources? Int J Urol 2014;21(12):1280–5.
30. Banas RA, Trumpower C, Bentlejewski C, et al. Immunogenicity and immunomodulatory effects of amnion-derived multipotent progenitor cells. Hum Immunol 2008;69(6):321–8.
31. Fazekasova H, Lechler R, Langford K, et al. Placenta-derived MSCs are partially immunogenic and less immunomodulatory than bone marrow-derived MSCs. J Tissue Eng Regen Med 2011;5: 684–92.
32. Matz EL, Thakker PU, Gu X, et al. Administration of secretome from human placental stem cell-conditioned media improves recovery of erectile function in the pelvic neurovascular injury model. J Tissue Eng Regen Med 2020;1–9. https://doi.org/10.1002/term.3105.
33. Peak TC, Anaissie J, Hellstrom WJG. Current Perspectives on Stem Cell Therapy for Erectile Dysfunction. Sex Med Rev 2016;4(3):247–56.
34. Lin C-S. Advances in stem cell therapy for the lower urinary tract. World J Stem Cells 2010;2(1):1–4.
35. Lin C-S, Xin Z-C, Wang Z, et al. Stem cell therapy for erectile dysfunction: a critical review. Stem Cells Dev 2012;21(3):343–51.
36. 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(1):201–10.
37. Dou L, Matz EL, Gu X, et al. Non-invasive cell tracking with brighter and red-transferred Luciferase for potential application in stem cell therapy. Cell Transplant 2019;28(12):1542–51.
38. Oliveira MS, Barreto-Filho JB. Placental-derived stem cells: culture, differentiation and challenges. World J Stem Cells 2015;7(4):769–75.
39. Kendirci M, Trost L, Bakondi B, et al. 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(4):1560–6.
40. Ryu JK, Kim DH, Song KM, et al. Intracavernous delivery of clonal mesenchymal stem cells restores erectile function in a mouse model of cavernous nerve injury. J Sex Med 2014;11(2):411–23.
41. Jayaraman P, Nathan P, Vasanthan P, et al. Stem cells conditioned medium: a new approach to skin wound healing management. Cell Biol Int 2013; 37(10):1122–8.
42. 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(10): 3331–40.
43. 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(10):2439–48.
44. 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(2):110–23.
45. Gu X, Shi H, Matz E, et al. Long-term therapeutic effect of cell therapy on improvement in erectile function in a rat model with pelvic neurovascular injury. BJU Int 2019;124(1):145–54.
46. Gu X, Thakker PU, Matz EL, et al. Dynamic Changes in Erectile Function and Histological Architecture After Intracorporal Injection of Human Placental Stem Cells in a Pelvic Neurovascular Injury Rat Model. J Sex Med 2020;17(3):400–11.
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 A 2011;17(15–16): 2123–32.
48. Wu S, Wang Z, Bharadwaj S, et al. Implantation of autologous urine derived stem cells expressing vascular endothelial growth factor for potential use in genitourinary reconstruction. J Urol 2011;186(2): 640–7.
49. Liu G, Wang X, Sun X, et al. The effect of urine-derived stem cells expressing VEGF loaded in collagen hydrogels on myogenesis and innervation following after subcutaneous implantation in nude mice. Biomaterials 2013;34(34):8617–29.
50. Wu S, Liu Y, Bharadwaj S, et al. Human urine-derived stem cells seeded in a modified 3D porous small intestinal submucosa scaffold for urethral tissue engineering. Biomaterials 2011;32(5):1317–26.
51. 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(2):165–78.
52. 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(1):15–24.
53. Bahk JY, Jung JH, Han H, et al. Treatment of diabetic impotence with umbilical cord blood stem cell intracavernosal transplant: preliminary report of 7 cases. Exp Clin Transplant 2010;8(2):150–60.
54. Yiou R, Hamidou L, Birebent B, et al. Safety of intracavernous bone marrow-mononuclear cells for post-radical prostatectomy erectile dysfunction: an open dose-escalation pilot study. Eur Urol 2016;69(6): 988–91.
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;5:204–10.
56. Levy JA, Marchand M, Iorio L, et al. Determining the feasibility of managing erectile dysfunction in humans with placental-derived stem cells. J Am Osteopath Assoc 2016;116(1):e1–5.
57. Melman A, Davies KP. Gene therapy in the management of erectile dysfunction (ED): past, present, and future. ScientificWorldJournal 2009;9:846–54.
58. Melman A, Rojas L, Christ G. Gene transfer for erectile dysfunction: will this novel therapy be accepted by urologists? Curr Opin Urol 2009;19(6):595–600.
