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### ABSTRACT

**Introduction:** Erectile dysfunction (ED) is a common sexual disorder that affects the lives of millions of male patients and their partners. Various medical and surgical therapies exist, with the most common being oral intake of phosphodiesterase 5 inhibitors. One therapeutic strategy in preclinical development to treat ED is stem cell transplantation.

**Aim:** To examine the studies that have investigated stem cells for the treatment of ED.

**Methods:** A literature review was performed through PubMed focusing on stem cells and ED.

**Main Outcome Measures:** An assessment of different types of stem cells and how they may be applied therapeutically in the treatment of ED.

**Results:** The stem cell types that have been investigated for the treatment of ED include bone marrow–derived mesenchymal, adipose–derived, muscle–derived, testes, urine–derived, neural crest, and endothelial progenitor. Depending on the cell type, research has demonstrated that with transplantation, stem cells exert a paracrine effect on penile tissue, and can differentiate into smooth muscle, endothelium, and neurons.

**Conclusion:** Multiple stem cell lines are currently being studied for their potential to treat ED. To date, stem cells have proven safe and effective in both animal and human models of ED. More research is needed to understand their full therapeutic potential.

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**Key Words:** Stem Cell Therapy; Erectile Dysfunction; Penile Tissue

### INTRODUCTION

Erectile dysfunction (ED) is a sexual disorder defined as the inability to initiate or maintain an erection that is satisfactory for sexual intercourse, impacting the lives of male patients and their partners.<sup>1</sup> It is estimated that at least 30 million men in the United States suffer from ED.<sup>2</sup> The Massachusetts Male Aging Study found that 52% of men between the ages of 40 and 70 have reported some degree of ED.<sup>3</sup> After the age of 70, that percentage grows.

A penile erection is a complex event that requires vascular, neural, endocrine, and psychological inputs. It requires the integration of signals from nerves, endothelium, and smooth muscle; and when this signaling pathway fails, the result is ED.<sup>4</sup> A broad arsenal of medical therapies has been developed for the treatment of ED, with phosphodiesterase 5 inhibitors (PDE5i) being the most commonly prescribed medications due to their

overall efficacy and few contraindications.<sup>5</sup> However, there are patients who do not respond to PDE5i, and still others who do not respond to any form of medical therapy, thus making the penile prosthesis the only viable option.

ED that is refractory to medical therapy is often observed in post-prostatectomy patients, diabetics, and those with veno-occlusive dysfunction due to severe Peyronie's disease. Nerve-sparing radical prostatectomy (RP) can lead to ED in up to 50% of patients, and is the result of damage to the neurovascular bundle or an indirect neuropraxia, all of which leads to hypoxia, fibrosis, and apoptosis of cavernosal nerve cells.<sup>6,7</sup> ED resulting from diabetes mellitus is caused by decreased nerve signaling, endothelial dysfunction, and increased oxidative stress.<sup>8</sup> In Peyronie's disease, an abnormal accumulation of fibrous plaques in the tunica albuginea often leads to penile malformations associated with veno-occlusive ED.<sup>9</sup> To explore the mechanisms and future treatments of these conditions, animal models have been developed to reproduce post-RP, diabetic, age-related and Peyronie's-related ED. One therapeutic strategy in preclinical and early clinical development is stem cell transplantation.

With the advent of stem cell use for the treatment of ED, researchers have evaluated a number of adult stem cells in experimental rat models. These stem cell types include bone

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marrow-derived mesenchymal stem cells (BM-MSCs), adipose-derived stem cells (ADSCs), muscle-derived stem cells (MDSCs), testes-derived stem cells (TDSCs), urine-derived stem cells (UDSCs), neural crest stem cells (NCSCs), and endothelial progenitor stem cells (EPCs).<sup>10</sup> A review of each lineage follows, along with the preclinical studies that have evaluated their use in treating ED. It is important to recognize that the mechanism through which these stem cells exert their effects may differ based on the particular cell lineage and the disease model being used. As such, examining the preclinical studies within the context of the cell type will serve to highlight the differing mechanisms that researchers have utilized to design effective therapies suitable for human trial.

