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Review

Stem cell assisted low-intensity shockwave for erectile dysfunction treatment: Current perspective

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ABSTRACT

Stem cell therapy and low-intensity extracorporeal shockwave (LI-ECSW) are recognized as potential restorative therapies and have been used in the treatment of erectile dysfunction (ED). Stem cell therapy is well-known due to its attributed regenerative ability and thus can help to improve erectile function in patients with vasculogenic ED. Besides, current evidence also shows that LI-ECSW therapy can help stimulate cell recruitment and proliferation and promote angiogenesis and vascularization in the damaged tissue. Hence, due to the therapeutic and restorative effects of both therapies, the success of ED treatment can be elevated through a combination therapy between stem cell therapy and LI-ECSW. In this review, a detailed description and efficacy discussion of combination therapies between different types of stem cells and LI-ECSW therapy are described. Besides, other potential cell types to use together with LI-ECSW are also listed in this review. Thus, this review provides better insight on the efficacy of combination therapy for ED treatment.

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1. Introduction

Erectile dysfunction (ED) refers to a medical condition of inability to achieve or maintain an erection sufficient for satisfactory sexual intercourse [1]. Globally, the prevalence of ED ranges from 3 % to 76.5 %. The condition becomes more prevalent with increasing age. However, it may also be related to common comorbidities such as diabetes mellitus, obesity, cardiovascular disease and hypertension or post-surgery complication like in the case of radical prostatectomy [2]. Effective treatment for ED is crucial since it has significant negative impact on the relationship of men and their life partners.

Initial treatment for ED is oral medication of phosphodiesterase type-5 inhibitors (PDE5i) and intracavernosal injections [3]. Although these treatment options show good efficacy among men with mild to moderate ED, there are still a handful of patients with hard-to-treat ED who are either unresponsive or cannot tolerate these medications [4]. Besides, side effects such as headaches, blushing and hearing impairment are reported for PDE5i medication, whereas pain, priapism and corpora cavernosa fibrosis are reported for intracavernosal injections [5]. For the group of patients with problems of unresponsive or refractory medication, surgical option in the form of penile prosthesis is recommended. Although penile prosthesis surgery has high patient satisfaction, it is invasive and several complications like penile pain and nerve injury have been reported [6]. With the suboptimal outcomes reported following these treatments, there is increasing demand in search of a less invasive therapy option that focuses on restoring spontaneous physiologic erection.

Restorative therapies, which are based on the concept of repairing or replacing diseased tissue by stimulating endogenous regenerative capabilities, have become an alternative option for ED treatment. Unlike oral medication and surgery that just focus on addressing disease symptoms, restorative therapies aim to restore the structure and function of damaged erectile tissue, which tends to be the core issue of ED. Some examples of restorative therapies include platelet rich plasma (PRP), stem cell therapy and low-intensity extracorporeal shockwave therapy (LI-ECSW) [7]. Although PRP has been shown to contain various growth factors such as platelet-derived growth factor (PDGF), insulin-like growth factor (ILGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF) that can induce mitogenesis and neoangiogenesis to reconstitute damaged tissue [8], it is still poorly studied. There are limited attempts made to investigate the efficacy of PRP on ED. Only two clinical trials evaluating efficacy of PRP for ED treatment are available [9,10].

As opposed to PRP, stem cell therapy is more widely studied and its application in the field of regenerative medicine is more widespread [1]. Stem cells are unspecialized and undifferentiated cells found in embryonic and adult tissues. Due to their precursory nature, stem cells possess self-renewal potential and the ability to differentiate into other cell lines. The regenerative effects of these cells are attributed to their ability to secrete growth factors, cytokines and chemokines; upregulate signaling pathways for inflammation reduction; inhibit apoptosis; accelerate wound healing; as well as induce angiogenesis and neurogenesis [11]. According to a systematic review by Irdam et al. on therapeutic effects of mesenchymal stem cells (MSC) on ED, functional outcomes were improved as observed through higher intracavernous pressure (ICP)/mean arterial pressure (MAP) ratio in animal studies following MSC therapy. An increase in cavernous endothelial cells, VEGF, nitric oxide synthase (NOS) and smooth muscle cells were also observed in the animal models. For studies involving human subjects, International Index of Erectile Function (IIEF-5) score and Erection Hardness Score (EHS) were improved and peak systolic velocity (PSV) was higher after MSC therapy [12]. Although stem cell therapy has been shown to be effective in mitigating ED, it has several limitations of unable to migrate to target tissue and low number of surviving cells post-intracavernous injection. A study reported the case of stem cells drifting away in the circulating system and migrated to bone marrow, leaving the target tissue after being injected into the corpora [13]. Fortunately, these limitations can be overcome by LI-ECSW. Hence, the idea of combining stem cell therapy with LI-ECSW to treat ED is developed.

