REVIEW

The Basic Science Behind Low-Intensity Extracorporeal Shockwave Therapy for Erectile Dysfunction: A Systematic Scoping Review of Pre-Clinical Studies

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ABSTRACT

Introduction: Despite recent promising clinical results, the underlying mechanism of action of low-intensity extracorporeal shockwave therapy (Li-ESWT) for erectile dysfunction (ED) is mostly unclear and currently under investigation.

Aim: To systematically identify and evaluate evidence regarding the basic science behind Li-ESWT for ED, discuss and propose a putative mechanism of action, address the limitations, and imply insights for further investigation in the field.

Methods: Using Cochrane's methodologic recommendations on scoping studies and systematic reviews, we conducted a systematic scoping review of the literature on experimental research regarding Li-ESWT for ED and other pathologic conditions. The initial systematic search was carried between January and November 2017, with 2 additional searches in April and August 2018. All studies that applied shockwave treatment at an energy flux density >0.25 mJ/mm^2 were excluded from the final analysis.

Main Outcome Measure: We primarily aimed to clarify the biological responses in erectile tissue after Li-ESWT that could lead to improvement in erectile function.

Results: 59 publications were selected for inclusion in this study. 15 experimental research articles were identified on Li-ESWT for ED and 44 on Li-ESWT for other pathologic conditions. Li-ESWT for ED seems to improve erectile function possibly through stimulation of mechanosensors, inducing the activation of neo-angiogenesis processes, recruitment and activation of progenitor cells, improving microcirculation, nerve regeneration, remodeling of erectile tissue, and reducing inflammatory and cellular stress responses.

Clinical Implications: Improving our understanding of the mechanism of action of Li-ESWT for ED can help us improve our study designs, as well as suggest new avenues of investigation.

Strengths & Limitations: A common limitation in all these studies is the heterogeneity of the shockwave treatment application and protocol.

Conclusion: Li-ESWT for ED, based on current experimental studies, seems to improve erectile function by inducing angiogenesis and reversing pathologic processes in erectile tissue. These studies provide preliminary insights, but no definitive answers, and many questions remain unanswered regarding the mechanism of action, as well as the ideal treatment protocol. **Sokolakis I, Dimitriadis F, Teo P, et al. The Basic Science Behind Low-Intensity Extracorporeal Shockwave Therapy for Erectile Dysfunction: A Systematic Scoping Review of Pre-Clinical Studies. J Sex Med 2019;16:168—194.**

INTRODUCTION

Shockwave therapy is a non-invasive treatment method that uses the passage of acoustic waves through tissue to induce the desired effects. It was originally introduced as a non-invasive treatment for kidney stones and has since been used in the management of many other conditions, including bone fractures, musculoskeletal disorders, wound healing, Peyronie's disease, and ischemic cardiovascular disorders.[1] In 2010, Vardi et al[2] proposed the use of low-intensity extracorporeal shockwave therapy (Li-ESWT) as a promising new treatment option for erectile dysfunction (ED). Since then, more robust data from randomized controlled trials, systematic reviews and meta-analyses strongly suggested that Li-ESWT improves erectile function in patients with vasculogenic ED and may have the potential to become a first-line, non-pharmacologic treatment for these patients.[3–5]

Despite these promising results, the underlying mechanism of action of Li-ESWT is mostly unclear and currently under investigation. Li-ESWT has been investigated in animal models in orthopedics, cardiology, wound healing, and sexual medicine (Peyronie's disease and ED). These studies showed, sometimes with conflicting results, that shockwave energy initiates multiple cascades of biologic responses, typically involving the release of vascular endothelial growth factor (VEGF), inducing cell proliferation, cell survival, antifibrotic effects, anti-inflammatory effects, and recruitment and activation of endogenous stem cells. These cellular responses result in angiogenesis, wound healing, and tissue regeneration.[1,3]

All of these studies used different treatment protocols (eg, devices, type of shockwaves, focus, energy flux density [EFD], number of shockwaves applied) on different cells, tissues, and disease models. Thus, there is high potential for different and sometimes even conflicting results. This leads to confusion and uncertainty when trying to understand the underlining mechanisms of action of Li-ESWT for ED. Moreover, translating these results into improvements in clinical safety and efficacy could be difficult.

Through a systematic scoping review of the literature, regarding experimental preclinical studies, we tried to investigate the mechanism of action of Li-ESWT for ED. By analyzing the experimental design, results, and limitations of different studies, we primarily aimed to clarify the biologic responses in erectile tissue after Li-ESWT that could lead to improvement in erectile function. Importantly, we also considered preclinical literature from other fields, such as orthopedics, wound healing, and cardiology, considering its effects on cell types that have not been fully investigated in the current Li-ESWT for ED literature (eg, fibroblasts, the immune system). Improving our understanding of the mechanism of action of Li-ESWT by cross-fertilization of ideas from other clinical fields, could help us improve our experimental designs for testing Li-ESWT for ED, as well as suggest new avenues of investigation. Finally, we aimed to summarize our results by proposing a holistic putative mechanism of action.

MATERIALS AND METHODS

Scoping studies comprise a further type of literature review that tends to address broader topics, where many different study designs might be applicable. In comparison, systematic reviews typically focus on a well-defined research question using the PICOS (Population, Intervention, Comparison, Outcome, Study design) approach. Furthermore, scoping studies do not assess the quality of included studies, because they may have different designs.[6,7] In our study, we tried to find evidence regarding a broader topic, such as the mechanism of action of Li-ESWT for ED. Therefore, we conducted a systematic scoping review of the literature using Cochrane's methodologic recommendations on scoping studies and systematic reviews.[6–9]

The results of our research are presented in the form of a flow chart (Figure 1). Between January 2017–November 2017, we performed a systematic search in the following databases: Medline, Embase, The Cochrane Library, Scopus and Web of Science. An additional literature research for new studies, to keep our review up-to-date, was made in April 2018, as well in August 2018. The keywords "shockwave(s)", "shock wave(s)," and "ESWT" were searched alone and in combination with other terms (eg, erectile, penis, corpora, angiogenesis, animal studies, stem cells, function, effect[s], mechanism, receptors). Additionally, the reference lists were tracked backward for further relevant articles, which were not listed in the databases mentioned above or were not identified during the research. Furthermore, we reviewed articles that were suggested by the "related citations in PubMed" option for the most recent articles. Our research was not restricted by language or date of publication.

After screening the title and the abstract (if available), all articles dealing with extracorporeal shockwave treatment and addressing experimental research or articles involving Li-ESWT for ED or other pathological conditions (except urolithiasis) were included for full text reviewing. The screening of full articles was conducted by 2 reviewers (I.S., P.T.) independently with predefined exclusion criteria. Finally, any discrepancies were discussed between the 2 reviewers to reach a consensus. If a disagreement occurred, a third author (D.H.) was designated to reach a consensus.

Articles in which the term "shockwave" was not used as the known physical term of acoustic waves produced by a shockwave generator were excluded. Other exclusion criteria were as follow:

- Shockwave treatment referring to extracorporeal shockwave lithotripsy for urinary stone disease.[10]
- Articles that did not provide sufficient information about the treatment protocol of Li-ESWT (eg, number of shockwaves applied)
- Articles that did not include information about the settings of the shockwave application (eg, energy flux density [EFD], maximum shockwave pressure at focus)
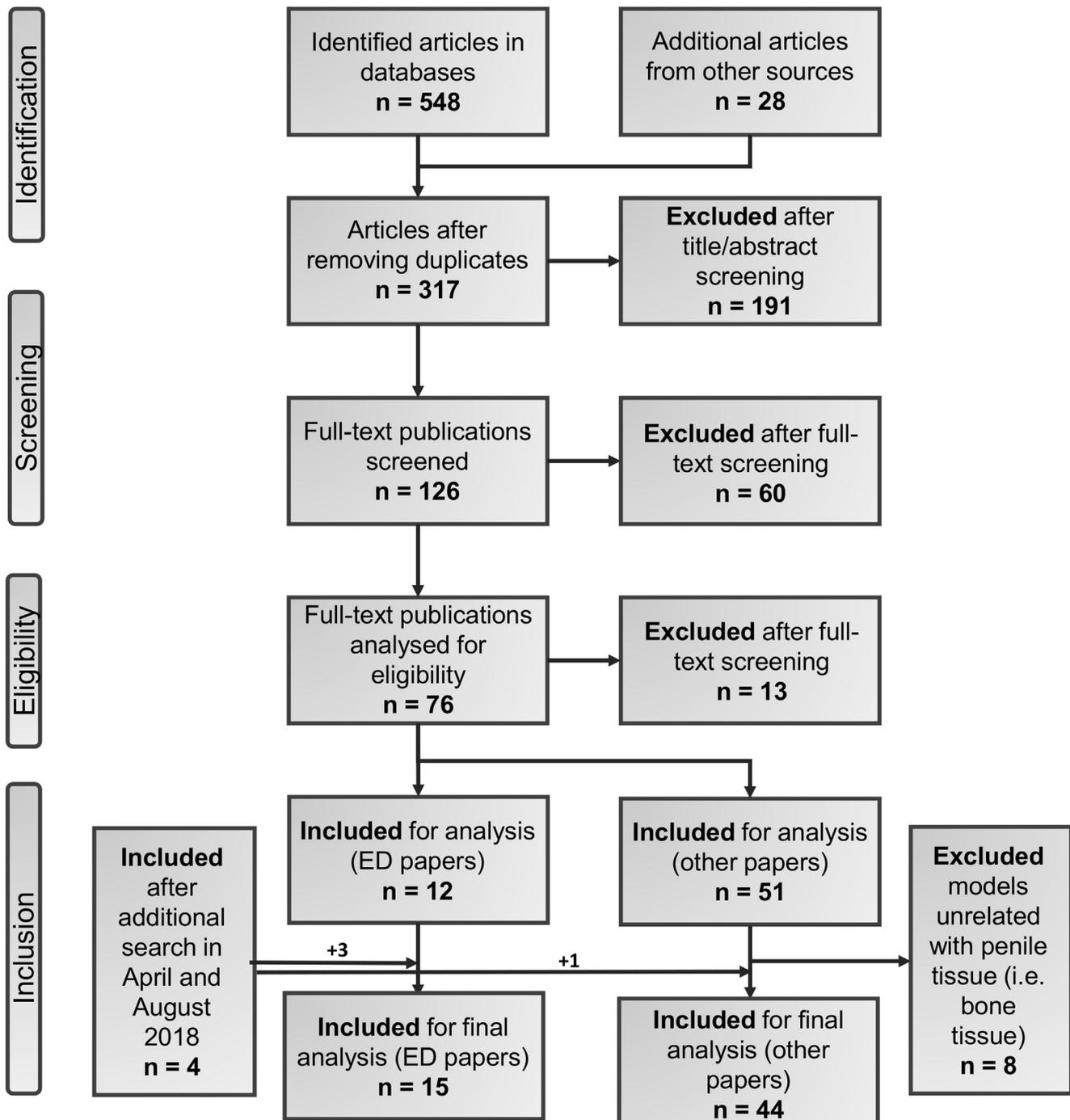
Figure 1. Flow chart of the review process.