### Bone Marrow-Derived Mesenchymal Stem Cells

BM-MSCs have served as the model cell type in the study of stem cell therapy for ED. This cell line, originally isolated and cultured *in vitro* by Friedenstein, differentiates into a wide range of cell types including osteocytes, chondrocytes, myocytes, adipocytes, and pancreatic islets cells.<sup>11–13</sup> To obtain this stem cell line, the bone marrow must be aspirated. Then the BM-MSCs can be isolated from the hematopoietic stem cells by way of their selective adherence in tissue culture flasks. This cell line in particular has received greater therapeutic interest because of its low immunogenicity. Chamberlain et al demonstrated that BM-MSCs lack costimulatory molecules required for T-cell activation.<sup>11</sup> Another attribute of BM-MSCs is their ability to secrete proteins that promote cellular differentiation and proliferation, as well as modify inflammatory responses through modulation of cytokine production and immune cell suppression.<sup>12</sup>

Fall et al evaluated the effects of intracavernous BM-MSC injection in the bilateral nerve crush (BCNI) rat model for post-prostatectomy ED.<sup>14</sup> They found that treatment decreased the number of cells undergoing apoptosis, increased the speed at which neuronal (nNOS) and endothelial (eNOS) nitric oxide synthase normalized, and partially increased intracavernosal pressures (ICPs) as compared to control. However, despite nNOS recovery, erectile responses remained impaired, most likely because full erectile recovery is dependent not only on nerve regeneration, but also the preservation of cavernosal tissue. There have been other studies that observed similar efficacy of BM-MSC in treating the nerve crush injury rat model.<sup>15–17</sup>

The diabetic rat model also has been used to evaluate BM-MSC therapies for ED. Qui et al transplanted BM-MSCs into the corpora cavernosa of streptozocin-induced diabetic rats. This demonstrated an increase in erectile function, as well as increases in smooth muscle and endothelium cell concentrations, as compared to diabetic control rats.<sup>18</sup> Furthermore, they found that 4 weeks after treatment, only a few stem cells remained, suggesting that a paracrine mechanism was at work. Instead of differentiating and incorporating into the tissue after transplantation, these stem cells acted through the release of signaling molecules, resulting in immunomodulation and inhibition of

fibrosis and apoptosis. In addition to their antiapoptotic effect within the corpora, Sun et al subsequently showed that BM-MSCs are capable of promoting neuroregeneration through the secretion of neurotrophins, specifically brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and vascular endothelial growth factor (VEGF).<sup>19</sup>

### Adipose-Derived Stem Cells

ADSCs have similarly shown considerable therapeutic potential in the treatment of ED. In 2001, Zuk et al performed suction-assisted lipectomy (liposuction) of human adipose tissue to isolate ADSCs.<sup>20</sup> These cells could then be induced to further differentiate *in vitro* into adipogenic, chondrogenic, myogenic, and osteogenic cells. ADSCs also can be harvested from bone marrow, but this is an extremely painful procedure and results in a much smaller yield. Studies have shown that adipose tissue may have up to 500 times more ADSCs than bone marrow, making subcutaneous and visceral fat the most efficient location for ADSC extraction.<sup>21</sup> Studies regarding the exact location of ADSCs within the adipose tissue have led most researchers to believe that the cells reside in a stromal stem cell niche, which can be found within the adipose vasculature.<sup>22</sup> Depending on the cells' environment, differentiating potential includes vascular smooth muscle, endothelial cells, adipose tissue, and a variety of mesenchymal cell lines.<sup>23</sup> Once the adipose tissue is obtained, it must be further digested using a collagenase to isolate the stem cells, resulting in the adipose-derived stromal-vascular fraction (AD-SVF).<sup>24</sup> Specific cellular markers then sort the stem cells.

The cavernosal nerve injury model has allowed researchers to gain insight into how ADSCs may be used to treat post-RP neuropraxia. This model has since been used to better understand the mechanism of action of ADSCs.<sup>25</sup> One month after BCNI and simultaneous treatment with ADSCs, lysed ADSCs (lysate), or control, erectile function and penile tissue were evaluated. Results showed that both ADSCs and lysate led to a significant recovery of erectile function when compared to controls, along with a significantly higher expression of nNOS, increased preservation of smooth muscle, and reduced fibrosis. Since both the ADSCs and the lysed ADSCs exerted similar effects on surrounding tissue, the authors concluded that the mechanism of action was not the previously held notion of cell-cell induction, but rather the release of growth factors and cytokines in a paracrine fashion, similar to that of BM-MSC. In support of this hypothesis, one study showed that after ADSC injection, only a small proportion of stem cells was detectable at 5 weeks, yet improvement in erectile function was observed.<sup>26</sup>