LI-ECSW is the application of low-intensity electric shock on targeted deep tissue to stimulate multiple biological changes such as tissue and nerve regeneration through the creation of transient micromechanical shear forces [14]. A clinical trial reported improvement in IIEF-EF score and a high rate of conversion from PDE5i non-responders to responders following LI-ECSW therapy [4]. Meanwhile, in vivo studies reported regeneration of endothelial cells and smooth muscle after LI-ECSW, which improved erectile function in animal models [15,16]. In addition, LI-ECSW is shown to stimulate expression of angiogenesis-related growth factors like VEGF, endothelial nitric oxide synthase (eNOS) and proliferating cell nuclear antigen (PCNA; endothelial cell proliferation factor). This indicates the importance of LI-ECSW in inducing neovascularization and angiogenesis [14]. Another study further demonstrated the ability of LI-ECSW to assist in the “homing” of stem cells into target penile tissue [17]. All this evidence suggested the fundamental mechanism of LI-ECSW in stimulating cell recruitment and proliferation, promoting angiogenesis and vascularization in the damaged tissue. This indicates the suitability of LI-ECSW to eliminate the limitations pertaining to stem cell therapy and improve the latter's therapeutic effects for ED treatment (Fig. 1). But, since research regarding combined therapy of LI-ECSW and stem cell is still preliminary and limited, this review aims to provide a detailed description on efficacy of this treatment regimen and discuss some potential cell types that could be used together with LI-ECSW. Hopefully, with the information on current advances of this combined therapy, further research will be conducted to fill existing knowledge gap and improve the method for effective ED treatment.

2. Combination therapy using low-intensity extracorporeal shockwave treatment (LI-ECSW) and adipose-derived stem cells (ADSC)

Adipose-derived stem cells (ADSC) are a stem cell source that can be isolated from adipose tissue. They have abundant sources found localized in the subcutaneous adipose tissue throughout the whole body. They can be used for autologous or allogenic transplantation in the body safely with limited foreign immune response [18]. Studies investigating the efficacy of combined therapy of ADSC with LI-ECSW on ED are also available. Similar to previous studies involving single therapy of ADSC, results from the combined therapy studies also showed promising outcomes regarding the use of LI-ECSW and ADSC.

For instance, Li et al. conducted an in vivo experiment using diabetes mellitus induced ED rat model to study the combined therapeutic efficacy of LI-ECSW and ADSC on ED treatment. A total of 98 male rats were randomized into five groups, including normal control group (receiving intracavernous injection of phosphate buffer solution), ED control group (receiving intracavernous injection of phosphate buffer solution), LI-ECSW group (receiving cavernous shockwave treatment), ADSC group (receiving intracavernous injection of ADSC suspension) and LI-ECSW + ADSC group (receiving cavernous shockwave treatment one day after injection of ADSC suspension). For the LI-ECSW treatment procedure, a total of 300 shocks were delivered at energy level of

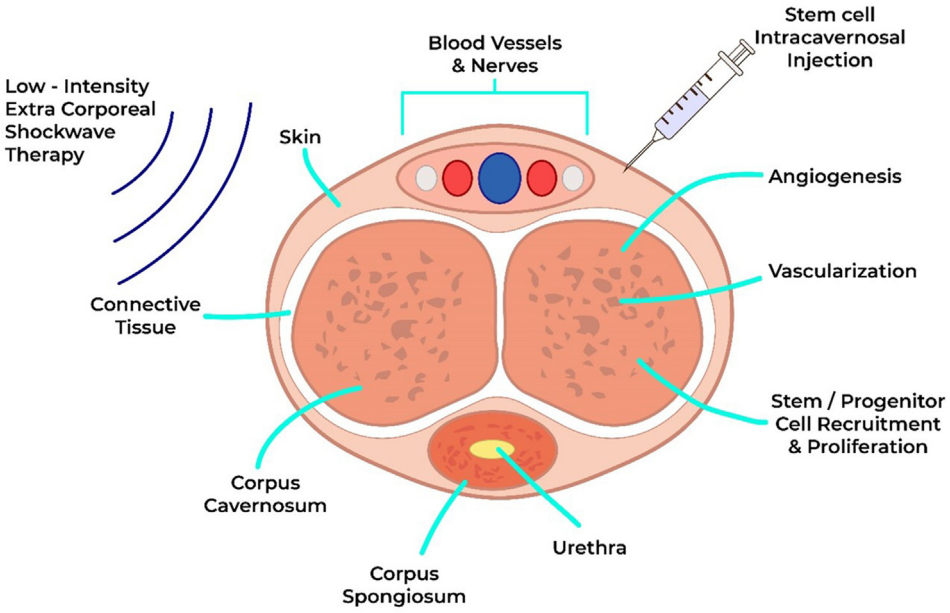

Fig. 1. Augmented therapeutic response by the combination therapy of LI-ECSW and stem cells [49].