- Because we intended to investigate Li-ESWT, all studies that applied shockwave treatment at an EFD level >0.25 mJ/mm^2 or maximum pressure >150 bars (>15 MPa) were also excluded from the final analysis.[11]
- All articles that were veterinary clinical research of Li-ESWT on animals.[12]
- Studies that discussed cell types or biologic processes that are not normally represented in erectile tissue (eg, osteoclasts and osteoblasts).[13]

- Publications on shockwaves for gene transfection, treating biofilms, or enhancing antimicrobial effects.[14,15]
- Abstracts only (no full text available or abstracts where the full text was already included in the review). In exception, we included unpublished data (conference abstract) from 1 of the authors of this review (F.G.), because it involves novel results of Li-ESWT in a rat model of hypertension-induced ED (not previously studied in the shockwave literature), and we have access to all methodologic details.[16]

- Grey literature (eg, reports of device manufacturers) without publication in a scientific journal.

RESULTS

Li-ESWT for Erectile Dysfunction

To date, 15 experimental research studies from 8 research groups have tried to address the question "How does Li-ESWT for erectile dysfunction really work?" (Tables 1 and 2).[16−30] Most of these studies were conducted on disease animal models, such as streptozotocin (STZ)−induced diabetic rats,[17−19,21−23] Goto-Kakizaki (GK) rats (a model of type II diabetes),[20] obesity rats (Zucker fatty rats),[25] and spontaneously hypertensive rats[16] mimicking ED of vascular origin. Additionally, 3 studies used bilateral cavernous nerve injury (BCNI) rat models,[27−29] mimicking postprostatectomy ED. 2 studies were conducted on normal Sprague-Dawley rats,[26,30] and 1 study on naturally aged Wistar rats.[24] Furthermore, some studies included in vitro application of Li-ESWT on cell cultures such as rat Schwann cells[17,27,28] and human umbilical vein endothelial cells (HUVECs).[25] Moreover, a few studies investigated the combination of Li-ESWT with transplantation of bone marrow mesenchymal stem cells[17] or adipose tissue−derived stem cells (ADSCs).[29]

Li-ESWT for Disease Models Other Than ED

44 experimental studies of Li-ESWT in the fields of orthopedic, neurologic, and cardiologic diseases, as well as wound healing processes, were included in the final analysis (Figure 1). These studies showed an even greater heterogeneity than ED studies regarding the shockwave applicator, the treatment protocol, the EFD, and other parameters[31−74] (Tables 3−7). On the other hand, careful review of these studies could confirm some mechanisms of action of Li-ESWT or suggest other possible mechanisms that have not been investigated in ED.

Improvement of Erectile Function

11 of the 15 studies in ED included in vivo assessment of erectile function, all by measuring the intracavernosal pressure (ICP) response after stimulation of the cavernous nerve.[16,17,19−23,25,28−30] 10 of the 11 studies used pathologic models of ED and showed an improvement of the ICP/mean arterial pressure (MAP) ratio in the Li-ESWT group in comparison to the control group.[16,17,19−23,25,28,29] The study by Müller et al[30] showed a decrease in ICP/MAP ratio in the Li-ESWT groups in comparison to controls. However, this study applied shockwaves on rats with normal erectile function, used a ballistic device, and applied the therapy at only 1 spot on the penis (dorsal midshaft).

Interestingly, in the study by Assaly-Kaddoum et al,[20] using a GK-diabetic rat model, erectile function was further investigated ex vivo by conducting organ bath studies of the erectile tissue. The organ bath results showed that Li-ESWT in these rats did not increase erectile tissue's reactivity neither to acetylcholine nor to non-adrenergic, non-cholinergic stimulation, and, hence, the improvement in erectile function observed was likely independent of the NO/cyclic guanosine monophosphate (cGMP) pathway.[20] Additionally, and in line with these findings, sildenafil, a phosphodiesterase-5 inhibitor, potentiated the improvement of the ICP/MAP ratio observed after Li-ESWT. Histologic examination of erectile tissue for endothelial nitric oxide (NO) synthase (eNOS) expression was not reported in this study.

Vasodilation/NO

Vasodilation in Organs Other Than Erectile Tissue

Many experimental studies showed that Li-ESWT increases the expression of eNOS.[31−37] It was also shown, using biochemical assays performed immediately after treatment, that Li-ESWT led to eNOS activation in HUVECs[31] and neuronal-NOS (nNOS) activation in glioma cells,[32] resulting in NO production in both cell types. This is direct evidence that Li-ESWT at an EFD as low as 0.03 mJ/mm^2 can stimulate NO release, via activation of eNOS or nNOS.

Goertz et al[38] showed that Li-ESWT applied to a normal mouse ear resulted in increased venular diameter ($+18\%$) and venous blood flow ($+50\%$), 10 minutes after treatment. However, arteriolar diameter was slightly reduced in these mice (-6%).[38] Additionally, Krokowicz et al[39] suggested that the observations on the microcirculation are short-term effects, and the positive long-term results are maintained through the anti-inflammatory action.

Vasodilation in the Corpora Cavernosa

Immunohistologic examination of erectile tissue from Li-ESWT-treated rats in STZ and BCNI models showed increased expression of eNOS and nNOS, as well as cGMP, at the time of erectile function evaluation, ≤4 weeks after Li-ESWT.[17−19,21−23,29] Taken together, the evidence suggests that Li-ESWT may result in enhanced NO production by activation and up-regulation of eNOS and nNOS. The contribution of this pathway to shockwave efficacy is yet to be determined outside the GK rat model, despite the results of the ex vivo organ bath experiments by Assaly-Kaddoum et al.[20] Early activation of the NO vasodilation cascade could explain why some patients report improved erectile function within 1 or 2 days of their first shockwave session.

Furthermore, in a recent study in naturally aged rats, Li-ESWT seems to alter the expression's ratio of adrenergic receptors in the corpora (increasing expression of α2-adrenergic receptor and simultaneously decreasing expression of α1-adrenergic receptor), indicating a possible decrease in sympathetic activity.[24] This may lead to easier smooth muscle relaxation through NO or other erectile agents, resulting in vasodilation and erection. The possible decrease in sympathetic activity wasn't proved with functional tests, which is a major limitation of this study.

Table 1. Experimental preclinical studies (in vivo + in vitro) on Li-ESWT for ED in erectile tissue of diabetic-ED models

Study	Model	Device	Treatment protocol (sessions × pulses, EFD)	Results	Erectile function	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Shan et al[17]	STZ-diabetic Sprague-Dawley rats	Shockwave applicator (Shenzhen Hyde Medical Equipment Co.)	• 6 × 300 (1,800) • 3×/wk for 2 wks, with 1-wk interval • EFD: 0.082 mJ/mm^2	↑ Survival of BMSCs ↑ ICP/MAP ratio ↑ CD31, SDF-1, VEGF, eNOS, α-SMA, muscle/collagen ratio • SWT + BMSC had greater effects	☑		☑	☑			☑	• Li-ESWT may be synergistic with stem cell transplantation
Ortec et al[18]	STZ-diabetic Sprague-Dawley rats	Electrohydraulic focused (Omnispec ED 1000, Medispec Ltd.)	• 6 × 300 (1,800) • 3×/wk for 2 wks • EFD: 0.1 mJ/mm^2	↑ The expression of eNOS and VEGF in erectile tissue after SWT		☑	☑					• Angiogenesis is a potential mechanism of action of Li-ESWT for ED
Jeong et al[19]	STZ-diabetic Sprague-Dawley rats	Electromagnetic focused (Urontech Co.)	• 6 × 300 (1,800) • 3×/wk for 2 wks • EFD: 0.1 mJ/mm^2	↑ ICP/MAP and AUC/MAP ↑ muscle/collagen ratio ↑ VEGF, nNOS, eNOS, PECAM-1, and cGMP	☑	☑	☑			☑	☑	• Improve erectile function • Restore erectile tissue components (nerves, endothelium, and smooth muscle)
Assaly-Kaddoum et al[20]	GK rats (type II diabetes model)	Electrohydraulic focused (Omnispec ED 1000, Medispec Ltd)	• 12 × 300 (3,600) • 2×/wk for 3 wks, 3-wk break, 2×/wk for 3 wks. • EFD: 0.09 mJ/mm^2	↑ Improved ICP/MAP and AUC/MAP ratio. ↑ Li-ESWT + PDE5i more effective • No change in ex vivo cavernosal strip response to ACh, SNP or NANC stimulation	☑							• Li-ESWT improves erectile function independently of NO/cGMP pathway • Li-ESWT + sildenafil potentiates the effect
Lei et al[21]	STZ-diabetic Sprague-Dawley rats	LIPUS (WBL-ED, WanBeiLi)	• 6 × 300 (1,800)	↑ ICP/MAP ↑ endothelium and smooth	☑	☑	☑			☑	☑	• LIPUS therapy (300 mW/cm^2) improved

(continued)

Table 1. Continued

Study	Model	Device	Treatment protocol (sessions × pulses, EFD)	Results	Erectile function	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
		Electrohydraulic focused (DermaGold, MTS Europe GmbH).	• 3×/wk for 2wks • 100, 200 or 300 mW/cm^2 (LIPUS) • EFD: 0.1 mJ/mm^2 (Li-ESWT)	muscle, collagen I/collagen III, elastic fibers, ↑ eNOS and nNOS expression ↓ TGF-b1/Smad/CTGF signaling pathway								erectile function and reversed pathologic changes in penile tissue of STZ-induced diabetic rats in equally to LI-ESWT (0.1 mJ/mm^2)
Qiu et al[22]	STZ-diabetic Sprague-Dawley rats	Electrohydraulic focused (DermaGold, MTS Europe GmbH).	• 6 × 300 (1,800) • 3×/wk for 2wks. • EFD: 0.1 mJ/mm^2	↑ ICP/MAP ↑ nNOS-positive nerves in the sinusoids, dorsal arteries, and nerves ↑ Endothelium and smooth muscle ↑ EdU+ cells	☑		☑	☑		☑	☑	↑ Partial restoration of erectile function and cavernosal nerves (nNOS), endothelium and smooth muscle ↑ Recruitment of endogenous MSCs
Liu et al[23]	STZ-diabetic Sprague-Dawley rats	Electromagnetic (HaiBing medical equipment limited Co.)	• • 6 × 100 (600) or 6 × 200 (1200) or •6 × 300 (1,800) • 3×/wk for 2 wks. • EFD: 0.057 mJ/mm^2	↑ ICP/MAP ↑ Smooth muscle and endothelial contents ↑ Expression of α-SMA, vWF, nNOS and VEGF ↓ The expression of RAGE • Better results with higher dose	☑		☑		☑	☑	☑	• Li-ESWT improves erectile function by restoring pathological changes of smooth muscle, endothelium and nerves in corpus cavernosum • The effect might relate to treatment dose positively

α-SMA = alpha-smooth muscle actin; ACh = acetylcholine; AUC = area under the curve; BMSC = bone marrow mesenchymal stem cells; CD31 = platelet and endothelial cell adhesion molecule−1; cGMP = cyclic guanosine monophosphate; CTGF = connective tissue growth factor; ED = erectile dysfunction; EdU = 5-ethynyl-20-deoxyuridine; EFD = energy flux density; eNOS = endothelial nitric oxide synthase; GK = Goto-Kakizaki; ICP = intracavernosal pressure; Li-ESWT = low-intensity extracorporeal shockwave therapy; LIPUS = low-intensity pulsed ultrasound; MAP = mean arterial pressure; NANC = non-adrenergic non-cholinergic; nNOS = neuronal nitric oxide synthase; PECAM-1 = platelet and endothelial cell adhesion molecule−1; RAGE = receptor for advanced glycation end products; SDF = stromal cell-derived factor; SMA = smooth muscle actin; Smad = protein-family that are the main signal transducers for receptors of the transforming growth factor beta (TGF-B) superfamily; SNP = sodium nitroprusside; STZ = streptozotocin; SWT = shockwave therapy; VEGF = vascular endothelial growth factor; vWF = von Willebrand factor.