To further clarify the mechanism-driving improvement in erectile function with ADSC, a study using EdU-labeled ADSC tracked cells at different time points after injection in a cavernosal nerve injury model.<sup>27</sup> It was found that stromal cell-derived factor-1 (SDF-1), which acts as a signal for recruitment for ADSCs, was upregulated in the major pelvic ganglion. This showed that cavernosal nerve injury leads to

upregulation of SDF-1, thereby attracting inter cavernously injected stem cells to the major pelvic ganglion to promote neuroregeneration and improvements in the smooth muscle/collagen ratio in the corpus cavernosum. Another study demonstrated that ADSCs produce angiogenic factors, as VEGF-A, hepatocyte growth factor, and angiopoietin-1 were detected.<sup>28</sup> Furthermore, ADSCs that had been cultured in a hypoxic environment showed increased expression of angiogenic cytokines when compared to ADSCs cultured in a normoxic environment.<sup>29</sup>

The use of ADSCs for ED has also been evaluated in an animal model of obesity due to type 2 diabetes.<sup>30</sup> Paragonadal ADSCs were harvested and then injected into the corpora, leading to improvement in erectile function. Elevated levels of nNOS expression were observed in rats treated with ADSC, accompanied by decreased levels of cell apoptosis when compared to controls. Echoing previous trials, a negligible amount of stem cells were detectable at 3 weeks. The use of AD-SVF has similarly been found to promote recovery of erectile function in diabetic mice, as well as in a Peyronie's disease model. Studies show that AD-SVF improves erectile function by stimulating neuroregeneration, restoring endothelial and smooth muscle content, enhancing the release of angiogenic factors, and upregulating eNOS.<sup>31</sup> This has important implications, as AD-SVF is easier and faster to isolate than ADSC, and exhibits less alteration of cellular characteristics.

The therapeutic benefit of ADSC has also been studied in a rat model of Peyronie's disease. While the literature on ADSC for Peyronie's disease predominantly pertains to tissues in the chronic, or "irreversible," stages of fibrosis, few studies also have explored the active/acute phase of Peyronie's disease. In 1 study, erectile function and penile tissue were evaluated 5 weeks after treatment with ADSC in an animal model of Peyronie's disease.<sup>26</sup> The ADSC treatment of rats with Peyronie's disease resulted in an increase in erectile function when compared to controls, which were accompanied by comparatively decreased levels of collagen III. Another study used an identical rat model to examine the use of ADSCs as a preventative option against corporal fibrosis.<sup>32</sup> The study showed that injecting ADSCs at the time of injury (prevention) showed similar improvement in erectile function when compared to conventional methods of stem cell therapy. Increased levels of the antifibrotic enzymes matrix metalloproteinase and decreased levels of the profibrotic enzymes tissue inhibitors of metalloproteinases added insight into the mechanism of action stem cells utilized to reduce corporal fibrosis.

### Muscle-derived Stem Cells

MDSCs also have been evaluated in treating the various disease processes responsible for ED. The use of MDSCs present a number of beneficial effects, such as their active and prolonged proliferation, low immunogenicity, and ability to convert to several cell lineages.<sup>33</sup> MDSCs are isolated from skeletal muscle, specifically from within the basal lamina of myofibers, and are

known to be multipotent, differentiating into myocytes, osteocytes, and adipocytes *in vitro*.<sup>34</sup> Normally MDSCs will not divide unless they are activated by weight-bearing exercises or other trauma to regenerate muscle. To isolate and culture MDSCs, the tissue from a muscle biopsy must be enzymatically degraded. The muscle cell extract is then preplated on collagen-coated flasks containing proliferation medium.<sup>35</sup>

Nolazco et al examined the ability of MDSCs to restore the corpora cavernosal smooth muscle and erectile function in an aged rat model.<sup>36</sup> After implanting stem cells in the corpora of rats, they found that erectile function increased to a level comparable with young adult rats serving as a control. Furthermore, it was confirmed that the stem cells replicated and converted into smooth muscle cells. This was shown by confocal immunofluorescence microscopy for proliferating cell nuclear antigen (PCNA),  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), and smoothelin, and by Western blot for  $\alpha$ -SMA and PCNA.