0.09 mJ/mm^2 and a frequency of 120/min during each session. The session was repeated three times per week with a day's break in between each session over a duration of three weeks. The erectile function of the rats was assessed based on ICP and MAP. According to the results, ICP-to-MAP ratio of LI-ECSW + ADSC group was higher than that of LI-ECSW and ADSC monotherapy groups, indicating the erectile function was significantly improved in the LI-ECSW + ADSC combined therapy group ($p < 0.05$). Immunohistochemistry showed the expression of α-smooth muscle actin (α-SMA), neuronal nitric oxide synthase (nNOS) and von Willebrand factor (vWF) in the corpus cavernosum of ADSC, LI-ECSW and LI-ECSW + ADSC groups were higher than that of the ED control group. Apart from the expression of α-SMA, which was significantly lower in ADSC group when compared to LI-ECSW group and LI-ECSW + ADSC group ($p < 0.05$), the differences in expression of nNOS and vWF were not significant among the three treatment groups. The findings suggested that both ADSC and LI-ECSW therapy can improve erectile function by increasing the expression of α-SMA, nNOS and vWF in the corpus cavernosum of the rats. A combination of ADSC and LI-ECSW therapy exhibits better effect on ED than when each applied individually [19].

Similarly, Jeon et al. tested the combination therapy of ADSC and LI-ECSW on rat model with post-prostatectomy ED. The rats were randomly divided into five groups: Control, BCNI (bilateral cavernous nerve injury), ADSC (BCNI group with 1×10^6 ADSCs (diluted in PBS) directly injected around the cavernous nerve), LI-ECSW (BCNI group with LI-ECSW on the corpus cavernosum), and ADSC/LI-ECSW (BCNI group with a combination of ADSCs and LI-ECSW). A total of 300 shocks were delivered at an energy level of 0.1 mJ/mm^2 and a frequency of 2 shocks/s during each LI-ECSW session. LI-ECSW was repeated three times per week with a one-day break in between each session, for a total duration of three weeks. The erectile function of the rats in terms of ICP was compared after four weeks among experimental groups undergoing single therapy of ADSC and LI-ECSW as well as combined therapy of LI-ECSW + ADSC. The cavernous nerves and penile tissue of the rats were also evaluated through immunostaining, western blotting and a cyclic guanosine monophosphate (cGMP) assay. It was found out that the ICP was significantly improved in the group receiving combined therapy of LI-ECSW + ADSC compared to other experimental groups ($p < 0.05$). The LI-ECSW + ADSC group was observed to have significantly increased level of α-SMA ($p < 0.05$), nNOS ($p < 0.05$), eNOS ($p < 0.05$) and cyclic guanosine monophosphate (cGMP) ($p < 0.05$) in the corpus cavernosum compared to the groups that received single therapy of LI-ECSW and ADSC. The apoptotic index in the corpus cavernosum was also reduced in the LI-ECSW + ADSC group. Results of the study showed that ADSC increased β-3 tubulin expression of the cavernous nerve while LI-ECSW enhanced the VEGF expression in corpus cavernosum. This indicates that ADSC can improve the recovery of injured cavernous nerve while LI-ECSW can improve angiogenesis in the corpus cavernosum. Therefore, the combined therapy of LI-ECSW + ADSC can treat post-prostatectomy ED in rats by improving the erectile function [20].

3. Combination therapy using low-intensity extracorporeal shockwave treatment (LI-ECSW) and mesenchymal stem cells (MSC)

MSC are useful cell sources for regenerative medicine, which possess extraordinary self-renewal abilities. Given their abilities to promote cell growth and prevent apoptosis, they are widely used for treatment involving nerve injury, trauma and inflammatory diseases [12]. Naturally, they can also be used as a good remedial option for ED treatment. In the mechanism of ED treatment, MSC therapy can stimulate endothelial cell proliferation and prevent their apoptosis through paracrine pathway, thus preventing the degeneration of penile tissues [21]. Meanwhile, LI-ECSW can stimulate angiogenesis and restore blood flow to the damaged penile tissues, thus promoting regeneration and repair [22]. Theoretically, by combining both LI-ECSW and MSC therapy, the condition of ED can be mitigated more effectively.

Indeed, evidence has shown the enhanced benefit of using combined therapy of LI-ECSW and MSC in treating ED. For example, a study by Zhu et al. reported efficient promotion of autophagy and angiogenesis by combined therapy of LI-ECSW and MSC for ED treatment. The study used diabetic ED rat as in vivo experimental model. The rats were randomly divided into four groups: (1) ED control group; (2) LI-ECSW group; (3) MSC group and (4) LI-ECSW + MSC group. For LI-ECSW therapy, 300 pulses with energy

flux density of 0.1 mJ/mm^2 with a frequency of 120/min was administered on the rat penis. The procedure was repeated three times per week with one day's break for a duration of four weeks. For MSC therapy, 200 μL phosphate buffer solution (PBS) containing 1×10^6 MSC was administered through bilateral intracavernous injection. Erectile function was evaluated based on ICP/MAP and changes in penile tissues were observed through histology and immunofluorescence staining. Results showed that the ratio of ICP/MAP was significantly higher in the LI-ECSW + MSC group compared to both the single treatment LI-ECSW or MSC groups ($p < 0.05$). Besides, the expression levels of nerve growth factor (NGF) and brain-derived neurotropic factor (BDNF) were also significantly increased in the LI-ECSW + MSC group compared to other experimental groups ($p < 0.01$). In vitro investigation observed that LI-ECSW increased the quantity of MSC in the corpus cavernosum. It also promoted the recruitment of MSC by inducing the penile tissues to express more platelet endothelial cell adhesion molecule (PECAM) and stromal derived factor-1 (SDF-1). Moreover, the MSC recruited were also induced by LI-ECSW to express more VEGF in the corpus cavernosum through activation of PI3K/AKT/mTOR and NO/cGMP signaling pathways. Hence, the overall findings proved a significant improvement of ED through the stimulation of angiogenesis, vasodilation and autophagy as well as inhibition of apoptosis in the corpus cavernosum by combined therapy of LI-ECSW + MSC [23].

