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REGENERATIVE MEDICINE (A ATALA, SECTION EDITOR)

Adipose Tissue-Derived Stem Cells for the Treatment of Erectile Dysfunction

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Abstract Although a spectrum of options is available for erectile dysfunction (ED) treatment, ED in diabetics, post-prostatectomy patients, and those with Peyronie's disease (PD) may be more severe in degree and less likely to respond to conventional medical therapies. Unfortunately, there have been limited breakthroughs in therapeutic options for severe ED during the past decade. However, one of the more fascinating strategies in preclinical development to treat ED is stem cell transplantation. Depending on the cell type, recent research has demonstrated that with transplantation, these stem cells can exert a paracrine effect on surrounding penile tissues and differentiate into smooth muscle, endothelium, and neurons. Adipose tissue-derived stem cells (ADSCs) have become a valuable resource because of their abundance and ease of isolation. It is evident that ADSCs may provide a realistic, therapeutic modality for the treatment of ED. In this review, we will cover the literature that has evaluated ADSCs in the treatment of ED.

Keywords Erectile dysfunction · Stem cell · Adipose tissue · Erection · eNOS · nNOS

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Introduction

Erectile dysfunction (ED) is a consequence of a number of common medical conditions that influences the lives of sufferers and their partners. ED is defined as the persistent inability to attain and/or maintain an erection sufficient to permit satisfactory sexual performance [1]. Epidemiological data have shown a high prevalence and incidence of ED worldwide. The Massachusetts Male Aging Study (MMAS), the first large community-based study of ED, reported an overall prevalence of ED in 52 % of men aged 40–70 years; specific prevalence for minimal, moderate, and complete ED was 17.2, 25.2, and 9.6 %, respectively [2]. After the age of 70, the percentage grows even larger. ED is caused by either neurogenic, endothelial, or smooth muscle dysfunction. For example, long-standing diabetes leads to endothelial dysfunction, decreased nitrergic nerve signaling, and increased oxidative stress in the penile tissues. This in turn results in decreased NO synthesis and smooth muscle relaxation, as well as cavernosal apoptosis and fibrosis [3]. Post-prostatectomy ED is the result of either direct damage to the neurovascular bundle or an indirect neuropraxia, all of which leads to penile hypoxia, fibrosis, and apoptosis [4, 5]. Peyronie's disease (PD) is characterized by a progressive fibroblastic proliferation of collagenous plaques of the tunica albuginea of the penis [6]. These plaques lead to penile morphological abnormalities and, when ED is present, veno-occlusive disease.

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It is well accepted that phosphodiesterase type 5 inhibitors (PDE5-i) are the first-line treatment option for men with ED. Many other therapies have been developed for the treatment of ED, including intracorporal injections, intraurethral suppositories, vacuum devices, and penile prostheses. Although the majority of ED cases can be treated successfully with PDE5-i, those associated with diabetes mellitus (DM), radical prostatectomy, or PD have poorer results and therefore provide a clinical challenge. Unfortunately, there has been slow progress in the treatment options for severe ED during the past decade. Animal models have been developed to reproduce diabetic, post-prostatectomy, and Peyronie's-related ED, enabling basic researchers to study the disease process and potential therapies. One exciting area in these models is the use of stem cells. Over the past 20 years, researchers have made significant progress in treating various medical conditions with stem cells, and now, this field has extended into the treatment of ED. In this review, we will cover the pertinent studies that have evaluated stem cells in the treatment of ED.

Stem Cells

The field of embryonic stem cell research began in 1981 with the discovery of mouse embryonic stem cells by two independent groups at the University of Cambridge and University of California San Francisco [7, 8]. In 1998, the Thomson group from the University of Wisconsin reported on human embryonic stem cells [9]. Next, in 2006, Takahashi and Yamanaka of Kyoto University revealed a method for reprogramming differentiated adult stem cells to behave as embryonic-like stem cells, and in turn, these altered cells were termed induced pluripotent stem cells [10]. The field of stem cell research has since expanded exponentially and has provided prodigious insight into disease processes and their potential treatments.

Stem Cell Populations

Stem cells possess the ability to self-renew and differentiate into defined cell types. Generally speaking, stem cells are divided into those derived from the early embryo, called the embryonic stem cells (ESC), and those derived from adult tissue, termed adult stem cells (ASC). There are two main advantages of ESCs versus ASCs, the first being their ability to proliferate for longer periods of time and the second being their ability to differentiate into a broader range of cell types [11]. However, due to the ethical conflict that surrounds ESCs, their use in preclinical and clinical research has been limited and, as such, is not considered a reliable, therapeutic approach in the near future. Therefore, ASCs have become the main focus of current research, and recent discoveries have shown a greater potential for self-renewal and differentiation in this cell type than initially projected.