A similar study by Woo et al evaluated the use of MDSCs for the treatment of ED in the cavernous nerve injury rat model.<sup>37</sup> After harvesting femoral muscle from the rats and performing bilateral cavernous nerve injury, labeled MDSCs were injected into the cavernosa. Four weeks after injection, it was determined through pharmacologically induced erection that the peak ICP raised to near normal levels in those rats treated with stem cells in comparison to control rats, i.e. those rats that only underwent nerve injury. In addition, after harvesting the tissue, researchers were able to identify stem cells still within the cavernosum. From these data, it was postulated that the MDSCs converted into muscle cells or neuronal cells, preventing and possibly reversing the fibrotic effect of nerve crush. This direct conversion stands in contrast to the ADSCs and BM-MSCs, which exert their effect through an indirect, paracrine mechanism on cells.

The neuronal effects of MDSCs in a BCNI model were explored by Kim et al.<sup>38</sup> The stem cells were transfected with a reporter gene at the outset of the experiment and stained with PGP 9.5, a neuronal cell marker after tissue harvesting. As in previous studies, they found that ICP improved over a sham-injected group at the 2- and 4-week intervals. They also found expression of the reporter gene within the cavernosa, indicative of MDSC survival. Furthermore, the density of PGP 9.5 was increased to higher levels than both the sham and the control groups. Kim et al hypothesized that the MDSCs produced neurotrophic factors that suppress Schwann cell death. Supporting this hypothesis, Geuna et al demonstrated overexpression of neuregulin-1 (NRG-1), a neurotrophic factor involved in synaptic plasticity, inside the muscle-vein graft used as a conduit for nerve regeneration.<sup>39</sup>

### Testes-derived Stem Cells

Mammalian testes consist of germ cells and various types of somatic cells, the most functionally important being Leydig and Sertoli cells. Past studies have discovered that stem cells exist within both these germ cell and somatic cell populations. These stem cells

were characterized as being of Leydig or spermatogonial origin, and demonstrated unipotential differentiation capacity.<sup>40,41</sup> Investigators obtained testicular tissue from organ donors and were able to reprogram these spermatogonial stem cells to pluripotency when given appropriate growth factors in the embryonic stem cell medium.<sup>42</sup> More recently, stem cells have been discovered within the testes that do not require reprogramming, but possess the intrinsic ability to differentiate into all 3 germ cell layers under similar conditions as embryonic stem cells.<sup>43</sup>

TDSCs are obtained by a simple biopsy of the testicle. The tissue biopsy is then washed and enzymatically degraded, separating the interstitial cells containing testicular somatic cells and somatic stem cells from the seminiferous tubules. The remaining interstitial cells are collected, filtered, and plated in gelatin-coated flasks and cultured in TDSC-specific medium.<sup>42,44</sup>

TDSCs have been found to have varying cellular biomarkers; this, in part, explains the differing replicative potential in studies. Spermatogonial stem cells, for example, are positive for GPR-125 and GFR $\alpha$ 1.<sup>42</sup> Two stem cell markers recently used to isolate the TDSCs with the highest replicating potential include CD34 and CD73. Choi et al evaluated this cellular subset, finding that CD34- and CD73-positive cells exhibited longer proliferative capacity and greater differentiation potential in cells of adipogenic, osteogenic, neuronal, and pancreatic lineages.<sup>44</sup> As a further beneficial result, these cells did not lead to the formation of teratomas in immunodeficient mice, as was seen in a previous study with stem cells isolated from the testes.<sup>43</sup>

Choi et al also has been the only study to evaluate the use of TDSCs in improving erectile function.<sup>44</sup> By injecting these CD34<sup>+</sup>/CD73<sup>+</sup> stem cells periprostatically into rats where the cavernous nerve had been crushed, they evaluated functional recovery based on nerve stimulation and intracavernous pressure. The researchers found that the mean ICP/MAP ratios were significantly lower in the injury group compared to the sham, and that the group injected with TDSCs exhibited partial recovery. In addition, when compared to a crush group treated with BM-MSc, both stem cell-treated groups demonstrated similar erectile capacities with no significant difference.

### Urine-derived Stem Cells

Researchers began looking at urine as a source of stem cells for use in urological tissue engineering, especially given how collection is convenient and noninvasive. In 2008, Zhang et al described an approach to expand single cells obtained from urine and bladder washes into a population expressing urothelial markers.<sup>45</sup> The urine was collected from mid- and late-stream samples, centrifuged, and cell pellets were plated in cell medium. The resulting cells stained positive for markers of stem/progenitor populations.

Their expression of differential cell markers was corroborated in a study in which UDSCs were successfully stimulated to differentiate into smooth muscle-like cells that expressed  $\alpha$ -smooth muscle actin, desmin, and myosin, and demonstrated

contractile function. In addition, these stem cells were able to differentiate into urothelial-specific cells.<sup>46</sup> They were also capable of extensive expansion and remained genetically stable. The quality of the cells studied was similar to those of biopsy-derived cells.