Meanwhile, another similar investigation featured the use of engineered MSC expressing SDF-1 combined with LI-ECSW to improve ED in streptozotocin-induced diabetic rats. Shin et al. constructed a biologically engineered MSC expressing SDF-1 in an attempt to improve ED [24]. LI-ECSW procedure was conducted by applying a total of 300 shocks at energy level of 0.1 mJ/mm^2 and frequency of 120 shocks/min to the penis of ED rats. The procedure was repeated three times a week with one day break for a duration of four weeks. The stem cell therapy was performed through bilateral intracavernous injection of SDF-1-engineered MSC (SDF-1 eMSC). Experimental results showed that the ratio of ICP/MAP was significantly higher in rats treated with LI-ECSW + SDF-1 eMSC than in rats treated with single therapy of either LI-ECSW or SDF-1 eMSC ($p < 0.05$). This indicated significant improvement in erectile function of ED rats. Furthermore, concentration of α-SMA exhibited the highest elevation in rats treated with LI-ECSW + SDF-1 eMSC, indicating improvement of smooth muscle contents in the corpus cavernosum. The combined treatment also increased expression of SDF-1, nNOS and NO/cGMP in the corpus cavernosum. Increased SDF-1 indicated improved vascularization in the corpus cavernosum through recruitment of EPC, while increased nNOS and NO/cGMP indicated improved vasodilation in smooth muscle vessels via activation of NO/cGMP signaling pathway. The study proved the effectiveness of combined therapy LI-ECSW + MSC compared to single therapy and that this regimen could be used as a potential synergistic treatment for ED [24].

Specifically, MSC derived from bone marrow, also known as bone marrow-derived stem cells (BMSC), are among the common stem cells used for ED treatment due to their abilities to improve erectile function and increase contents of endothelium and smooth muscle as well as anti-fibrotic properties [25]. A study therefore combined BMSC transplantation with LI-ECSW therapy to improve erectile function of ED rats. For the LI-ECSW treatment, a total of 300 shocks were delivered at energy level of 11.08 MPa and frequency of 60 shocks/min. The procedure was repeated three times weekly for two weeks at a one-week interval. One day after LI-ECSW treatment, the ED rats were injected with 500 μL of PBS containing 1×10^6 BMSC at the cavernous bodies for intracavernosal stem cell transplantation. According to the results, the ratio of ICP/MAP among rats treated with LI-ECSW + BMSC improved more effectively than those treated with LI-ECSW or BMSC alone, indicating improvement in erectile function four weeks after treatment. Observation on changes in the number of BMSC showed a significant amount of BMSC still remained in the cavernous body of rats treated with LI-ECSW + BMSC (23.00 ± 2.02) compared to rats treated with BMSC only (4.00 ± 0.60; $p < 0.001$). Four weeks after treatment, number of BMSC present in the cavernous body increased by 10-fold for the LI-ECSW + BMSC group whereas almost no BMSC was observed in the BMSC group ($p < 0.001$). This suggested that LI-ECSW + BMSC treatment enhanced the survival and proliferation of BMSC in the cavernous body. Besides, the LI-ECSW + BMSC group exhibited elevated expressions of SDF-1, VEGF, CD31 (vascular endothelial marker) and eNOS in the cavernous tissues compared to groups receiving only one treatment. This indicated more improvement in angiogenesis and endothelial functions by the combined therapy than single therapy. Furthermore, the combined therapy also exerted positive effects on muscle restoration and fibrosis degree, as demonstrated by increase in α-SMA expression and smooth muscle/collagen ratio respectively. Overall, the findings suggested a combination of LI-ECSW and BMSC transplantation could improve erectile function of ED rats better than single therapy of either LI-ECSW or BMSC. This was because LI-ECSW would improve the survival of transplanted BMSC in the cavernous tissues due to elevated expression of SDF-1 and enhancement of angiogenesis [26].