Table 2. Experimental preclinical studies (in vivo + in vitro) on Li-ESWT for ED in erectile tissue of non-diabetic ED models

Study	Model	Device	Treatment protocol (sessions × pulses, EFD)	Results	Erectile Function	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Giuliano et al[16]	Spontaneously hypertensive rats	Electromagnetic (Dornier Aries, Dornier MedTech)	• 12 × 2,000 (24,000) • 2×/wk for 6 wks • EFD: 0.06 mJ/mm²	↑ ICP/MAP ratio, with additive effect of acute sildenafil ↑ smooth muscle/collagen ratio ↑ CD31 • No change in nNOS	☑	☑				☒	☑	• Li-ESWT improves erectile function • Li-ESWT + sildenafil potentiates the effect • Li-ESWT increases corporal smooth muscle and endothelial content
Sokolakis et al[24]	Naturally aged rats	Electrohydraulic focused (Omnispec ED1000, Medispec)	• 6 × 300 (1,800) • 3×/wk for 2 wks • EFD: 0.09 mJ/mm²	↑ VEGF, eNOS ↑ α2-adrenergic receptor/α1-adrenergic receptor ratio • No change in nNOS or NGF		☑				☒		• Li-ESWT induces angiogenesis • Possible decrease in sympathetic activity • Partially reverse changes associated with aging
Ruan et al[25]	ZF rats (ZUC-Leprᶠᵃ 185)	Electromagnetic defocused (LiteMed Inc, Taipei, Taiwan)	• 8 × 500 (4,000) • 2×/wk for 4 wks • EFD: 0.02 mJ/mm²	↑ ICP/MAP ratio ↑ smooth muscle/collagen • reverses endothelium damage (RECA-1) ↓ lipid accumulation • activates (EdU+) progenitor/stem cells	☑			☑			☑	• Li-ESWT restored erectile function and diminished obesity-related pathologic changes in ZF rats • Enhanced endogenous stem/progenitor cell proliferation and differentiation
Lin et al[26]	• Healthy male Sprague-Dawley rats • Rat Schwann cells and HUVECs	Electromagnetic semi-focused (LiteMed Inc, Taipei, Taiwan,	• 1 × 500 with • EFD: 0.057 mJ/mm2 • 1 × 300 with	↑ EdU+ cells in the sub-tunical region ↑ Schwann cell numbers, Ki-		☑	☑			☑		• Li-ESWT can activate the local penile progenitor cells in situ, with greater activation in younger mice

(continued)

Table 2. Continued

Study	Model	Device	Treatment protocol (sessions × pulses, EFD)	Results	Erectile Function	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
			• EFD: 0.02 mJ/mm^2	67 positivity, pERK1/2 ↑ tube formation of HUVECs in Matrigel								• Li-ESWT activates Schwann cells and endothelial cells in vitro
Wang et al[27]	• BCNI-model Sprague-Dawley rats • Rat Schwann cells	Electromagnetic unfocused (LiteMed, Taipei, Taiwan)	• Rats: 1 × 500 • EFD: 0.06 mJ/mm^2 • Cells: 1 × 0−1,000 • EFD: 0.01 mJ/mm^2	↑ BDNF expression, in vivo and in vitro • BDNF up-regulation in Schwann cells dependent on activation of PERK/ATF4 pathway						☑		• Li-ESWT may stimulate the expression of BDNF through activation of PERK/ATF4 signaling pathway
Li et al[28]	• BCNI-model Sprague-Dawley rats • Primary Schwann cells	Electrohydraulic focused (DermaGold, MTS Europe GmbH).	• 4 × 300 (1,200) • EFD: 0.06 mJ/mm^2 • 4 × 1000 (4,000) • EFD: 0.09 mJ/mm^2 • 1 × 200 with • EFD: 0.02 mJ/mm^2(cells)	↑ ICP/MAP ratio and AUC ↑ number of blood vessels ↑ nNOS+ nerves ↑ EdU+ cells ↑ SDF-1 expression ↑ p75 and p-Erk1/2 expression in nerves ↑ Schwann cell proliferation, pERK and p75	☑		☑	☑		☑	☑	• Improve erectile function • Improve vascular and neuronal tissue recovery • Potential mechanism through the recruitment of endogenous progenitor cells, angiogenesis and activation of Schwann cells

(continued)

Table 2. Continued

Study	Model	Device	Treatment protocol (sessions × pulses, EFD)	Results	Erectile Function	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Jeon et al[29]	BCNI-model Sprague-Dawley rats	Piezoelectric (PiezoWave2, Richard-Wolf GmbH)	• 9 × 300 (2,700) • 3×/wk for 3 wks • EFD: 0.1 mJ/mm²	↑ The ICP/MAP, α-SMA, nNOS, eNOS, cGMP ↑ β-III tubulin ↓ Apoptosis index ↑ VEGF	☑	☑	☑			☑		• Application of h-ADSCs improves nerve recovery • Li-ESWT induces angiogenesis • The combination has better results as each therapy alone
Müller et al[30]	75 Sprague-Dawley rats	Ballistic (Storz MP100, Storz Medical)	• 1 or 2 or 3 × 2,000 • EFD: 0.11 mJ/mm² • 3 × 1,000 (3,000) • EFD: 0.055 mJ/mm² • 1×/wk for 1 −3 wks	↓ ICP/MAP and smooth muscle/collagen ratio ↑ apoptotic index • Low energy showed less reduction in function	☒						☒	• SWT resulted in a time- and treatment-dependent reduction of ICP/MAP ratios, which might be mediated partly through apoptosis and collagenization of corporal smooth muscle

ADSC = adipose tissue—derived stem cell; ATF4 = activating transcription factor 4; BCNI = bilateral cavernous nerve injury; BDNF = brain-derived neurotrophic factor; CD31 = platelet and endothelial cell adhesion molecule—1; cGMP = cyclic guanosine monophosphate; ED = erectile dysfunction; EdU = 5-ethynyl-20-deoxyuridine; EFD = energy flux density; eNOS = endothelial nitric oxide synthase; HUVEC = human umbilical vein endothelial cell; ICP = intracavernosal pressure; Li-ESWT = low-intensity extracorporeal shockwave therapy; MAP = mean arterial pressure; NGF = nerve growth factor; nNOS = neuronal nitric oxide synthase; PERK = protein kinase RNA-like endoplasmic reticulum kinase; RECA = rat endothelial cell antigen—1; SDF-1 = stromal cell—derived factor 1; SWT = shockwave therapy; VEGF = vascular endothelial growth factor; ZF = Zucker fatty.

Table 3. Experimental preclinical studies (in vivo + in vitro) on Li-ESWT on neuronal tissue (without studies on penile tissue)

Study	Model	Treatment protocol (device, sessions × pulses, EFD)	Results	Neuronal function	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Ciampa et al[32]	Rat glioma cell line C6 cultures	• Electromagnetic (MODULITH SLK, Storz Medical) • 1 × 500 • EFD: 0.03 mJ/mm² to 0.11 mJ/mm²	↑ nNOS activity and NO production		☑			☑			Vasodilation Anti-inflammatory activity
Yahata et al[43]	Sprague-Dawley rats with SCI	• Electromagnetic (Duolith SD-1, Storz Medical) • 9 × 200 in 2 spots (3,200) • EFD: 0.25 mJ/mm²	↑ VEGF expression in neurons, astrocytes, and oligodendrocyte ↑ CD31 and α-SMA ↑ 5-HT—positive axons ↓ Apoptosis (TUNEL)			☑			☑	☑	Promotes VEGF expression in various neural cells and enhances angiogenesis in the injured spinal cord
Lee et al[56]	Sprague-Dawley rats with spinal cord injury	• Ballistic (Dolor-Clast, EMS) • 1 × 1,000 • EFD: 0.04 mJ/mm²	↑ SDF-1, CXCR4, VEGF and neuro-trophic factors (BDNF)				☑		☑	☑	Causes alterations of the microenvironment for the cell therapy
Lobenwein et al[66]	Spinal cord ischemia model in mice + Spinal slice cultures ex vivo	• Electrohydraulic (OrthoGold, TRT) • 1 × 500 or 1 × 300 (cell cultures) • EFD: 0.1 mJ/mm² or 0.08 mJ/mm² (cell cultures)	↓ Degenerating neurons. ↑ Expression VEGF and HIF-1a ↓ Inflammatory response. • The effect is dependent on TLR3 and not TLR4	☑				☑	☑		Protects from neuronal degeneration and improves functional outcome and survival in spinal cord ischemia
Lee et al[67]	Rats with sciatic nerve-crushing damage	• Device N/A • 1 × 300 • EFD: 0.09 mJ/mm²	↑ Expression of neu-rotrophin-3 • Facilitated the activity of macro-phages and Schwann cells					☑	☑		Improves the survival and regeneration of neurons
Yamaya et al[68]	Sprague-Dawley rats with SCI	• Device N/A • 9 × 200 (1,800) • EFD: 0.25 mJ/mm²	↑ Expression of VEGF and Flt-1 in spinal cord without any detrimental effect	☑				☑	☑		Enhances the neuroprotective effect of VEGF, reducing secondary injury and increasing locomotor recovery

Table 3. Continued

Study	Model	Treatment protocol (device, sessions × pulses, EFD)	Results	Neuronal function	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Kenmoku et al[69]	Sprague—Dawley rats	• Ballistic (Dolor-Clast, EMS) • 1 × 2000 • EFD: 0.18 mJ/mm^2	• Application induced degeneration of acetylcholine receptors • CMAP amplitude of the treated muscles was significantly decreased	☒					☒		Transient dysfunction of nerve conduction at neuromuscular junctions
Hausner et al[70]	Sprague—Dawley rats with sciatic nerve injury and autologous graft	• Orthowave, (MTS, Switzerland) • 1 × 300 • EFD: 0.1 mJ/mm^2	• Electrophysiological observations revealed marked values of amplitude and compound nerve action potential ↑ Myelinated nerve fibers • No difference in vessels	☑		☒			☑		Improves the rate of axonal regeneration, probably involving faster Wallerian degeneration and improved removal of degenerated axons

5-HT = 5-hydroxytryptamine; α-SMA = alpha-smooth muscle actin; BDNF = brain-derived neurotrophic factor; CD31 = platelet and endothelial cell adhesion molecule—1; CMAP = compound muscle action potential; CXCR4 = C-X-C chemokine receptor type 4; EFD = energy flux density; EMS = Electro Medical Systems; Flt-1 = Flt-1: vascular endothelial growth factor receptor-1; HIF-1a = hypoxia inducible factor-1a; Li-ESWT = low-intensity extracorporeal shockwave therapy; nNOS = neuronal nitric oxide synthase; NO = nitric oxide; SCI = spinal cord injury; SDF-1 = stromal cell—derived factor 1; TLR3 = Toll-like receptor 3; TLR4 = Toll-like receptor 4; TRT = Tissue Regeneration Technologies; VEGF = vascular endothelial growth factor.