It is important to understand that beyond their pluripotency, self-renewal, and differentiation capacity, stem cells are defined by specific cell surface markers and transcription factors. For example, surface markers for ESCs include stage-specific embryonic antigens 3 and 4 (SSEA-3, SSEA-4) and tumor rejection antigens 1–60 and 1–81 (TRA-1-60, TRA-1-81). The first ASCs discovered were hematopoietic stem cells (HSC) derived from bone marrow. This was followed by the detection of mesenchymal stem cells (MSC). MSCs are multipotent stem cells that can be found in bone marrow, adipose tissue, umbilical cord blood, placenta, and dental pulp. Like ESCs and HSCSs, MSCs express several cell markers, including CD105, CD73, stromal antigen 1 (stro-1), CD44, CD166, CD54/CD102, and CD49; they lack the expression of surface markers that are characteristic of HSCs, such as CD14, CD34, CD45, CD11a, and CD31. Researchers now recognize that many more organs within the human body possess a population of ASCs that can serve to replace damaged and injured tissues [11]. Over the years, investigators have discovered these populations in cardiac tissue, adipose tissue, skeletal muscle, testicular cells, endothelium, intestine, brain, and even urine.

The Stem Cell Niche

Exactly how these stem cells interact with their tissue environment and exert their proliferative effects has become a topic of major interest. Researchers now understand that these interactions occur within a cell-specific niche within the tissue. The stem cell niche serves as the microenvironment in which the above-mentioned multi- and pluripotent stem cells reside (Fig. 1) [12].

Stem Cell Niches are divided into two classes: the stromal niche and the epithelial niche. The stromal niche is defined by localized anatomical locations that contain specialized support cells that maintain stem cell activity. Cell adhesion and extracellular matrix molecules anchor stem cells to the niche and allow for efficient communication between cells through secreted, soluble cytokines, and growth factors [13•]. An example that has become increasingly important today is the vascular wall, which has been shown to contain adipose-derived stem cells (ADSCs), HSCs, neural stem cells, intestinal stem cells, and testicular stem cells [14]. In contrast, the epithelial niche does not contain this system of support cells, but instead lies at the basement membrane and directly adjacent to mature progeny [13•]. An example of the epithelial niche would be muscle-derived stem cells. An understanding of these niches is important for isolating and culturing stem cells for implantation into diseased tissue. The process of isolation and culture differs for each stem cell type. All stem cells must be cultured in a specific stem cell medium, and then flow cytometry and