Apart from MSC, MSC-derived exosomes therapy is also applied in combination with LI-ECSW to improve erectile function in ED patients [27]. In vitro analysis on MSC-derived exosomes therapy have shown promising outcomes on penile tissue regeneration [28,29]. Theoretically, when applied in combination with LI-ECSW, effects on angiogenesis, vascularization, activation and proliferation of MSC would be enhanced. A prospective clinical study in Ukraine investigated the therapeutic model of combined therapy of MSC-derived exosomes and LI-ECSW on a contingent of 38 male patients suffering from severe organic ED. The treatment regimen involved intracavernous injection of 2.5 ml MSC-derived exosomes in each peduncle of penis after LI-ECSW session. The LI-ECSW procedure involved 3000 shocks delivered at frequency of 3 Hz with a total power up to 0.35 mJ/mm^2 on 6 standard penile areas (Fig. 2). Results showed significant improvement in terms of IIEF-5 score ($p < 0.01$), EHS ($p < 0.01$) and pharmaco-doppler-sonography data ($p < 0.01$) for patients who received LI-ECSW + MSC-derived exosomes therapy. Mean PSV in stimulation for the group increased significantly from 23.4 ± 0.3 cm/s before treatment to 29.6 ± 0.3 cm/s after treatment ($p < 0.01$). While mean EDV in stimulation decreased distinctly from 5.4 ± 0.2 cm/s pre-treatment to 4.5 ± 0.3 cm/s post-treatment ($p < 0.01$). Among patients who received only LI-ECSW therapy, significant improvements were observed after treatment in terms of IIEF-5 scores ($p < 0.01$), EHS ($p < 0.01$) and mean PSV in stimulation ($p < 0.01$) but not mean EDV in stimulation ($p = 0.24$). The mean PSV in stimulation for the LI-ECSW group increased significantly from 23.4 ± 0.3 cm/s before treatment to 25.4 ± 0.3 cm/s after treatment ($p < 0.01$). When compared between the LI-ECSW + MSC-derived exosomes combined therapy group and LI-ECSW single therapy group, significant improvement was only observed in mean PSV during stimulation ($p < 0.01$). Although the LI-ECSW + MSC-derived exosomes group recorded greater improvements in IIEF-5 score, EHS and mean EDV in stimulation than the LI-ECSW group, the differences were not significant ($p > 0.05$). Nonetheless, the study showed a combination of LI-ECSW and MSC-derived exosomes therapy exhibited significant positive impact on erectile function of patients with severe ED. However, it suggested a perspective of further comparison studies on larger population, longer observation periods and

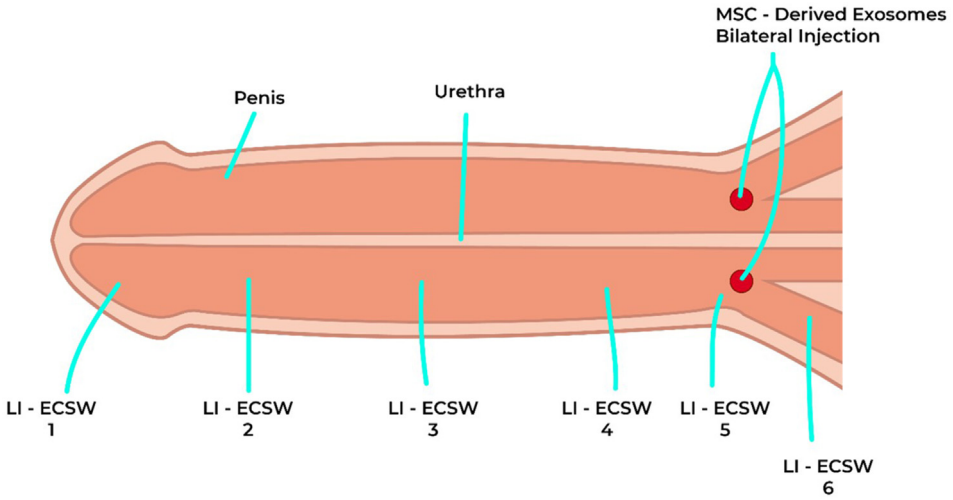

Fig. 2. Application of MSC-derived exosomes injection and LI-ECSW therapy [27].

less severe forms of ED due to some insignificant differences observed between the dual and single treatment groups [27].

4. Combination therapy using low-intensity extracorporeal shockwave treatment (LI-ECSW) and umbilical cord-derived Wharton's Jelly (UCWJ)

Another type of stem cells that can be used for ED treatment are umbilical cord-derived Wharton's Jelly (UCWJ). UCWJ is a mucous connective tissue of umbilical cord present between umbilical vessels and amniotic epithelium. Due to the presence of clinically significant amounts of regenerative substances such as growth factors, cytokines, hyaluronic acid, collagen, sulphated proteoglycans and extracellular vesicles, and its ease of harvest, UCWJ becomes a promising biologic source for tissue repair [30]. For instance, a randomized, single-blinded, controlled clinical trial had been registered to evaluate the efficacy and safety of utilizing a combination of LI-ECSW and UCWJ to treat moderate to severe ED in male patients. A total of 60 patients with moderate to severe ED would be enrolled and treated with LI-ECSW and intracavernosal injection of UCWJ over a period of seven weeks. Patients were randomized and enrolled into either the treatment arm or the control arm. Upon randomization, subjects received DualStim therapy using both radial and focused LI-ECSW without intracavernosal injections during weeks 1 and 2. During week 3, both the treatment and control arms received DualStim therapy. The treatment arm received 2.5 ml intracavernosal injections of GeneXSTEM (Wharton's Jelly) into each corpus cavernosum, while the control arm received 2.5 ml intracavernosal injections of sterile saline in the same manner. Both treatment and control arms continued to receive DualStim therapy during weeks 5 and 6. In week 7, both arms received DualStim therapy, with the treatment arm receiving 2.5 ml intracavernosal injections of Wharton's Jelly in each corpus cavernosum, and the control arm receiving 2.5 ml intracavernosal injections of sterile saline in each corpus cavernosum. Sexual function and intercourse experience would be evaluated at baseline, 1-, 3- and 6-months follow-up post-treatment based on the International Index of Erectile Function – Erectile Function (IIEF-EF) score, Sexual Encounter Profile (SEP), Global Assessment Questionnaire (GAQ) and EHS. The occurrence of any adverse event would also be recorded following the treatment. The study would provide an insight into the efficacy and safety of combined therapy LI-ECSW + UCWJ through the determination of immediate and short-term efficacy of the treatment in restoring erectile function of patients with moderate/severe ED. The study was still ongoing, and the expected date of complete recruitment was on 31 July 2022 [31].