Table 4. Experimental pre-clinical studies (in vivo + in vitro) on Li-ESWT on stem cells (without studies on penile tissue)

Study	Model	Treatment protocol (device, sessions × pulses, EFD)	Results	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Zhang et al[37]	EPCs	• Electrohydraulic (Orthospec, Medispec Ltd) • 1 × 140−500 • EFD: 0.04 - 0.13 mJ/mm^2	↑ Expressions of eNOS, Ang-1, Ang-2, and Bcl-2 ↓ IL-6, FGF-2, C-X-C chemokine receptor type 4, VEGF, Bcl-2-associated × protein, and caspase 3	☑	☑	☑	☑			The shock intensity ranging from 0.10−0.13 mJ/mm^2 and shock number ranging from 200−300 impulses were the optimal parameters
Sheu et al[45]	Male mini-pigs. myocardial infarction (AMI) models. Li-ESWT + BMDMSCs	• Device N/A • 1 × 300 • EFD: 0.12 mJ/mm^2	↑ Protein expression of SDF-1α, CXCR4, VEGF, angiopoietin and four other pro-angiogenic factors. ↑ Cells positive for CD31, CXCR4, VEGF, and vWF ↓ Protein expression of MMP-9, TNF-α, and NF-κB ↓ Expression of NOX-1, NOX-2, oxidized protein mitochondrial Bax, cleaved caspase 3, and PARP	☑	☑	☑				Inhibits inflammatory stimuli, oxidative stress and enhances angiogenesis
Aicher et al[57]	Athymic nude rats + injection of xenogenic human EPCs	• Electromagnetic (Dornier) • 1 × 500 or 1 × 1,000 or 1 × 2,000 • EFD: 0.05 mJ/mm^2	↑ SDF-1 mRNA ↑ Number of VEGF+ cells	☑	☑					Mediates preconditioning on EPC recruitment
Di Meglio et al[58]	Fisher-344 rats as models of AMI	• Electromagnetic (Duolith Vet, Storz) • 3 × 100 (300) • EFD: 0.25 mJ/mm^2	↑ c-kit-positive, Ki67-positive, orthochromatic cells, corresponding to cardiac primitive cells		☑				☑	Enhances tissue regeneration and myocardial regeneration

(continued)

Table 4. Continued

Study	Model	Treatment protocol (device, sessions × pulses, EFD)	Results	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Zhao et al[59]	Cultured rat BMDMSC	• Device N/A • 1 × 100 • EFD: 0.09 mJ/mm²	↑ VEGF, CXCL-5, and PCNA ↑ The growth of major pelvic ganglia neurites		☑	☑		☑		Enhances the secretion and proliferation of BMSC, promotes angiogenesis and nerve regeneration
Yip et al[60]	BMDMNCs	• Device N/A • 1 × 140 or 1 × 280 or 1 × 560 • EFD: 0.09mJ/mm²	↑ VEGF ↑ The formation of BMDMNCs into CD31+ cells		☑	☑				Accelerates the differentiation of BMDMNCs into endothelial phenotype cells

AMI = acute myocardial infarction; Ang-1 = Angiopoietin 1; Ang-2 = Angiopoietin 2; BAX = bcl-2–like protein 4; Bcl-2 = B-cell lymphoma 2; BMDMNC = bone marrow–derived mononuclear cells; BMSC = bone marrow mesenchymal stem cells; CD31 = platelet and endothelial cell adhesion molecule–1; CXCL-5 = C-X-C chemokine receptor type 5; CXCR4 = C-X-C chemokine receptor type 4; EFD = energy flux density; eNOS = endothelial nitric oxide synthase; EPC = endothelial progenitor cells; FGF-2 = fibroblast growth factor 2; IL-6 = interleukin-6; Li-ESWT = low-intensity extracorporeal shockwave therapy; MMP-9 = Matrix metallopeptidase 9; mRNA = messenger RNA; NF-κB = nuclear factor 'kappa-light-chain-enhancer' of activated B-cells; NOX-1 = NADPH oxidase 1; NOX-2 = NADPH oxidase 2; PARP = poly-(ADP-ribose) polymerase; PCNA = proliferating cell nuclear antigen; SDF-1α = stromal cell–derived factor 1α; TNF-α = tumor necrosis factor–α; VEGF = vascular endothelial growth factor; vWF = von Willebrand factor.

Neoangiogenesis

Neoangiogenesis in Organs Other Than Erectile Tissue

Up-regulation of angiogenic growth factors and increase in endothelial cell number or capillary density after Li-ESWT is a remarkably consistent finding across all investigated disease models and tissue types.[33,40–54] Within 24 hours of Li-ESWT, up-regulation of growth factors such as VEGF-A, its receptor VEGFR-2, and angiopoietin, are frequently reported.[33,40,41,45–48,50,53,54] A positive effect of Li-ESWT on lymphangiogenesis has also been shown, with increased expression of VEGF-C and its receptor VEGFR-3.[49,52] Increased expression of phosphatidylinositol-glycan biosynthesis class F protein, which amplifies the activity of VEGF, has also been demonstrated.[46]

Li-ESWT–induced neoangiogenesis is physiologically significant, as shown by its enhancement of survival of skin or musculocutaneous flaps[33,42,55–57]. All of these studies reported a positive effect of Li-ESWT on microcirculation. They reported increased tissue perfusion,[33,54,56,57] with neovascularization and increased capillary density,[34,54,56,57] but also dilation of pre-existing vessels, possibly through the expression of eNOS and NO.[34,38,42]

In vitro studies on HUVECs showed that Li-ESWT induced phosphorylation and, hence, activation of pro-angiogenic effectors, such as extracellular signal–regulated kinases 1/2, protein kinase B, phosphatidylinositol 3-kinase, and eNOS.[41,75] These effects are dependent on the expression of mechanosensory proteins, such as the caveolin-1 and beta-1-integrin (focal adhesion pathway)[43] or well-described sensors of fluid shear stress VEGFR-2, VE-cadherin, and platelet and endothelial cell adhesion molecule–1.[75] Significantly, Li-ESWT can restore angiogenic signaling, even in cells subjected to pro-atherosclerotic oscillatory flow conditions.[75] This suggests that, in addition to forming new endothelium, Li-ESWT may have protective, anti-atherosclerotic effects on existing endothelial cells.[76,77]

Neoangiogenesis in the Corpora Cavernosa

Increased expression of VEGF in the penis after Li-ESWT is a consistent finding in almost all studies, together with increased expression of endothelial markers such as platelet and endothelial cell adhesion molecule–1 (also known as CD31; constitutively expressed on endothelium), rat endothelial cell antigen-1 (constitutively expressed on endothelium), and von Willebrand factor (high levels indicate activated endothelium or angiogenesis[78]).[16–19,23–25,29] In STZ and BCNI rats, erectile tissue histology showed greatly reduced endothelial cell content in the cavernosal sinusoids in untreated animals, with a significant recovery after Li-ESWT.[22,28]

HUVECs form tubular networks within 6 hours of seeding in Matrigel. Li-ESWT–treated HUVECS formed more robust networks, with tube length and branch points increased by 42% and 43%, respectively, compared with control HUVECs.[26]

More progenitor cells were present in the erectile tissue after Li-ESWT in a BCNI model.[28] The contribution of these

Table 5. Experimental pre-clinical studies (in vivo + in vitro) on Li-ESWT on blood and lymphatic vessels (without studies on penile tissue)

Study	Model	Treatment protocol (device, sessions × pulses, EFD)	Results	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Mariotto et al[31]	HUAVEC cell cultures	• Electromagnetic (MODULITH SLK, Storz) • 1 × 500–1,500 • EFD: 0.03 mJ/mm^2	↑ eNOS activity and NO production	☑			☑			Vasodilation Anti-inflammatory activity
Goertz et al[38]	Fluorescent microscopy in the ears of hairless mice	• Electromechanic (Dornier AR2) • 1 × 500 • EFD: 0.015 mJ/mm^2 (low) • EFD: 0.04 mJ/mm^2 (high)	↓ The arteriolar diameter ↑ Venular diameter ↑ Venular red blood cell velocity ↑ Edema formation	☑						ESWT on the tissue seems to cause an initial slight mechanical trauma
Krokowicz et al[39]	Lewis rats focused on cremaster muscle	• Electrohydraulic (EvoTron, SanuWave) • 1 × 500 • EFD: 0.10 mJ/mm^2	• The changes in the vascular diameter observed in microcirculation are short-term	☑			☑			The positive and long-term result of action of ESWT is its anti-inflammatory action
Hatanaka et al[41]	Cultured HUVECs	• Electromagnetic (Duolith SD-1, Storz) • 1 × 800 • EFD: 0.03 mJ/mm^2	↑ Expression of VEGF and eNOS ↑ Phosphorylation of eNOS, Erk1/2 and Akt, and FAK ↑ Phosphorylation of caveolin-1 and the β1-integrin activity. • Knockdown of either caveolin-1 or β1-integrin suppressed the upregulation of VEGF and eNOS		☑					• Different pathways mediated the upregulation of VEGF and eNOS • Angiogenic signaling pathways through mechano-transduction proteins (caveolin-1 and β1-integrin)
Caron et al[44]	Hypertensive male Sprague–Dawley rats, though L-NAME	• Electrohydraulic (Medispec) • 12 × 400 (4,800) • EFD: 0.09 mJ/mm^2	• Peritubular capillaries and eNOS, VEGF, VEGF-R, SDF-1 gene expressions did not increase	☒	☒					Does not improve renal repair and angiogenesis in a hypertensive nephropathy model