While the therapeutic potential of UDSC is being explored in the treatment of a number of urinary tract diseases, there has been only 1 preclinical study evaluating the use of UDSC in treating ED.<sup>47</sup> Investigators evaluated the use of either UDSCs or UDSCs genetically modified with FGF2 in treating ED in the type 2 diabetic rat model. They found that the UDSCs expressed MSC markers and secreted proangiogenic growth factors, including VEGF, FGF2, and platelet-derived growth factor. Furthermore, both implanted UDSCs and UDSCs-FGF2 groups displayed a statistically significant increase in the ICP and ICP/MAP ratio, as compared to control animals, after intracavernous injection of apomorphine to stimulate an erection. Interestingly, as has been seen in other studies of other stem cell lineages, a few UDSCs were detected within the implanted sites at the end of the study. There also was increased expression of endothelial and smooth muscle markers within the cavernous tissue, indicating that the UDSC exerted a paracrine effect that increased endothelial expression and smooth muscle content.

### Neural Crest Stem Cells

Neural crest cells are a part of a transient, embryonic structure in the vertebrate that migrate from the dorsal aspect of the neural tube and through embryonic tissues to stop at elected sites where they differentiated into various cell types. These cell types include melanocytes, adrenal chromaffin cells, craniofacial cartilage and bone, smooth muscle, peripheral and enteric neurons, and glia. It has been established that neural crest cells contain pluripotent progenitors, before and during their migration. When isolated in vitro, neural crest cells are heterogeneous in their differentiation potential, with some containing all of the phenotypes in the neural crest progeny, and others giving rise to only a single cell type.<sup>48</sup>

There has only been 1 study evaluating the use of NCSCs for the treatment of ED. Song et al transplanted human NCSCs created by the previously described technique into the rat penile corpus cavernosum and evaluated the histological change in the penile tissue samples 2 weeks after transplantation.<sup>49</sup> The researchers found expression of CD31 and von Willebrand factor protein, both being specific cell-type markers for endothelial cells, in the transplanted NCSCs in penile sections. There was also expression of specific markers for smooth muscle cells, including SMA, desmin, and calponin in the stem cells, indicating that they differentiated into smooth muscle cells in the penile corpus cavernosum. These results indicated that the stem cells, when transplanted into the microenvironment of the cavernosa, were able to respond to growth factors and differentiate into endothelial and smooth muscle cells. With future studies



evaluating not just tissue but erectile function as well, there is hope that NCSCs may effectively treat ED.

### Endothelial Progenitor Stem Cells

Endothelial progenitor stem cells (EPCs) are noteworthy, not only for their potential to treat ED, but for their capacity to act as a marker for endothelial damage and vascular disease. EPCs are derived from the bone marrow and circulate in the blood stream with the capacity to proliferate and differentiate into mature endothelial cells.<sup>50</sup> Investigators have also localized them in the cavernosal sinusoidal endothelial space, serving as a local reservoir for vascular repair.<sup>51</sup> EPC were originally studied as a risk factor for patients with cardiovascular disease, with a reduction in cell number indicating an increased risk for the disease.<sup>52</sup>

The initial study evaluating the relationship between EPCs and ED was conducted by Foresta et al.<sup>53</sup> They found that there was an inverse relationship between the number of CD34<sup>+</sup>/CD133<sup>+</sup> EPCs and ED. Baumhakel and Esposito then substantiated these results.<sup>54,55</sup> Interestingly, Esposito demonstrated that CD34<sup>+</sup>/KDR<sup>+</sup> EPC levels were not only significantly lower in men with ED, but also correlated with the severity of ED.

Only 1 study has actually evaluated the efficacy of EPC in treating ED.<sup>56</sup> Gou et al transfected EPC with VEGF165 and transplanted the stem cells into the corpora of diabetic rats. They found that the ICP increased significantly in the treated group as compared to the control group. Furthermore, histological staining of the tissue revealed neovascularization in the corpora cavernosa. Through the use of fluorescence microscopy, they demonstrated that the EPC survived and differentiated into mature endothelial cells that integrated into the corpora.