5. A combination of multiple stem cells therapy with low-intensity extracorporeal shockwave treatment (LI-ECSW)

While the above studies feature application of a single type of stem cell in combination with LI-ECSW for ED treatment, utilization of multiple types of stem cells together with shockwave therapy had also been reported. For example, a study conducted by Lander and Berman used autologous stromal vascular fraction (SVF) containing multiple stem cells combined with LI-ECSW to treat ED in male patients [17]. SVF is a heterogeneous population of cells obtained from enzymatic digestion of lipoaspirate, which generally consists of ADSC, MSC, hematopoietic stem cells (HSC), endothelial progenitor cells (EPC), pericyte progenitor cells (PPC), macrophages, red blood cells, platelets, T-regulatory cells and growth factors [17]. In the study, 52 male patients with multifactorial ED were treated with LI-ECSW and intracavernosal injection of autologous SVF obtained from lipoaspirate. For the LI-ECSW procedure, 6000 shocks set at frequency of 15 Hz were delivered along the lateral aspect of penile shaft before intracavernosal injection of SVF. Retrospective review of treatment efficacy was evaluated with IIEF Score and EHS. Results reported that 71 % (37 out of 52 patients) of patients provided positive responses regarding overall improvement following the treatment. Mean IIEF score improved significantly from baseline at 10.21 to 18.40 at 6 months ($p = 0.0008$), whereas mean EHS score improved significantly from 1.34 at baseline to 2.17 at 6 months ($p = 0.012$). Besides, none of the patients experienced adverse reactions following LI-ECSW or harvesting and injection of SVF. This showed that combination therapy of LI-ECSW and SVF is safe and can mitigate ED. It also demonstrated the added regenerative abilities offered by the diverse types of stem cells and progenitor cells present in SVF. The ADSC, MSC and HSC population in the SVF showed their abilities to differentiate along the mesenchymal lines into other cell types, whereas the EPC and PPC acted as precursors for new blood vessels formation [17].

6. Other potential cell types that can be used in combination with low-intensity extracorporeal shockwave treatment (LI-ECSW)

6.1. Penile progenitor cells

The penis contains many different types of cells, including terminally differentiated cells and progenitor cells. Most penile smooth muscle cells and fibroblasts are terminally differentiated and therefore cannot be activated to proliferate. Penile progenitor cells, on the other hand, including subtunical penile progenitor cells, para-sinusoidal penile progenitor cells, Schwann cell progenitor cells and endothelial progenitor cells, can be activated to proliferate and differentiate into mature penile cells [32]. As such, penile progenitor cells also become an important therapeutic target concerning ED treatment through LI-ECSW since shockwave therapy has been shown to improve erectile function [33]. However, compared to other stem cells mentioned before, research on penile progenitor cells is still preliminary and no report investigating the efficacy of combined therapy LI-ECSW with penile progenitor cells is available to date. Nonetheless, studies showing beneficial effects of LI-ECSW on penile progenitor cells are available.