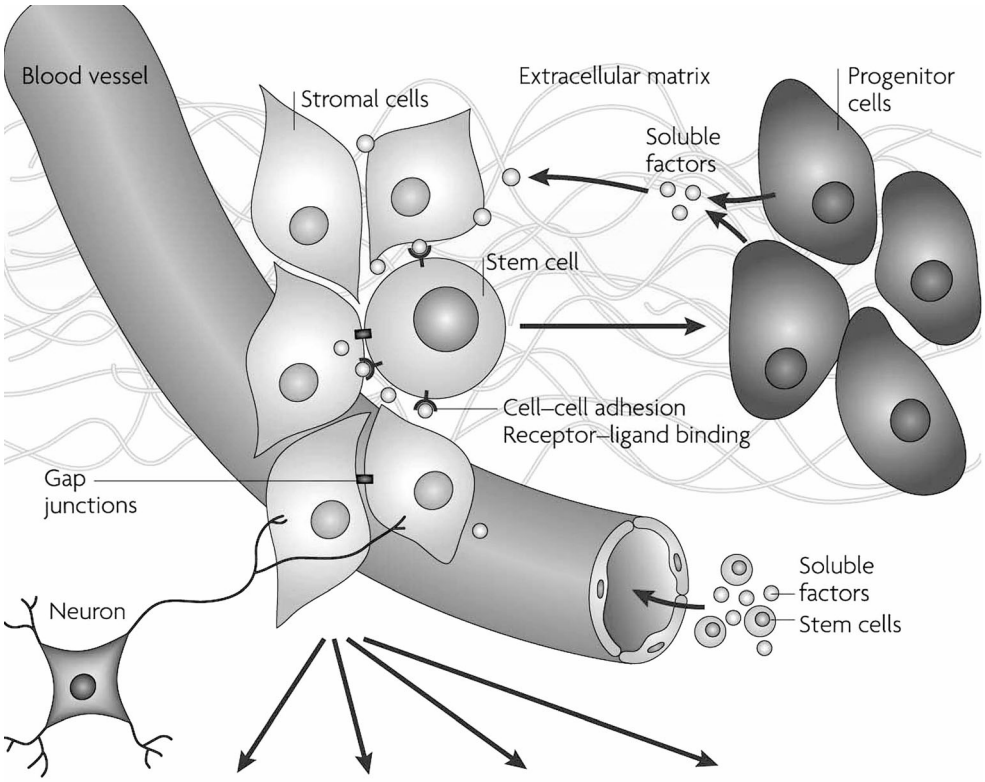

Fig. 1 The stem cell niche represents the microenvironment in which stem cells are able to secrete and respond to soluble factors in order to regulate cell proliferation and growth (reprinted with permission [12])

immunohistochemistry are used to detect the cell surface markers specific to that population.

With the advent of stem cell use for the treatment of ED, researchers have evaluated a number of ASCs in experimental animal models. These stem cell types include bone marrow-derived mesenchymal stem cells, ADSCs, muscle-derived stem cells, testicular stem cells, urine-derived stem cells, neural crest stem cells, and endothelial progenitor stem cells [15].

Adipose-Derived Stem Cells

Adipose-derived stem cells (ADSCs) and their regenerative potentials were discovered in 2001 by Zuk et al. [16••]. By performing suction-assisted lipectomy (liposuction) of human adipose tissue under general anesthesia, the group was able to isolate and culture cells that differentiated in vitro into adipogenic, chondrogenic, myogenic, and osteogenic cells in the presence of induction factors. While ADSCs can also be obtained from within the bone marrow, this is an extremely painful procedure that yields few cells. In fact, the frequency of these cells in adipose tissue is 100- to 500-fold higher than in bone marrow [17]. Therefore, subcutaneous and visceral fat are the simple and most practical locations to obtain these cells. As demonstrated by Zuk et al. and others, subcutaneous and visceral fat extraction allows for a safe, minimally invasive surgical procedure with a larger tissue sample [18].

Despite having proven the existence of ADSCs, identifying exactly where in this adipose tissue they reside has been more difficult. We now know that they reside in a stromal stem cell niche. More specifically, research has revealed that they are located within a microenvironment of the adipose vasculature [19]. Based on experimental results, it is believed that ADSCs exist in adipose tissue as a population of vascular stem cells that vary in their differentiating potential. Depending on the specific environment in which these cells are located, their potential ranges from vascular smooth muscle and endothelial cells to adipose tissue and the other mesenchymal cell types [20••].

Once obtained, the adipose tissue must be digested using a collagenase to dissolve the structural components of the tissue, leaving the stem cells, also known as the stromal-vascular fraction (SVF) [21]. Afterwards, the stem cells must be sorted based their specific, cellular markers. Defining those cellular markers has been difficult because of the differing differentiation potentials within a sample of these stem cells. As such, different studies have isolated ADSCs of varying potential and reactivity. Characteristic markers that are often used for sorting include CD29, CD34, CD73, CD90, and CD105 [22]. To illustrate that their cellular pluripotency exceeds that of most other stem cell types, ADSCs have been cultured in various media to stimulate their differentiation into endothelial, smooth muscle, and neuronal cells [23–25].

ADSCs Treatment for ED

ADSCs have shown great regenerative potential in preclinical experiments treating ED (Table 1). ADSCs have been documented to improve erectile function after crush injury to the

Table 1 Preclinical trials using ADSCs to treat erectile dysfunction

Study	Animal model	Stem cell therapy	Reference
Albersen et al.	CNI rat	ADSC vs stem cell lysate	[26•]
Fandel et al.	CNI rat	Adipose-derived SVF	[27•]
Song et al.	CNI rat	Adipose-derived SVF	[28]
Castiglione et al.	PD rat	ADSC	[29•]
Gokce et al.	PD rat	ADSC	[31]
Gokce et al.	PD rat	ADSC vs genetically modified ADSCs with IFN α-2b	[32]
Ma et al.	ED rat	ADSC-seeded SIS	[33]
Qui et al.	XRT rat	ADSC	[34]
Garcia et al.	Diabetic rat	ADSC	[35]
Das et al.	Diabetic rat	Adipose-derived SVF	[36]
Wang et al.	Diabetic rat	ADSC in hypoxic environment	[37]
Liu et al.	Diabetic rat	ADSC + VEGF	[38]
Piao et al.	CNI rat	ADSC + BDNF/PGLA membrane	[39]
Lee et al.	CNI Rat	ADSC + BDNF/PLGA membrane + bFGF hydrogel	[40]