### Endogenous Stem Cells

There is now strong evidence to suggest that endogenous stem cells exist within the corpus cavernosum. Upon staining the penile tissue sections of young rat penises, Nolzco et al detected mesenchymal stem cells extensively distributed along the tunica albuginea and interspersed in scattered clusters in the corpora cavernosa.<sup>36</sup> In a more recent study, Lin et al found numerous stem cells in the penis of neonatal rats, distributed in the subtunic and perisinusoidal spaces.<sup>57</sup> Researchers have now begun to explore the possibility of mobilizing endogenous stem cells within the corpora to enhance self-healing.<sup>58</sup> By using low energy extracorporeal shock wave therapy (LESWT), Qiu et al demonstrated an increased number of identifiable stem cells in the penis using a diabetic rat model.<sup>59</sup> Furthermore, this cellular proliferation correlated with improved erectile function, as well as regeneration of nNOS-positive nerves, endothelium, and smooth muscle. Xu et al activated the p38 mitogen-activated protein kinase (MAPK) pathway to stimulate endogenous stem cells.<sup>60</sup> The p38 MAPK pathway is thought to be integral in stem cell activation and differentiation and as such may serve alongside LESWT as another means of activating these endogenous cells.

### Stem Cells with the Greatest Potential

Researchers must answer many more questions before stem cells become a reliable therapeutic option. The first of these questions may be how to define which stem cell population offers the greatest therapeutic potential, provided there is a balance of the associated risks, side effects, and costs of implanting these stem cells. As of yet, no study has directly compared the efficacy of 2 or more stem cell lines in an animal model. However, it is safe to say that there is strong evidence to consider BM-MSCs, ADSCs, and MDSCs as reliable lines. Moreover, the ease with which ADSCs and MDSCs are obtained from patients makes them superior to the BM-MSCs. Going further, UDSCs could possibly offer the least invasive means of recovering stem cells for culture and transplantation. A number of studies using UDSC have highlighted advantages over other adult stem cell lines.<sup>61</sup> As previously mentioned, these cells can be obtained with ease and without ethical controversy, making preclinical studies and future clinical trials much more feasible and cost-effective. Also, isolation of these cells does not require enzymatic digestion. Furthermore, UDSCs have proven to have greater pluripotency and to differentiate with higher efficiency. Studies have shown that UDSCs secrete more angiogenic growth factors and anti-fibrotic cytokines in vitro.<sup>62</sup> However, there is far less evidence as to their efficacy in preclinical trials.

### Delivery Methods of Stem Cells

An examination of the literature shows there are numerous routes to administer the stem cell population. For human trials to prove useful, it will be important to determine the most effective route of administration, particularly as it relates to the cavernous nerve injury model. While most of the previously mentioned studies have utilized intracavernosal injection (ICI), others have transplanted stem cells periprostatically, into the major pelvic ganglion, and onto a scaffold.

Few studies have systematically compared the efficacy of each of these delivery methods. One group used ADSCs to compare ICI delivery with periprostatic delivery in a rat model of cavernous nerve injury.<sup>63</sup> They found that although both methods produced a similar recovery in erectile function, the mechanism underlying this recovery differed significantly. ICI administration principally led to the prevention of corporeal smooth muscle deterioration with less significant nerve regeneration. On the other hand, periprostatic administration led to a greater degree of nerve regeneration compared to that of ICI, with less smooth muscle recovery. These differences must depend, in part, on the different microenvironments that exist within the corporal bodies and periprostatic region/MPG. Whereas apoptosis and fibrosis primarily occur in the cavernous bodies, Wallerian degeneration dominates within the periprostatic region/MPG. Furthermore, it is believed that with periprostatic delivery, which is similar in strategy to delivery of stem cells into the major pelvic ganglion, there is a more direct, paracrine effect of secreted neurotrophins at the site of nerve

injury. It should be noted that their results indicated no further improvement in erectile function when the 2 methods were combined. In agreement with the above findings, an interesting meta-analysis of 12 published pre-clinical trials noted that no significant differences in erectile function were observed using either the ICI or periprostatic approaches.<sup>64</sup>

Implanting the stem cells while fixated to a biocompatible scaffold may offer another means of increased cell survival. Various biomaterials have been used to construct these scaffolds, including an adipose matrix, collagen matrix, vein graft, PLGA membrane, hyaluronic acid-based hydrogel, and alginate gel sponge sheet. Regardless of the specific polymer being used, it is hypothesized that these scaffolds improve cell survival through cellular engraftment into tissue and prevention of the implanted stem cells from migrating outside the injured area. Of particular interest, the lumen of the vein graft is posited to simultaneously act as a barrier to inflammation and serve as a channel that guides the direction of new nerve growth.<sup>65</sup> The previously mentioned meta-analysis found that acellular scaffolds promoted cavernous nerve regeneration but were less effective for smooth muscle cell recovery, possibly explaining why there was no difference in overall erectile function.<sup>64</sup>