Table 5. Continued

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Study	Model	Treatment protocol (device, sessions × pulses, EFD)	Results	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Serizawa et al[49]	Male Sprague-Dawley rats lymphoedema model	• Electromagnetic (Duolith SD-1, Storz) • 4 × 500 (2,000) • EFD: 0.25 mJ/mm²	↑ VEGF-C expression ↑ newly formed lymphatic vessels	☑					☑	Induces lymphangiogenesis and improves secondary lymphedema
Kubo et al[52]	A rabbit ear model of lymphedema	• Device N/A • 12 × 200 (2,400) • EFD: 0.09 mJ/mm²	↑ VEGFR-3 and the density of lymphatic vessels	☑					☑	Promotes lymphangiogenesis and ameliorates secondary lymphedema
Shao et al[62]	Male Sprague-Dawley rats, model of carotid artery injury	• R05 (HealthTronics) • 1 x 181 • EFD: 0.011 mJ/mm²	↓ Macrophages ↓ IL-18 and CD40 expression				☑			Attenuates inflammation in rat carotid artery
Tepekoylu et al[63]	Male 12- to 14-wk-old C57BL/6 mice with aortic xenograft	• Electrohydraulic (Orthogold, TRT) • 1 × 500 • EFD: 0.1 mJ/mm²	↑ Increase Macro-phage migration inhibitory factor and macrophage inflammatory protein 1β ↓ CD40 ligand and complement component C5/C5a ↑ TNF-α and IL-6 ↑ Macrophage infiltration ↑ Polarization -> M2 macrophages				☑		☑	Reduces the calcification of subcutaneously implanted decellularized xenografts via the modulation of the acute macrophage-mediated inflammatory response and improves the in vitro repopulation

Akt = protein kinase B; CD40 = Cluster of differentiation 40; EFD = energy flux density; eNOS = endothelial nitric oxide synthase; ERK1/2 = extracellular signal—regulated kinases 1/2; ESWT = extracorporeal shockwave therapy; FAK = focal adhesion kinase; HUVEC = human umbilical vein endothelial cells; IL-18 = interleukin-18; Li-ESWT = low-intensity extracorporeal shockwave therapy; L-NAME = L-NG-Nitroarginine Methyl Ester; NO = nitric oxide; SDF-1 = stromal cell—derived factor 1; VEGF = vascular endothelial growth factor.

Table 6. Experimental pre-clinical studies (in vivo + in vitro) on Li-ESWT on ischemic tissue (eg, myocardial infarct, skin burns, limp ischemia) (without studies on erectile tissue)

Study	Model	Treatment protocol (device, sessions × pulses, EFD)	Results	Tissue function	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Zhang et al[33]	Sprague-Dawley rats Ischemic skin flaps	• Electrohydraulic (Orthospec, Medispec) • 1 × 300 • EFD: 0.13 mJ/mm²	↑ Capillary density. ↑ Blood perfusion. ↑ vWF+ cells ↑ Expressions of chemotactic and angiogenic factors	☑	☑		☑				Improving the survival of ischemic skin flaps
Tao et al[34]	Domestic pigs, model of acute myocardial infarct (AMI)	• Device N/A • Treatment sessions N/A • EFD: 0.1 mJ/mm²	↑ Number of capillaries ↑ Expression of angiogenic factors (eg, VEGF)	☑	☑	☑				☑	Improvement in micro-vascular circulation and reconstruction of ischemic myocardial region
Goerzt et al[40]	Full-thickness burns to the ears of hairless mice	• Electromagnetic (Dornier AR2) • 3 × 500 (1500) • EFD: 0.04 mJ/mm² or 0.015 mJ/mm²	↑ Angiogenesis ↓ Non-perfused areas ↑ Number of rolling and sticking leukocytes as a part of an improved metabolism	☑		☑				☑	• Higher intensity (0.04 mJ/mm²) showed better results. • Healing through angiogenesis and improved metabolism
Holfeld et al[46]	Male adult C57/BL6 mice. Induced hind limb ischemia	• Electrohydraulic (OrthoGold, TRT) • 1 × 300 • EFD: 0.1 mJ/mm²	↑ Expression of VEGF-A, PlGF their receptors ↑ VEGFR phosphorylation showed a nearly five-fold increased activation of VEGFR-2 ↑ CD31+ cells	☑		☑					Biologic induction of neovascularization in addition to surgical or interventional revascularization could improve the outcome of ischemic tissue repair
Tepekoylou et al[47]	Hind limb ischemia model Sprague-Dawley rats	• Electrohydraulic (OrthoGold, TRT) • 1 × 300 • EFD: 0.1 mJ/mm²	↑ Circulating EPCs. ↑ VEGF-A ↑ Capillary density			☑					Induction of local angiogenesis in the ischemic muscle
Mittermayr et al[50]	Ischemic epigastric flap model in S-D rats	• Device N/A • 1 × 300 • EFD: 0.1 mJ/mm²	↑ Flap perfusion, microvessel number, and survival, irrespective of the timing of shockwave	☑		☑					Improves skin flap survival through neovascularization and early upregulation of

(continued)

Table 6. Continued

Study	Model	Treatment protocol (device, sessions × pulses, EFD)	Results	Tissue function	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
			treatment (pre-ischemia vs postischemia) ↑ VEGFR-2 expression								angiogenesis-related growth factors
Kuo et al[54]	Dorsal skin random flap model in 36 DS rats	• Device N/A • 1 × 500 or 2 × 500 (1,000) • EFD: 0.15 mJ/mm²	↑ Blood perfusion, VEGF and PCNA ↓ Leukocyte infiltration and TNF-α	☑				☑		☑	Rescues ischemic zone by increasing tissue perfusion and suppression of inflammatory response
Abe et al[65]	Male Sprague-Dawley rats. AMI model	• Electromagnetic (Storz Medical) • 3 × 200 (600) • EFD: 0.1 mJ/mm²	• Induced a polarity shift of the macrophage phenotype from M1 to M2. ↓ Expression of TGF-β1 and of proinflammatory cytokines (IL-1α, IL-4, IL-6, IL-12p70, IL-13, IL-17, and IFN-γ)					☑			Exerts anti-Inflammatory effects in a rat model of acute myocardial infarction
Yu et al[71]	H9c2 myoblast cell culture	• Electromagnetic (Modulith SLC, Storz) • 1 × 500 • EFD: 0.06 or 0.09 or 0.12 mJ/mm²	↑ Increased phosphorylation of AKT, which indicates the activation of the PI3K-AKT pathway ↓ expression of apoptosis-molecules					☑		☑	Protective effect against I/H-induced cell death, potentially by preventing the activation of components of the mitochondrial-dependent intrinsic apoptotic pathway
Lei et al[72]	25 domestic pig as model of AMI	• Electromagnetic (Storz Medical) • 3 × 200/spot × 9 spots (5400) • EFD: 0.09 mJ/mm²	• Ameliorates myocardial fibrosis in terms of collagen area fraction ↓ fibrocytes	☑						☑	Ameliorates myocardial fibrosis after AMI in pigs

AKT = protein kinase B; AMI = acute myocardial infarction; CD31 = platelet and endothelial cell adhesion molecule—1; EFD = energy flux density; IL = interleukin; INF-γ =interferon—γ; Li-ESWT = low-intensity extracorporeal shockwave therapy; PI3K = phosphatidylinositol 3-kinase; PIGF = phosphatidylinositol-glycan biosynthesis class F protein; TGF-β1 = transforming growth factor—β1; TNF-α = tumor necrosis factor—α; VEGF = vascular endothelial growth factor; VEGFR = vascular endothelial growth factor receptor; vWF = von Willebrand factor.

Table 7. Experimental preclinical studies (in vivo + in vitro) on Li-ESWT on wound healing (without studies on erectile tissue)

Study	Model	Treatment protocol (device, sessions × pulses, EFD)	Results	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Kuo et al[35]	STZ-induced diabetes Wistar rats + skin defect	• Defocused (MTS CP155, MTS) • 1 × 800 or 2 × 800 (1,600) or 3 × 800 (2,400) • EFD: 0.09 mJ/mm²	↑ Blood perfusion (laser Doppler) ↓ Inflammatory response ↑ PCNA, VEGF, eNOS	☑	☑		☑			Enhances wound healing
Yan et al[36]	Cranially based random-pattern flap model	• Device N/A • 1 × 750 • EFD: 0.09 mJ/mm²	↑ Increased blood perfusion ↑ expression of NO and VEGF ↑ Vasodilatation of pre-existing vessels at early stage ↑ Neovascularization at late stage	☑	☑					Improves the survival of skin flaps
Nacak et al[42]	Wistar rats transverse rectus abdominis musculocutaneous flap	• Ballistic (Elettronica Pagani SRL) • 1 × 500 • EFD: 0.1 mJ/mm²	↑ Capillary density and dilatation of microvessels ↓ Inflammation and interstitial edema ↑ Neovascularization and dense collagen fibrils	☑	☑		☑			Increases tissue perfusion and enhance skin flap survival
Hayashi et al[48]	eNOS-KO mice and normal C57BL/6 mice	• Electromagnetic (Duolith SD-1, Storz) • 1 × 100 • EFD: 0.25 mJ/mm²	↑ eNOS-dependent VEGF expression in skin wound tissues	☑						Accelerates the wound healing process in diabetic mice and is dependent on eNOS
Zins et al[51]	Mice with full-thickness excisional wound	• Electrohydraulic (Derma-Gold, TRT) • 1 × 200 • EFD: 0.1 mJ/mm²	↑ Expression of PECAM-1		☑					Promotes angiogenesis in wounds
Meier et al[53]	Sprague-Dawley rats epigastric skin flap model	• Electrohydraulic (Evotron, Sanuwave) • 1 × 500 • EFD: 0.11 mJ/mm²	↑ VEGF expression but not FGF2		☑					Acts via induction of specific growth factors
Kamelger et al[55]	Murine skin flap model in Sprague-Dawley rats	• Device N/A • 1 × 200 or 1 × 500 or 1 × 1,500 or 1 × 2,500 or 1 × 5,000 • EFD: 0.11 mJ/mm²	↓ Percentages of necrotic zones	☑					☑	Treatments between 500—2,500 shocks increases flap survival