59. Christ G, Spray D, Brinkt P. Characterization of K currents in cultured human corporal smooth muscle cells. J Androl 1993;14(5):319–28.
60. Melman A, Bar-Chama N, McCullough A, et al. hMaxi-K gene transfer in males with erectile dysfunction: results of the first human trial. Hum Gene Ther 2006;17(12):1165–76.
61. Champion HC, Bivalacqua TJ, D'Souza FM, et al. Gene transfer of endothelial nitric oxide synthase to the lung of the mouse in vivo. Circ Res 1999;84(12):1422–32.
62. Bivalacqua TJ, Deng W, Kendirci M, et al. Mesenchymal stem cells alone or ex vivo gene modified with endothelial nitric oxide synthase reverse age-associated erectile dysfunction. Am J Physiol Heart Circ Physiol 2007;292(3):1278–90.
63. Magee TR, Ferrini M, Garban HJ, et al. Gene therapy of erectile dysfunction in the rat with penile neuronal nitric oxide synthase. Biol Reprod 2002;67(3):1033–41.
64. Garbán H, Marquez D, Magee T, et al. Cloning of rat and human inducible penile nitric oxide synthase. Application for gene therapy of erectile dysfunction. Biol Reprod 1997;56(4):954–63.
65. Bennett N, Kim J, Wolfe D, et al. Improvement in erectile dysfunction after neurotrophic factor gene therapy in diabetic rats. J Urol 2005;173(5):1820–4.
66. Markus A, Patel TD, Snider WD. Neurotrophic factors and axonal growth. Curr Opin Neurobiol 2002; 3:523–31.

67. Palma CA, Keast JR. Structural effects and potential changes in growth factor signalling in penis-projecting autonomic neurons after axotomy. BMC Neurosci 2006;7:1–12.
68. Kato R, Wolfe D, Coyle CH, et al. Herpes simplex virus vector-mediated delivery of neurturin rescues erectile dysfunction of cavernous nerve injury. Gene Ther 2009;16(1):26–33.
69. Kato R, Wolfe D, Coyle CH, et al. Herpes simplex virus vector-mediated delivery of glial cell line-derived neurotrophic factor rescues erectile dysfunction following cavernous nerve injury. Gene Ther 2007;14(18):1344–52.
70. Lee M-C, El-Sakka AI, Graziottin TM, et al. The effect of vascular endothelial growth factor on a rat model of traumatic arteriogenic erectile dysfunction. J Urol 2002;167(2 Pt 1):761–7.
71. Choe S, Kalmanek E, Bond C, et al. Optimization of Sonic hedgehog delivery to the penis from selfassembling nanofiber hydrogels to preserve penile morphology after cavernous nerve injury. Nanomedicine 2019. https://doi.org/10.1016/j.nano.2019.102033.
72. Matz EL, Pearlman AM, Terlecki RP. Safety and feasibility of platelet rich fibrin matrix injections for treatment of common urologic conditions. Investig Clin Urol 2018;59:61–5.
73. Cui T, Kovell R, Brooks D, et al. A Urologist's Guide to Ingredients Found in Top-Selling Nutraceuticals for Men's Sexual Health. J Sex Med 2015;12(11):2105–17.
74. Shahinyan RH, Amighi A, Carey AN, et al. Direct-to-consumer internet prescription platforms overlook crucial pathology found during traditional office evaluation of young men with erectile dysfunction. Urology 2020. https://doi.org/10.1016/j.urology.2020.03.067.
75. Houman JJ, Eleswarapu SV, Mills JN. Current and future trends in men's health clinics. Transl Androl Urol 2020;9(I):S116–22.
76. Krader CG. Marketing used by men's health clinics is cause for concern. Urol Times 2020.
77. America SMS of N. POSITION STATEMENT : ED Restorative (Regenerative) Therapies (Shock Waves , Autologous Platelet Rich Plasma , and Stem Cells).; 2020.
78. Chen KK, Hsieh JT, Huang ST, et al. ASSESS-3*: a randomised, double-blind, flexible-dose clinical trial of the efficacy and safety of oral sildenafil in the treatment of men with erectile dysfunction in Taiwan. Int J Impot Res 2001;13(4):221–9.
79. Mulhall JP, Carlsson M, Stecher V, et al. Predictors of Erectile Function Normalization in Men With Erectile Dysfunction Treated With Placebo. J Sex Med 2018;15(6):866–72.
80. Stridh A, Pontén M, Arver S, et al. Placebo responses among men with erectile dysfunction enrolled in phosphodiesterase 5 inhibitor trials: a systematic review and meta-analysis. JAMA Netw Open 2020;3(3):e201423.