### Enhancing the Efficacy of Stem Cells

A number of approaches have been used to enhance the efficacy of transplanted stem cells. Most notably, researchers have employed gene therapy. When used alone, gene therapy raises the possibility of an inflammatory response and random expression of the transgene in other cell types. However, stem cells have the advantage of avoiding this inflammatory response and being able to express the gene over long periods of proliferation in a specific subset of cells.<sup>66</sup> Kim et al injected BM-MSCs transfected with human BDNF and assessed changes in erectile function.<sup>67</sup> In the study, a positive, synergistic effect in erectile function and smooth muscle content was observed, as well as eNOS and nNOS expression. This study underscores the important role of combined stem cell and gene therapy for the treatment of ED and, more specifically, revealed the strong neuromodulatory function of BDNF. Other investigators have further demonstrated the potential synergistic effects of gene transfer with stem cell therapy, finding significant improvement in erectile function after transfecting the *eNOS* and *VEGF* genes in ED rat models.<sup>66,68,69</sup>

Another method that has shown success is the use of hydrogels and cellular scaffolds coated with growth factors to enhance ADSC efficacy. ADSCs, when combined with BDNF-immobilized polylactic-co-glycolic (PLGA) membrane, improved nerve regeneration and erectile function in the cavernous nerve injury rat model when compared to ADSCs or BDNF+PLGA alone.<sup>70</sup> When BDNF/PLGA and ADSCs were combined with a basic fibroblast growth factor (bFGF) hydrogel, erectile function was restored to near normal capacity, even more so than the groups treated with ADSCs and BDNF/PLGA membrane or bFGF alone.<sup>71</sup>

A study performed by Kovanecz et al combined the current treatment strategy for postprostatectomy ED, the daily use of a PDE5i, with stem cells.<sup>72</sup> Specifically, they investigated the effects of sildenafil at low doses, alone or in combination with MDSCs or the nitric oxide donor, molsidomine, in treating veno-occlusive disease in the bilateral cavernous nerve resection rat model. The results of the study indicated that a sustained administration of sildenafil at medium and low doses prevents veno-occlusive disease after nerve resection, and reduces collagen deposition in the corpora cavernosa. When administered in combination with molsidomine, the treatment was no more effective than the drugs alone. Furthermore, when MDSCs were implanted, they too prevented veno-occlusive dysfunction and reduced collagen. However, supplementation with a very low dose of sildenafil did not enhance the effects in comparison to MDSCs administered alone.

Most recently, Lin et al employed magnetic nanoparticles to prevent ADSC migration away from the injury site of a BCNI rat model.<sup>73</sup> These biocompatible magnetic nanoparticles, a technology known as NanoShuttle, can be attached to the cell membranes in a process that does not affect cell proliferation or viability. Researchers can then direct aggregates of stem cells using magnetic forces. In their study, cell tracking showed that magnetized ADSCs were successfully retained in the corpora for up to 3 days, whereas most control ADSCs were washed out by the first day of intracavernous injection. Furthermore, erectile function was improved significantly for those rats treated with magnetized ADSCs, in comparison to all other groups. These results, in conjunction with those of gene therapy, further highlight the advantages of incorporating other biotechnological advances to optimize stem cell delivery and therapeutic efficacy.

### Clinical Trials

At this juncture, there have been few studies evaluating stem cell use in human patients. Bahk et al evaluated the efficacy of transplanting umbilical cord blood stem cells in the corpora cavernosa of seven diabetic patients with ED refractory to medical therapy.<sup>74</sup> These investigators found that morning erections returned in all participants except for 1 by the third month, and by adding a PDE5i before intercourse, 2 subjects were able to achieve penetration and orgasm.