Table 7. Continued

Study	Model	Treatment protocol (device, sessions × pulses, EFD)	Results	Vasodilation	Angiogenesis	Stem cells	Anti-inflammatory	Nerve regeneration	Tissue remodeling	Clinical interpretation
Chen et al[61]	Collagenase-induced tendonitis model S-D rats	• Electrohydraulic (MTS) • 1 × 200 • EFD: 0.16 mJ/mm^2	↑ PCNA, intensive TGF-β1 and IGF-I expression in tenocytes				☑		☑	Stimulates cell proliferation and tissue regeneration of tendon
Yang et al[64]	SZT-induced diabetic Wistar rat model	• Defocused (MTS CP155, MTS) • 1 × 100 × 8 areas (800) • EFD: 0.09 mJ/mm^2	↑ Up-regulation of haptoglobin ↓ Down-regulation of vitamin D—binding protein expression				☑			Enhances diabetic wound healing
Yang et al[73]	Diabetic S-D rats with incisional wound	• Electrohydraulic (Ortho-spec, Medispec) • 100/cm of wound • EFD: 0.11 mJ/mm^2	↑ Wound hydroxyproline content ↑ Expression of TGF-β1 ↑ Fibroblasts						☑	Improves the healing of incisional wound in diabetic rats
Cui et al[74]	Dermal fibroblasts derived from human hypertrophic scar tissue	• Electromagnetic (Duolith SD-1, Storz) • 1 × 1000 • EFD: 0.03 or 0.10 or 0.30 mJ/mm^2	• GAPDH and β-actin not affected • Bax protein and bcl-2, apoptotic factor not affected ↓ TGF-β1, α-SMA and vimentin ↓ Collagen 1a1, collagen-I protein, fibronectin, N-cadherin						☑	Li-ESWT induces anti-fibrotic effects

αSMA = alpha-smooth muscle actin; bcl-2 = B-cell lymphoma 2; EFD = energy flux density; eNOS = endothelial nitric oxide synthase; KO = knock-out; FGF2 = fibroblast growth factor 2; GAPDH = Glyceraldehyde 3-phosphate dehydrogenase; IGF-I = Insulin-like growth factor 1; Li-ESWT = low-intensity—extracorporeal shockwave therapy; MTS = MTS medical systems; NO = nitric oxide; PCNA = proliferating cell nuclear antigen; PECAM-1 = platelet and endothelial cell adhesion molecule—1; S-D = Sprague-Dawley; STZ = streptozotocin; TGF-β1 = transforming growth factor—β1; VEGF = vascular endothelial growth factor.

progenitors to neoangiogenesis is not determined. However, STZ rats receiving autologous bone marrow mesenchymal stem cell infusion combined with Li-ESWT showed greater improvement in erectile function and greater CD31 content (by computerized densitometry) than Li-ESWT alone.[17] Angiogenesis may be due to proliferation of existing endothelial cells, activation of local progenitor cells, or recruitment and activation of circulating endothelial progenitor cells (EPCs).[17,26,28]

Recruitment and Activation of Progenitor Cells

Recruitment or Activation of Progenitor Cells in Organs Other Than Erectile Tissue

Various studies reported the recruitment and activation of progenitor cells, especially EPCs, after Li-ESWT. Increased progenitor cell numbers after Li-ESWT (with and without exogenous stem cell therapy) have been reported in diverse tissues such as the penis (to be discussed later), spinal cord,[56] skin flaps,[33,59] and skeletal muscle.[57] Recruitment is likely due to up-regulation of stem cell chemokine stromal cell—derived factor 1 (SDF-1a) and its receptor C-X-C chemokine receptor type 4.[44,45,56,57,59] Proliferation/differentiation may be via up-regulation of proliferating cell nuclear antigen and multiple growth factors such as VEGF.[35,54,59] Li-ESWT—induced stem cell recruitment/activation has been linked to angiogenesis and tissue regeneration in spinal cord,[56] myocardium,[58] and skin wounds.[33] In vitro studies on bone marrow—derived mesenchymal cells showed that Li-ESWT could activate and accelerate the proliferation and differentiation of these cells into endothelial type cell, as well as promote angiogenesis and nerve regeneration.[59,60] Zhang et al[37] investigated the optimal dose of Li-ESWT on EPCs. They concluded that EFD doses from $0.10-0.13$ mJ/mm^2, with a number of shockwave pulses ranging from $200-300$, resulted in anti-inflammatory, angio-genetic, anti-apoptotic, and chemotactic alterations.[37]

Recruitment and Activation of Progenitor Cells in the Corpora Cavernosa

6 studies have investigated the effect of Li-ESWT on stem cells in erectile tissue.[17,22,25,26,28,29] 2 of these studies marked progenitor cells in newborn rats by injecting 5-ethynyl-20-deoxyuridine (EdU), treated with Li-ESWT at 12 weeks, and found greater numbers of these progenitors in the penis after Li-ESWT.[22,28] EdU incorporates into newly synthesized DNA and marks cells that have undergone cell division, marking progenitor/stem cells (EdU+). Ruan et al[25] injected intraperitoneal EdU into Zucker fatty rats (ZUC-Lepr^{fa} 185) at birth, showing that EdU+ progenitor/stem cells were activated after Li-ESWT. Lin et al[26] pulsed healthy young (12 weeks) and middle-aged (36 weeks) rats with EdU, followed by Li-ESWT, and harvested penile tissue at 48 hours and 1 week after Li-ESWT. Li-ESWT increased EdU+ cells in both age groups, with greater increase in the young rats. EdU+ cells were located in the subtunical (70—80%), para-sinusoid (10—19%), penile

blood vessels (3.8—6.7%), and penile nerve areas (1.9—5.3%). 2 other studies combined Li-ESWT with stem cell transplantation (ADSCs or bone marrow mesenchymal stem cells) and showed that Li-ESWT could recruit progenitor cells in the erectile tissue, increase transplanted stem cell survival, and enhance the pro-erectile effects of stem cell transplantation.[17,29] These studies imply that the recruitment or local activation of progenitor cells could be an important mechanism of action of Li-ESWT for ED.

Anti-Inflammatory Activity and Reduction in Cellular Stress

Anti-Inflammatory Activity, Reduction in Cellular Stress in Organs Other Than Erectile Tissue

A well-documented effect of Li-ESWT, which is not particularly investigated on erectile tissue, is its anti-inflammatory activity. In many studies, a reduced inflammatory response was observed after Li-ESWT, with fewer inflammatory cells and less interstitial edema.[35,42,54,61,62] Pro-inflammatory mediators such as tumor necrosis factor—α, transforming growth factor—β1 (TGF-β1), interleukin 1α (IL-1α), IL-4, IL-6, IL-12, p70, IL-13, IL-17, and interferon-γ, are reportedly down-regulated after Li-ESWT.[37,45,54,61—65] On the other hand, Goertz et al[40] reported that Li-ESWT applied after burn injury led to increased edema and sticking leukocytes compared with non-treated controls. However, the increase in inflammation markers in this study did not affect burn healing in Li-ESWT—treated mice, which still demonstrated accelerated angiogenesis compared with untreated controls.

1 possible mechanism by which Li-ESWT exerts its immune suppression effect is via NO. Low levels of NO (produced by eNOS and nNOS) is an immunosuppressant, but large bursts of NO (produced by iNOS) generated in response to immune stimuli results in formation of free radicals, cytotoxicity, and tissue damage.[79] Li-ESWT has been shown in vitro to activate eNOS and nNOS and suppress LPS-induction of iNOS,[31,32] thereby reducing the immune response and immune-related oxidative damage. Other markers of oxidative stress response are also reduced after Li-ESWT, including NADPH oxidase 1 (NOX-1), NOX-2, oxidized protein mitochondrial Bax, cleaved caspase 3, and poly-(ADP-ribose) polymerase.[45]

Chronic wounds exhibit a disrupted repair process (eg, due to aging, diabetes, vascular insufficiency) and typically remain in a prolonged inflammatory state. A key effector cell in wound healing is the macrophage, which may exhibit an inflammatory (M1) or wound healing (M2) phenotype. Li-ESWT has been shown in vitro to induce a shift from "inflammatory" M1 macrophages, toward the "wound healing" M2 phenotype.[63,65] An interesting study with decellularized aortic xenografts showed reduced graft rejection biomarkers in the animals treated with Li-ESWT after transplantation, with reduction of calcification and increased polarization toward M2-macrophages.[63]

Anti-Inflammatory Activity, Reduction in Cellular Stress in the Corpora Cavernosa

In the penis, it was observed that Li-ESWT decreased the expression of the receptor for advanced glycation end products (RAGE).[23] RAGE is up-regulated in the presence of advanced glycation end products, and both are highly associated with inflammation and the pathogenesis of diabetes.[80–82] Additionally, in the study by Jeon et al,[29] a decrease in the apoptotic index in erectile tissue after Li-ESWT was also observed. The terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (TUNEL) assay and 4′,6-diamidino-2-phenylindole staining of the nuclei were used for the evaluation of apoptosis. On the other hand, in the study by Müller et al,[30] a decrease in smooth muscle/collagen ratio and increase in apoptotic index were reported. Taken together, the evidence suggests that Li-ESWT may reduce inflammation and other oxidative stresses in the tissue microenvironment, resulting in increased cell survival and tissue repair.

Nerve Regeneration

Nerve Regeneration in Organs Other Than Erectile Tissue

In the last few years, an interest in the regenerating effects of Li-ESWT on nerves, especially after nerve injury, has emerged. Several studies proposed different mechanisms of action that could result in enhanced nerve recovery and regeneration after injury.[43,53,66–70] Many of these studies suggest that VEGF plays a crucial role in nerve regeneration, through a directly neuroprotective effect (reduced neuronal degeneration) and an improvement of the neuronal microenvironment (angiogenesis).[43,53,66,68] Some of these studies have also recorded amelioration of functional and electrophysiological outcomes on neuronal activity after Li-ESWT.[66,68,70] Another proposed mechanism for the improved rate of axonal regeneration involves a faster Wallerian degeneration, with increased removal of degenerated axons, providing a greater capacity of the injured axons to regenerate.[70] Furthermore, a direct effect on nerve regeneration by enhancing the expression of neurotrophin-3 and neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and increasing activity and proliferation of Schwann cells or 5-hydroxytryptamine–positive axons has been also observed.[42,53,67] Moreover, an anti-inflammatory effect of Li-ESWT through Toll-like receptor 3 could also be involved in the neuroprotective result.[66] On the other hand, a study on the effects of Li-ESWT on neuromuscular junctions showed a transient dysfunction of nerve conduction by degeneration of acetylcholine receptors.[69] In a study using a spinal cord injury model, a positive alteration of the microenvironment for cell therapy was shown through increased expression of SDF-1, VEGF, C-X-C chemokine receptor type 4, and neurotrophic factors such as BDNF.[56]

Nerve Regeneration in the Corpora Cavernosa

5 studies investigated the effects of Li-ESWT (in vivo and in vitro) on penile nerves.[22,26–29] In 2 of these studies, an increase and restoration of nNOS-positive nerve fibers in the sinusoids, dorsal arteries, and cavernous nerves after Li-ESWT were observed.[22,28] Furthermore, Jeon et al[29] showed an increase in β-III tubulin expression in the cavernous nerves of a BCNI rat models, indicating nerve regeneration after Li-ESWT in combination with ADSC transplantation.