For example, Lin et al. investigated the feasibility of LI-ECSW in activating in situ penile progenitor cells for ED treatment. The in vivo experiment was performed on 30 young and middle-aged male rats. The rats were randomized into two groups: control group and LI-ECSW group. Both groups of rats received an intraperitoneal injection of 50 mg/kg 5-ethylnyl-2′-deoxyuridine (EdU) to mark the penile progenitor cells. Two levels of acoustic energy were performed for the LI-ECSW procedure: very low level (L2, 0.02 mJ/mm^2 at 3 Hz for 300 pulses) and low level (L6, 0.057 mJ/mm^2 at 3 Hz for 500 pulses). Changes of penile progenitor cells were observed through histological analysis and immunofluorescence staining. It was reported that LI-ECSW significantly increased the penile progenitor cells in the penile erectile tissues at 48 h and Week 1 ($p < 0.01$). Very low energy LI-ECSW activated more progenitor cells in the penile nerves and blood vessels compared to low energy LI-ECSW ($p < 0.01$). Besides, LI-ECSW significantly increased penile progenitor cells in young rats at 48 h and Week 1 compared to middle-aged rats ($p < 0.01$). The difference in penile cells activation was also dependent on energy level of LI-ECSW. Among young rats, low energy LI-ECSW activated more penile progenitor cells than very low energy LI-ECSW. Further in vitro investigation of LI-ECSW on rat primary Schwann cells demonstrated that the cells were significantly proliferated at 48 h after shockwave treatment ($p < 0.01$). It was found out that low energy LI-ECSW increased ERK1/2 phosphorylation and that Schwann cell proliferation was promoted through ERK1/2 pathway. These findings proved the feasibility of LI-ECSW in mitigating ED by not only promoting the activation of in situ penile progenitor cells but also enhancing erectile function through Schwann cells proliferation. However, the study also emphasized the major concern regarding the effectiveness of LI-ECSW in improving erectile function among old individuals due to decreased number and quality of penile progenitor cells following aging. This was observed in the study where fewer penile progenitor cells were activated by LI-ECSW in middle-aged rats compared to young rats [33].

Another study combined LI-ECSW with a Korean herbal formulation named Ojayeonjonghwan to treat diabetes mellitus-associated ED rats. According to the study, the herbal formulation Ojayeonjonghwan (KH-204) has antioxidant properties, which can improve ED in aged diabetic rats [34]. So, the team intended to investigate whether a combination of LI-ECSW and KH-204 can accelerate ED treatment while exploring the mechanism behind this combined treatment. In vivo experiment showed that LI-ECSW + KH-204 improved ICP in the ED rats. ED rats treated with the combined therapy were observed to have improved expression of SDF-1 and PECAM-1. In vitro experiment showed that LI-ECSW stimulated penile progenitor cells to migrate to penile tissue, while KH-204 protected the cells in the corpus cavernosum. So, combined therapy of LI-ECSW + KH-204 protected the penile progenitor cells (recruited to the corpus cavernosum by LI-ECSW) from apoptosis via its antioxidant activity. As such, oxidative stress was relieved by the combined therapy, which occurred through increased expression of nuclear factor erythroid 2 related factor (Nrf2)/heme oxygenase-1 (HO-1) and superoxide dismutase (SOD) as well as decreased 8-hydroxy-2′-deoxyguanosine (8-OHdG). The findings indicated that a combination of LI-ECSW and KH-204 conferred protective and proliferative effects on penile progenitor cells, which were then prevented from apoptosis. This would help with the mitigation of ED [35]. Therefore, based on the above evidence, penile progenitor cells may be a suitable choice for stem cell therapy applied in combination with LI-ECSW.

6.2. Endothelial progenitor cells (EPC)

Apart from penile progenitor cells, LI-ECSW is also shown to have positive effects on EPC, which plays an important role in angiogenesis and vascularization [17]. This can help restore vascular flow and improve erectile function in ED patients. Application of LI-ECSW on damaged cavernous nerves had shown to induce EPC recruitment for formation of new blood vessels, penile angiogenesis and tissue restoration [36,37]. Another study also reported that the application of LI-ECSW and SDF-1 expressing MSC enhanced the recruitment of EPC into the injured penile tissues, thus accelerating angiogenesis and improving erectile function [24]. Given these favorable outcomes of LI-ECSW on EPC, the cells may serve as another potential option for stem cell therapy applied together with shockwave treatment for ED, especially diabetic related ED caused by endothelial dysfunction [38].

6.3. Cells other than mesenchymal stem cells

From the aspects of stem cells, several types of cells have been used for ED treatment. Although these cells are less popular compared to MSC, they showed promising results in mitigating ED. These cells include human urine-derived stem cells (HUDSC), muscle-derived stem cells (MDSC) and bone marrow-derived mononuclear cells (BM-MNC).

Ouyang et al. showed that the use of HUDSC enhanced with FGF-2 for ED treatment in rats improved their erectile function as observed through the improvement of ICP/MAP ratio [39]. Another study reported improvement in ICP and elevated expression of eNOS, nNOS and endothelial markers in the penile tissues of ED rats following treatment with HUDSC [40]. Similarly, Zhang et al. reported improved cavernosal endothelium through upregulated autophagic activity following HUDSC treatment in ED rats [41].

Meanwhile, an in vivo study investigated the efficacy of MDSC in treating diabetic ED rats. The results showed MDSC therapy decreased collagen and fat infiltration while upregulated nNOS and eNOS expression, thus improving erectile function in the rats [42]. Another similar study reported MDSC were able to proliferate and differentiate into muscle cells and neuronal cells, thus reversing the ED caused by cavernous nerve crush injury in rat model. However, the study highlighted the need for a longer period of observation and ways to prolong the functional effect of MDSCs [43].