cavernous nerves, serving as a model for post-prostatectomy neuropraxia. Albersen et al. used this model in one particular study to demonstrate the effects and identify the mechanisms of action of ADSCs and ADSC-derived lysate [26•]. Four weeks after bilateral cavernous nerve crush and treatment with ADSCs, stem cell lysate, or vehicle, erectile function was evaluated and penile tissue was collected for histology. These researchers observed that both ADSCs and lysate treatments resulted in significant recovery of erectile function, as compared to vehicle treatment. In addition, there was a significantly higher expression of nNOS, increased preservation of smooth muscle content, and less fibrosis in these treatment groups, as compared to control. The fact that the lysate produced comparable results as ADSCs supports the argument that it is not the incorporation of stem cells into the native tissue that promotes growth and regeneration, but is in fact the release of soluble factors contained in the stem cells exerting a paracrine effect.

Fandel et al. evaluated whether autologous adipose tissue-derived SVF therapy could replicate the effects of autologous ADSC treatment to improve on erectile recovery following cavernous nerve crush [27•]. nNOS positivity was significantly greater in ADSC and SVF-treated animals in comparison to control animals. However, there was no significant difference in nNOS positivity or ICP/MAP ratio between ADSC and SVF-treated animals. Based on these results, it was suggested that the positive neuronal response involved regeneration of nNOS containing nerve fibers. To further clarify the mechanism behind the ADSC-dependent improvement in erectile function, these investigators used EdU-labeled ADSCs and tracked these cells at different time points. They observed that stromal cell-derived factor-1 (SDF-1) was upregulated in the major pelvic ganglion (MPG), providing a signal for ADSC recruitment toward the MPG. Neuroregeneration was observed in the group that received ADSCs, along with beneficial effects on the smooth muscle/collagen ratio in the corporus cavernosum.

In conjunction with neurotrophic factors observed above, Song et al. evaluated the production of angiogenic factors as a component in the restoration of erectile function after cavernous nerve injury. Two weeks after nerve crush and intracavernous injection of SVF, they noted a dose-dependent response in the nerve-stimulated erectile response. Furthermore, they found increased expression of angiogenic factor proteins, including angiopoietin-1 (Ang-1), vascular endothelial growth factor-A (VEGF-A), and hepatocyte growth factor [28].

Castiglione et al. evaluated the use of intratunical injection of human ADSCs to prevent fibrosis and improve erectile function in a rat model of PD [29•]. The team evaluated the prevention of the acute, inflammatory phase of PD, which contrasted with previous studies that had only examined replacing irreversibly damaged tissue. Five weeks post-treatment, erectile function was evaluated, and the penile tissue was harvested and analyzed for protein expression. The results indicated that the PD group treated with stem cells had statistically significant increases in ΔICP, ICP/MAP, and tumescence slopes in comparison to the PD group with no treatment. Furthermore, Western blot analysis revealed that the increased collagen III and elastin found in the PD group was reduced in the group treated with ADSC. Finally, a small proportion of stem cells was detected at 5 weeks, supporting the hypothesis that there is a paracrine mechanism of action causing the above findings. Additional studies have provided further support for this paracrine mechanism. For example, it was discovered that when cultured in vitro, stem cells release cytokines and growth factors into their environment, similarly to stem cells injected in vivo, suggesting the use of stem cell conditional media as a treatment option [26•, 30].

Gokce et al. used the same rat model to evaluate the use of ADSCs as a preventative measure and treatment strategy [31]. In conjunction with previous studies, Gokce et al. observed that both therapeutic strategies were effective at increasing ICP and ICP/MAP values. Clarifying the possible molecular mechanism of these beneficial effects, they also found increased levels of antifibrotic enzymes, matrix metalloproteinases (MMP), and decreased levels of the profibrotic enzymes, tissue inhibitors of metalloproteinases (TIMP). This provides evidence for the mechanism behind the decreased collagen and elastin found by Castiglione. In a very recent study, Gokce et al. also reported that transplantation of genetically modified ADSCs with or without IFN α-2b attenuated Peyronie's like changes and enhanced erectile function in a rat model of PD. It appears that the penile application of ADSCs has a potential role in preventing the formation of Peyronie's plaque or at least limiting the natural progression of PD [32]. In another study, it was demonstrated that seeding syngeneic ADSCs onto porcine small intestinal submucosal (SIS) grafts resulted in significant cavernosal tissue preservation and maintenance of a normal erectile function [33]. SIS grafting alone induced transcriptional upregulation of inducible NOS and downregulation of eNOS, nNOS, and VEGF, an effect that was restored by seeding ADCSs on the SIS graft. The results of this study further support the concept of regeneration of damaged tunica albuginea as a treatment option for PD.