In vitro studies with Schwann cell cultures showed that Li-ESWT activates Schwan cell proliferation, with increased expression of p75 and Ki-67 and phosphorylation of extracellular signal–regulated kinases 1/2 pathways.[26,28] Wang et al[27] showed in vivo and in vitro that Li-ESWT stimulates the expression of BDNF. In vitro, Schwann cell BDNF production was dependent on the activation of the protein kinase RNA-like endoplasmic reticulum kinase/activating transcription factor 4 pathway. The involvement of the protein kinase RNA-like endoplasmic reticulum kinase/activating transcription factor 4 pathway suggests that Li-ESWT may result in minor protein misfolding, thereby activating the endoplasmic reticulum stress response.

Therefore, it appears that Li-ESWT may support nerve recovery and regeneration by directly stimulating neuronal proliferation, or indirectly via activation of supporting functions such as Schwann cells and angiogenesis.

Fibrosis Reduction/Tissue Remodeling

Fibrosis Reduction/Tissue Remodeling in Organs Other Than Erectile Tissue

Li-ESWT has been shown to reduce fibrosis and improve physiological function after injury.[64,71] In models of myocardial infarction and myoblast cell cultures, regeneration of myocardial tissue and reduction in myocardial fibrosis were observed after Li-ESWT. Reduced numbers of fibrocytes, activation of primitive cardiac cells, and suppression of mitochondrial-dependent apoptotic pathways were reported, along with activation of the phosphatidylinositol 3-kinase-Akt pathway.[58,71,72]

Interestingly, in wound-healing, tendinopathy and cartilage-damage models, Li-ESWT seems to induce fibroblast/tenocyte/chondrocyte activation and proliferation, with production of TGF-β1 and different collagen subtypes.[42,61,64,65,72] Application of Li-ESWT to chronic wounds seems to accelerate and improve wound healing through the reduction of inflammation, promotion of angiogenesis, and proliferation of fibroblasts.[64,73] Conversely, Li-ESWT on fibroblasts from hypertrophic scars resulted in reduction in TGF-β1 and collagen production, allowing the fibroblasts to restore physiological function.[74]

Taken together, Li-ESWT to injured tissue results in stimulation of fibroblasts and collagen production but does not appear to result in hypertrophic scar formation. In fact, it may induce scar remodeling and improve tissue function.

Cavernous Tissue Remodeling

Using Masson's trichrome, immunohistochemistry, or immunofluorescence, it was observed that Li-ESWT increased smooth muscle/collagen ratio and promoted cavernous tissue

remodeling.[16,17,19,21,23,25] Furthermore, Lei et al[21] showed, using Hart's elastin stain, an increase in elastin fibers after Li-ESWT. Lei et al[21] also used Picrosirius red to describe the changes in collagen I/collagen III ratio; however, the use of this technique as a method to distinguish type I from type III collagen has been called into question.[83] The TGF-β1/Smad/connective tissue growth factor signaling pathway, which plays an important role in the fibrogenic process, was observed to be down-regulated in the study of Lei et al,[21] showing also an antifibrotic effect of Li-ESWT. Conversely, in the study by Müller et al,[30] a decrease of smooth muscle/collagen ratio, resulting in "collagenization" of the corpora cavernosa, has been reported. This is consistent with their finding of decreased erectile function, although this study has major limitations.[30]

A recent study by Ruan et al[25] highlighted intracavernous lipid accumulation as a consequence of obesity in leptin-deficient ZF rats. Lipid accumulation in the corpora has previously been described in orchiectomized rabbits[84] and human patients with difficult penile prosthesis insertion [85]. Intriguingly, 12-week-old ZF rats that received 8 sessions of Li-ESWT over 4 weeks had increased cavernosal endothelial and smooth muscle content, as well as decreased cavernosal lipid accumulation, as shown by immunohistochemistry.[25]

Summarizing, Li-ESWT may partially reverse fibromuscular pathologic changes of the smooth muscle of corpora cavernosa and restore the elasticity/expandability of the erectile tissue, as well as diminish obesity-related pathologic changes.

DISCUSSION

In this systematic scoping review, we identified numerous studies that investigated the effects of Li-ESWT on various tissues, including erectile tissue. Summarizing the results, we observe that Li-ESWT may improve ED via 5 main mechanisms: (i) circulation improvement; (ii) stem cell recruitment and activation; (iii) immune regulation; (iv) fibrosis reduction; (v) nerve repair.

We therefore propose the following model. Although the molecules and pathways have not all been verified using knockouts, knock downs, or inhibitors in vivo, the end results in terms of functional improvement and changes in tissue structure and cellular content appear to be fairly robust and reproduceable.

Circulation Improvement and Stem Cell Activation

It is known that the negative pressure phase of shockwaves can result in formation of microbubbles in the vasculature and tissue. Collapse of these "cavitation bubbles" could cause mild disruption of the endothelium and trigger repair mechanisms. In endothelial cells, shockwaves activate, perhaps through shear stress, signaling of transmembrane proteins such as caveolin-1 and beta-1-integrin. These membrane proteins, acting as mechanosensors, lead to up-regulation of VEGF and eNOS expression. NO is produced, resulting in vasodilation and improved circulation. Additionally, stem cell chemoattractant SDF-1 is released, attracting circulating endothelial progenitors, which contribute to the angiogenic process. Resident and newly recruited progenitor cells become activated and may further assist in repair of damaged erectile tissue. The end result is the restoration of damaged endothelium in diabetics, and possibly creation of healthy collateral vessels to bypass atherosclerotic vessels. Inflammation and an oxidative microenvironment has been postulated as the link between diabetes and tissue damage. Data from non-ED models show that Li-ESWT reduces inflammation, with down-regulation of cytokines such as IL-1, IL-6, and interferon-γ, and support of "wound-healing" M2 macrophages. In the penis, a decrease in RAGE after Li-ESWT will likely lead to a decrease in oxidative stress. Coupled with stem cell activation and improved blood flow, this environment results, with time, in reduced cavernosal fibrosis and restoration of smooth muscle content, perhaps via down-regulation of the TGF-β1/Smad/CTGF signaling pathway. Additionally, Li-ESWT might also trigger the endoplasmic reticulum stress response and enhance Schwann cell–mediated nitrergic-nerve repair after injury (Figure 2). These 5 mechanisms likely work in synergy to produce the functional improvements seen in various models of erectile function. In fact, the cells involved (endothelium, stem cells, immune cells, fibroblasts, nerves) are present in almost every tissue, and dysfunctions of these cells are the basis of multiple pathologic conditions.

Although Li-ESWT seems to stimulate fibroblasts and collagen production, it does not appear to result in hypertrophic scar formation. This data appears to contradict the claims that Li-ESWT reduces scarring, because hypertrophic scar tissue and keloids are due to an overproduction of fibroblasts and excessive collagen deposition. However, it can be reconciled by the fact that the wound-healing process consists of different stages. In normal wound healing, the initial inflammatory phase results in clot formation and recruitment of immune cells and fibroblasts. The second stage is proliferation, where granulation tissue is formed due to growth of new blood vessels, fueling fibroblast proliferation, differentiation into myofibroblasts, and collagen deposition. In the maturation stage, when tissue integrity is sufficiently restored, the myofibroblasts disappear in a wave of apoptosis, leaving a minimal scar. Failure to transition from the inflammatory to the proliferation stage results in chronic wounds; failure to transition from the proliferation stage to the maturation stage results in hypertrophic scar formation.[86] Application of Li-ESWT to chronic wounds could promote the transition from inflammatory to proliferation and maturation stage,[64,73] possibly by up-regulation of anti-scarring factor fibroblast growth factor-2.[87]

A common observed limitation in all studies of Li-ESWT for ED is the heterogeneity of the shockwave treatment. They used different types of shockwave applicators ranging from electro-hydraulic[18,20–22,24,28] and electromagnetic[16,17,19,23,25–27] to
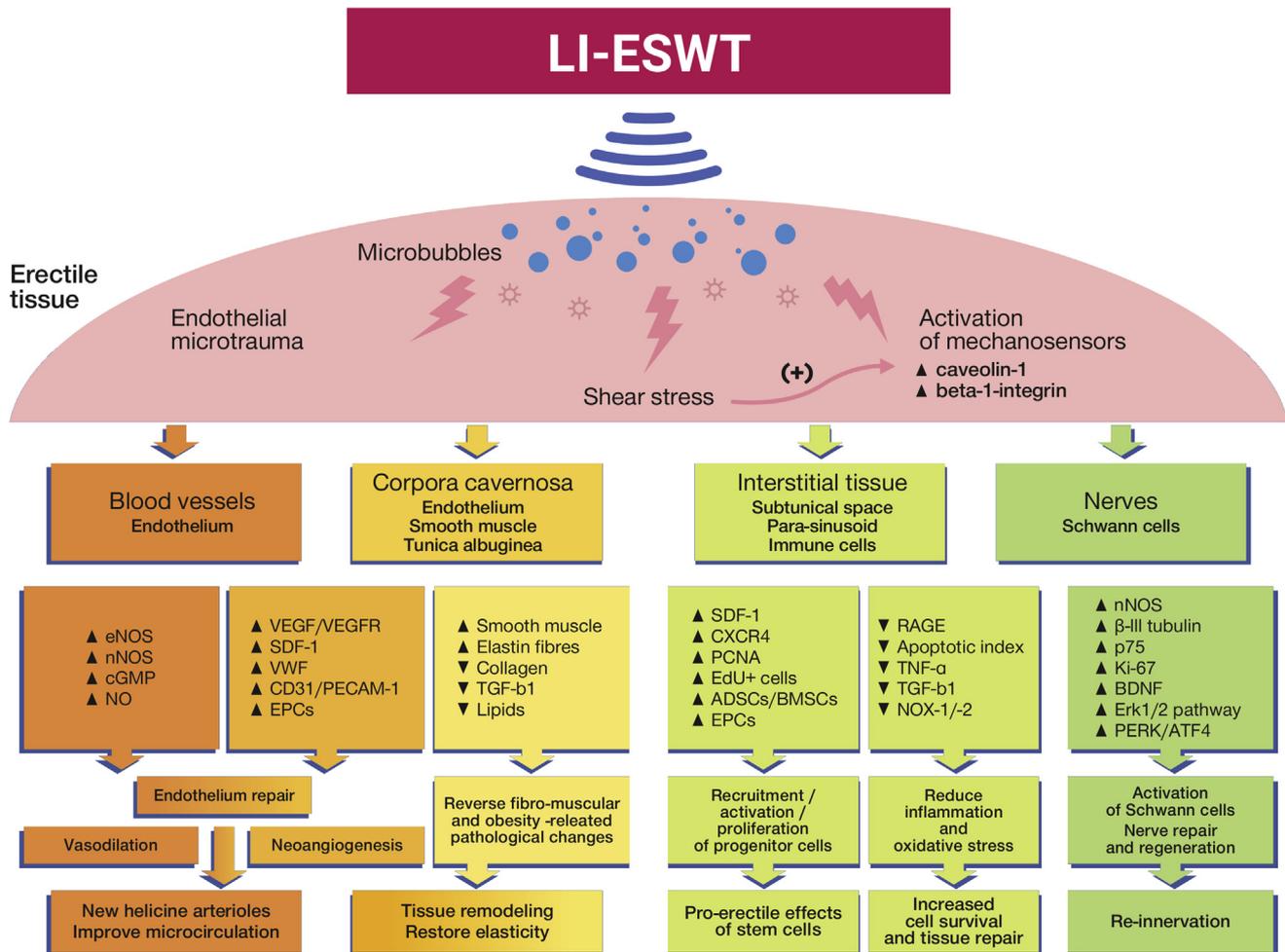
Figure 2. Putative mechanism of action of Li-ESWT for ED. ED = erectile dysfunction; Li-ESWT = low-intensity extracorporeal shockwave therapy. Figure 2 is available in color online at www.jsm.jsexmed.org.

piezoelectric[29] and ballistic/pneumatic,[30] whereas 1 study compared the use of low-intensity pulsed ultrasound to electrohydraulic Li-ESWT[21]. Furthermore, except for the electrohydraulic applicators, which all produce focused shockwaves, the other applicators produce focused, semi-focused, or unfocused shockwave forms, which results in different distribution of the energy in area and depth.