In terms of clinical investigation involving human patients, a phase I/II clinical trial that used BM-MNC as stem cell treatment for ED was conducted. A group of 12 radical prostatectomy patients experiencing ED were divided into 4 groups. The groups received

BM-MNC with doses varying from 2×10^7 to 2×10^9 cells. Results showed significant improvement in IIEF domains of intercourse satisfaction (6.8 vs 3.9; $p = 0.044$) and erectile function (17.4 vs 7.3; $p = 0.006$) as well as increased erection hardness (2.6 vs 1.3; $p = 0.008$) at 6 months follow-up compared to baseline. Patients who received higher doses of BM-MNC exhibited greater improvement in spontaneous erections [44].

Judging from the promising results shown by these cells when used as single therapy, combining these cells with LI-ECSW would theoretically produce enhanced therapeutic effects on ED treatment, as LI-ECSW has been shown to assist "homing" of stem cells to target penile tissues and enhance proliferation of the cells [45]. Therefore, these cells could be potential candidates for stem cell therapy applied in combination with shockwave therapy.

6.4. Stem cell-based conditioned medium

In addition to the stem cells and progenitor cells stated above, stem cell-based conditioned medium (CM) therapy may also be used together with LI-ECSW for ED treatment. Unlike stem cell therapy, CM therapy has the advantage of utilizing the paracrine action of stem cells while avoiding the possibility of tumor development as it possesses low immune response. CM can also be stored after production, thus offering added convenience for medical usage [46]. MSC-CM is an example of stem cell-based CM therapy that can be applied in combination with LI-ECSW due to its regenerative properties provided by the angiotrophic and neurotrophic factors. According to an in vivo study conducted to examine the therapeutic effects of human MSC-CM injection on ED treatment in rats, the 3D spheroid culturing method of MSC-CM resulted in a 278-fold increase of total protein content of the CM. The protein concentration of CM increased by 19 times upon increasing the centrifugation time for cutoff filtering. With the increasing protein contents, MSC-CM contained elevated amount of angiogenic, neurotrophic and anti-inflammatory factors, including ANGPT-1, ANG, VEGF, BDNF, bFGF, TNF-α, IL-1ra and IL-4. These biological factors were essential for the modulation of angiogenesis and functioned to confer positive neurotrophic effects on the injured cavernous nerve of ED rats, thus improving the penile erectile function, as shown through improvement of ICP/MAP ratio. Besides, treatment with MSC-CM also alleviated the progression of fibrosis and reduction in smooth muscle content of cavernosal tissue caused by cavernous nerve injury in ED rats. With the increasing concentration of CM, collagen density decreased and proportion of smooth muscle increased, reducing the effect of fibrosis in the tissue [47]. Currently, there is no research available that focuses on efficacy of combined therapeutic effects of LI-ECSW and MSC-CM on ED treatment. But, judging from the promising outcomes MSC-CM offers for erectile function improvement, combining it with LI-ECSW therapy would theoretically enhance its therapeutic effects. Hence, this would be another promising treatment option for ED.

7. Concluding remarks and perspective

Combined therapy of LI-ECSW and stem cells is more effective in treating ED than either one of these treatments applied alone. Despite the positive interactions shown between LI-ECSW and stem cells discussed above, multiple knowledge gaps still exist concerning this combined treatment. These gaps include the potential side effects (short- and long-term) of the combined therapy, its extent of effectiveness in treating ED caused by various other less-studied factors (particularly post-surgical trauma from radical prostatectomy that is difficult to treat) [45], differences in treatment outcomes when different energy intensity of LI-ECSW are applied [33], and whether its therapeutic effects are affected by age since reports have shown reduced cell proliferation as a result of decreased number and quality of penile progenitor cells due to aging [33,48]. All these gaps need to be filled by conducting further research on the combined therapy. In addition to in vivo studies, more clinical trials should be conducted, not just to examine the differential therapeutic effects on human due to physiological differences between human and animals, but also to observe short- and long-term efficacy of the treatment.

However, the major challenge for further research is the absence of regulatory approval for LI-ECSW and stem cell therapy except for experimental settings. The US Food and Drug Administration (FDA) have not approved the application of LI-ECSW or stem cell therapy for ED treatment since they are believed to be investigational and should only be conducted while strictly adhering to research protocols in compliance with the Institutional Review Board approval at little or no cost to the patient. For stem cell therapy, there is another challenge which involves presence of heterogeneous cell sources and formulations in the isolates that require individual evaluation as drug before approval can be granted [1,49]. But, even though LI-ECSW and stem cell therapy have not been approved by FDA, they are recognized treatment options for vasculogenic ED approved by the European Association of Urology (EAU) as stated in the latter's guidelines since 2013 [50]. So, although the progress of research on this topic is slow, with modern technology and advanced medical knowledge, this combined therapy will be an alternate treatment option for ED in the near future.

Author contributions

Conceptualization, D.D., and A.N.; Methodology, D.D., Y.L., and A.N.; Investigation, D.D., Y.L., and A.N.; Writing – Original Draft, Y.L.; Writing – Review & Editing, D.D., and A.N.; Resources, D.D., Y.L., and A.N.; Supervision, D.D., and A.N. All authors have reviewed and consented to the published version of the manuscript.

Data availability statement

This is a review article, and no new data were generated or analyzed in this study. Therefore, data sharing does not apply in this article.

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Declaration of competing interest

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