Radiation therapy for prostate cancer is another treatment modality recognized to cause significant ED. ADSCs may serve to ameliorate ED in this patient population. Qui et al. investigated the efficacy of using ADSCs to enhance erectile function in rats with radiation-induced ED [34]. As expected, they noted that radiation of the prostate significantly diminished in erectile function, expression of nNOS, and cavernous smooth muscle content. However, when irradiated rats were injected with ADSC, erectile function was restored, as well as nNOS expression, and smooth muscle content.

Garcia et al. evaluated the use of ADSCs to treat ED in the obese type 2 diabetic rat model [35]. By harvesting paragonadal adipose tissue for isolation and culture, and injecting autologous stem cells into the corpora of the rat penis, these researchers noted improved erectile function and decreased cell apoptosis in the treated group, as compared to control. Furthermore, nNOS levels were elevated and more diffuse in those rats treated with ADSC. As in previous studies, only a small population of stem cells was visible within the tissues at 3 weeks, emphasizing the concept of the paracrine release of cytokines and growth factors.

Transplantation of human adipose-derived SVF has also been found to enhance recovery of erectile function in diabetic mice. It has been shown that SVF improves erectile function by restoring endothelial and smooth muscle content, enhancing the release of angiogenic factors, stimulating phosphorylation of eNOS, and restoring penile nerve fibers [36]. Interestingly, ADSCs that had been preconditioned in a hypoxic environment induced increased expression of angiogenic cytokines and improved erections in rats, as compared to ADSCs cultured in a normoxic environment [37].

Researchers have employed other strategies to enhance the efficacy of ADSCs, just as in bone marrow-derived mesenchymal stem cells (BM-MSCs). One in particular that has proven to be effective in the experimental setting is the use of genetic engineering in stem cell-based therapy. Liu et al. transfected ADSCs with the VEGF gene and implanted them into the diabetic rat model of ED [38]. Their results revealed a significantly improved erectile response with the use of stem cells with targeted gene expression, as compared to using either therapy alone. Furthermore, Liu et al. demonstrated increased expression of endothelial, smooth muscle, and pericyte markers in the combination therapy group as compared to either therapy alone. This study demonstrates that combined therapy better restores the VEGF signaling pathway, as well as endothelial and smooth muscle function, required for the erectile mechanism.

Another strategy that has been used to enhance ADSCs is the use of hydrogels and biodegradable membranes that are coated with growth factors. Piao et al. evaluated the efficacy of ADSC and brain-derived neurotrophic factor (BDNF) immobilized poly-lactic-co-glycolic (PLGA) membrane in the cavernous nerve injury rat model [39]. These researchers noted improved nerve regeneration and erectile function when using PLGA membrane as compared to groups treated with either ADSC or the BDNF/PLGA membrane alone. Piao et al. then combined the BDNF/PLGA membrane and ADSC with a basic fibroblast growth factor (bFGF) hydrogel [40]. bFGF is known to enhance smooth muscle growth and recovery, and as such would be effective in treating ED. The results demonstrated that this treatment strategy improved erectile function to near normal capacity, significantly more so than the groups treated with ADSC +BDNF/PLGA membrane or bFGF alone.

At present, ADSCs is the only cell type that can be isolated and autologously transplanted on a same-day basis. Additionally, many devices for automated isolation of ADSCs are available commercially. Thus, in terms of cost, risks, ethics, expediency, and effectiveness, ADSCs compete very favorably with the alternatives [41].

Conclusion

It is evident that ADSCs provide a realistic, therapeutic modality for the future treatment of ED. The preclinical works using animal models for the various disease processes responsible for ED have provided evidence supporting stem cell differentiation and cavernosal tissue incorporation. With more investigations supporting stem cell safety and efficacy, human

clinical trials for the treatment of ED are anticipated in the near future.

Compliance with ethical standards

Conflict of Interest Ahmet Gokce, Taylor C. Peak, and Asim B. Abdel-Mageed each declare no potential conflicts of interest.

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- • Of importance
- •• Of major importance

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