Yiou et al investigated cell therapy with injection of bone marrow mononucleated cells to treat postprostatectomy ED.<sup>75</sup> In this experiment, they injected stem cells into the corporal bodies of 12 postprostatectomy patients who were resistant to all medical therapy. The results were subsequently compared to baseline at 6 and 12 months post-injection. At the primary endpoint, no serious side effects were observed. In addition, they found significant improvements in intercourse satisfaction and erectile function domains of the International Index of Erectile Function-15 and Erection Hardness Scale, and a significantly greater improvement in spontaneous erections with the higher doses. Clinical benefits were associated with improvement of

**Table 1.** Summary of Preclinical Trials Using Stem Cells to Treat Erectile Dysfunction

Study	Animal Model	Stem Cell Therapy	Reference
Fall et al	CNI rat	BM-MSC	14
Kendirci et al	CNI rat	BM-MSC	15
You et al	CNI rat	BM-MSC	16
Ryu et al	CNI rat	BM-MSC	17
Qui et al	Diabetic rat	BM-MSC	18
Sun et al	Diabetic rat	BM-MSC	19
Bivalacqua et al	Aged rat	BM-MSC + eNOS	66
Kim et al	CNI rat	BM-MSC + BDNF	67
Qui et al	Diabetic rat	BM-MSC + VEGF	68
Albersen et al	CNI rat	ADSC vs stem cell lysate	25
Castiglione et al	PD rat	ADSC	26
Fandel et al	CNI rat	Adipose-derived SVF	27
Song et al	CNI rat	Adipose-derived SVF	28
Wang et al	Diabetic rat	ADSC in hypoxic environment	29
Garcia et al	Diabetic rat	ADSC	30
Das et al	Diabetic rat	Adipose-derived SVF	31
Gokce et al	PD rat	ADSC	32
Liu et al	Diabetic rat	ADSC + VEGF	69
Piao et al	CNI rat	ADSC + BDNF/PLGA Membrane	70
Lee et al	CNI rat	ADSC + BDNF/PLGA Membrane + bFGF Hydrogel	71
You et al	CNI rat	ADSC: Perioprostatic Implantation vs Intracavernosal Injection	63
Ying et al	CNI rat	ADSC + Vein Graft	65
Nolazco et al	Aged rat	MDSC	36
Woo et al	CNI rat	MDSC	37
Kim et al	CNI rat	MDSC	38
Kovanecz et al	CNI rat	MDSC + sildenafil	72
Choi et al	CNI rat	TDSC	44
Ouyang et al	Diabetic rat	UDSC + FGF2	47
Song et al	Adult rat	NCSC	49
Gou et al	Diabetic rat	EPSC	56
Qiu et al	Diabetic rat	Endogenous stem cells	59
Xu et al	CNI rat	Endogenous stem cells	60

peak systolic velocity and of percent penile nitric oxide release test and sustained after 1 year. Furthermore, 9 of the 12 patients reported successful intercourse with vaginal penetration while on medication.

A small clinical trial evaluated the use of placental-matrix derived mesenchymal stem cells in 5 patients with ED

secondary to Peyronie's disease.<sup>76</sup> After injection of the stem cell solution into the plaque and at the base of the corporal bodies, patients were followed up at 6-week, 3-month, and 6-month intervals to assess changes in plaque volume, penile curvature, and erectile function status. Their results indicated statistically significant increases in peak systolic velocity at all time points. Of the 10 plaques that were managed, 7 had disappeared completely at the 3-month follow-up. Changes in end-diastolic velocity, stretched penile length, and penile girth were not statistically significant.

According to [clinicaltrials.gov](http://clinicaltrials.gov), there are currently 9 active clinical trials investigating stem cell therapy for the treatment of ED. With growing evidence supporting its safety and efficacy, we anticipate these clinical trials to reveal promising results and more insight into how to effectively utilize this newfound therapy. Furthermore, the ultimate aim of this research is to move the treatment of ED from palliative to curative.

## CONCLUSION

It is evident that stem cells can provide a realistic therapeutic modality for the treatment of ED. The preclinical work using rat models for the various disease processes responsible for ED has enabled researchers to elucidate the mechanisms that underlie their therapeutic potential (Table 1). BM-MSCs and ADSCs have demonstrated a paracrine effect on surrounding smooth muscle, neurons, and endothelium, promoting regeneration. MDSCs, on the other hand, have been shown to improve erectile function through stem cell differentiation and cavernosal tissue incorporation. UDSCs enhance erectile function through a unique paracrine effect, which may offer the most convenient and noninvasive source for future studies. As for TSCs, NCSCs, and EPCs, it is still unclear what specific role they might have in a field dominated by BM-MSCs and ADSCs. The next step, however, will be to find the most efficient means of utilizing gene transfer, growth factors, acellular scaffolds, and even the endogenous stem cells of the penis to create a maximally effective therapy.

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