Another important heterogeneity of these studies is the different treatment protocols, ranging from 300 shockwaves[26] to 2,000 shockwaves[16,30] per session, and with energy flux density (EFD) ranging from 0.02 mJ/mm^2[25,26] to 0.11 mJ/mm^2.[30] 4 of these studies also conducted a comparison study between different treatment protocols, which showed that an EFD around 0.10 mJ/mm^2 could have better results, and that administering increased total number of shockwaves (around 4000) could also have better results, which also gives rise to the question of a possible saturation effect of Li-ESWT.[23,26,28,30]

Although these studies provide scientific evidence that Li-ESWT for ED works, there are many unanswered questions. First of all, there are different types of shockwaves, but no data about whether all types of shockwaves are equal in terms of

biologic effects. A study of waveforms produced by electrohydraulic and electromagnetic lithotripters, by Cleveland et al,[88] showed that the basic shapes of both waveforms are very similar, consisting of a shock front, a compressive phase, and a tensile tail; however, the exact physical parameters, such as the peak pressure, the focus size, EFD, and total energy, typically vary. Are the ballistic/pneumatic devices, mostly used in orthopedics, equally effective? A comparison study between different type of applicators is needed. In addition to the applicator type, the different wave forms (focused, semi-focused, or unfocused) should also be compared.

Furthermore, different Li-ESWT protocols should be investigated to identify the ideal EFD, number of sessions (including interval and frequency of the treatment) and total number of shockwaves to be used in different scenarios. For example, does it require more energy to stimulate nerves compared with endothelium? Does 100 pulses at 0.05 mJ/mm^2 = 50 pulses at 0.10 mJ/mm^2? How do we account for the 3-dimensional focal zone of the machines? To compare the different protocols and devices, new comparison indexes that would include the above-mentioned parameters should emerge, calculating the

"biological effective energy" of each protocol and device. Thus, it could be investigated whether there is a saturation effect of repeated treatment, and whether there is an upper limit of shockwaves or "energy" that can be safely applied.[89] Because we currently believe that the effect is energy-dependent, perhaps different treatment protocols should be applied, depending on the severity or the type of erectile dysfunction.

Additionally, the mechanism of action of Li-ESWT should be further investigated. Most of the studies are performed in animal models where ED induction (eg, STZ injection, cavernous nerve injury) is immediately followed by Li-ESWT, where ED is not allowed to be established. This is in contrast to clinical situation where we see patients not at the beginning of ED pathogenesis but much later when the dysfunction has settled. Therefore, future studies should aim to understand better the reversibility of ED with Li-ESWT. There are also many pathways and mechanisms involved in the pathophysiology of ED, which need to be investigated. For example, how does the sympathetic nervous system respond to Li-ESWT[90]? What is the role of anti-inflammatory activity and reduction in cellular oxidative stress in Li-ESWT for ED[91]? Furthermore, the effects of Li-ESWT for post-prostatectomy ED need further investigation in better animal models. The effect of Li-ESWT on aged erectile tissue is poorly investigated until now. Because ED is correlated with increased age and with specific pathophysiological consideration, further research on aged erectile tissue is needed[92]. Another important mechanism to investigate is the effect of Li-ESWT in combination treatment modalities, such as Li-ESWT + PDE5i, and how Li-ESWT can turn PDE5i non-responders to responders.

CONCLUSIONS

Li-ESWT seems to improve impaired erectile function in a variety of animal models of ED, possibly through stimulation of mechanosensors, inducing the activation of neoangiogenesis processes, recruitment and activation of progenitor cells, improvement of microcirculation, nerve regeneration, remodeling of erectile tissue with increase in the muscle/collagen ratio, and reducing inflammatory and cellular stress responses. These studies provide preliminary insights but no definitive answers, and many questions remain unanswered regarding the mechanism of action, the experimental setting for testing Li-ESWT, as well as the ideal treatment protocol.

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REFERENCES

1. Fode M, Hatzichristodoulou G, Serefoglu EC, et al. Low-intensity shockwave therapy for erectile dysfunction: is the evidence strong enough? Nat Rev Urol 2017;14:593-606.

2. Vardi Y, Appel B, Jacob G, et al. Can low-intensity extracorporeal shockwave therapy improve erectile function? A 6-month follow-up pilot study in patients with organic erectile dysfunction. Eur Urol 2010;58:243-248.

3. Clavijo RI, Kohn TP, Kohn JR, et al. Effects of low-intensity extracorporeal shockwave therapy on erectile dysfunction: A systematic review and meta-analysis. J Sex Med 2017; 14:27-35.

4. Lu Z, Lin G, Reed-Maldonado A, et al. Low-intensity extracorporeal shock wave treatment improves erectile function: A systematic review and meta-analysis. Eur Urol 2017;71:223-233.

5. Angulo JC, Arance I, de Las Heras MM, et al. Efficacy of low-intensity shock wave therapy for erectile dysfunction: A systematic review and meta-analysis. Actas Urol Esp 2017; 41:479-490.

6. Hutton B, Salanti G, Caldwell DM, et al. The PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-analyses of Health Care Interventions: Checklist and explanations. PRISMA Extension for Network Meta-analysis. Ann Intern Med 2015;162:777-784.

7. Armstrong R, Hall BJ, Doyle J, et al. "Scoping the scope" of a Cochrane Review. J Public Health 2011;33:147-150.

8. Arksey H, O'Malley L. Scoping studies: Towards a methodological framework. Int J Soc Res Methodol 2005;8:19-32.

9. Levac D, Colquhoun H, O'Brien KK. Scoping studies: Advancing the methodology. Implement Sci 2010;5:69.

10. Handa RK, Evan AP. A chronic outcome of shock wave lithotripsy is parenchymal fibrosis. Urol Res 2010;38:301-305.

11. Kisch T, Wuerfel W, Forstmeier V, et al. Repetitive shock wave therapy improves muscular microcirculation. J Surg Res 2016; 201:440-445.

12. Leeman JJ, Shaw KK, Mison MB, et al. Extracorporeal shockwave therapy and therapeutic exercise for supraspinatus and biceps tendinopathies in 29 dogs. Vet Rec 2016;179:385.

13. Huang HM, Li XL, Tu SQ, et al. Effects of roughly focused extracorporeal shock waves therapy on the expressions of bone morphogenetic protein-2 and osteoprotegerin in osteoporotic fracture in rats. Chin Med J (Engl) 2016;129:2567-2575.

14. Wanner S, Gstöttner M, Meirer R, et al. Low-energy shock waves enhance the susceptibility of staphylococcal biofilms to antimicrobial agents in vitro. J Bone Joint Surg Br 2011; 93:824-827.

15. Ha CH, Lee SC, Kim S, et al. Novel mechanism of gene transfection by low-energy shock wave. Sci Rep 2015; 5:22843.

16. Giuliano F, Assaly-Kaddoum R, Laurin M, et al. Low intensity-shockwave therapy (Li-ESWT) delivered by Aries improves erectile function and decreases cavernosal fibrosis of spontaneously hypertensive rats (SHR). Eur Urol Suppl 2018; 17:e1384.

17. Shan HT, Zhang HB, Chen WT, et al. Combination of low-energy shock-wave therapy and bone marrow mesenchymal stem cell transplantation to improve the erectile function of diabetic rats. Asian J Androl 2017;19:26-33.

18. Ortaç M, Küçükergin C, Salabaş E, et al. Effect of low-energy shockwave therapy on angiogenic factors in the penile tissue of diabetic rats. Turk J Urol 2017;43:130.

19. Jeong HC, Jeon SH, Qun ZG, et al. Effects of next-generation low-energy extracorporeal shockwave therapy on erectile dysfunction in an animal model of diabetes. World J Mens Health 2017;35:186-195.

20. Assaly-Kaddoum R, Giuliano F, Laurin M, et al. Low intensity extracorporeal shock wave therapy improves erectile function in a model of type II diabetes independently of NO/cGMP pathway. J Urol 2016;196:950-956.

21. Lei H, Xin H, Guan R, et al. Low-intensity pulsed ultrasound improves erectile function in streptozotocin-induced type I diabetic rats. Urology 2015;86; 1241.e11—1241.e18.

22. Qiu X, Lin G, Xin Z, et al. Effects of low-energy shockwave therapy on the erectile function and tissue of a diabetic rat model. J Sex Med 2013;10:738-746.

23. Liu J, Zhou F, Li GY, et al. Evaluation of the effect of different doses of low energy shock wave therapy on the erectile function of streptozotocin (STZ)-induced diabetic rats. Int J Mol Sci 2013;14:10661-10673.

24. Sokolakis I, Dimitriadis F, Psalla D, et al. Effects of low-intensity shock wave therapy (LiST) on the erectile tissue of naturally aged rats. Int J Impot Res 2018 Aug 17. https://doi.org/10.1038/s41443-018-0064-0 [Epub ahead of print].

25. Ruan Y, Zhou J, Kang N, et al. The effect of low-intensity extracorporeal shock wave therapy in an obesity-associated erectile dysfunction rat model. BJU Int 2018;122:133-142.

26. Lin G, Reed-Maldonado AB, Wang B, et al. In situ activation of penile progenitor cells with low-intensity extracorporeal shockwave therapy. J Sex Med 2017;14:493-501.

27. Wang B, Ning H, Reed-Maldonado AB, et al. Low-intensity extracorporeal shock wave therapy enhances brain-derived neurotrophic factor expression through PERK/ATF4 signalling pathway. Int J Mol Sci 2017;18:433.

28. Li H, Matheu MP, Sun F, et al. Low-energy shock wave therapy ameliorates erectile dysfunction in a pelvic neurovascular injuries rat model. J Sex Med 2016;13:22-32.

29. Jeon SH, Shrestha KR, Kim RY, et al. Combination therapy using human adipose-derived stem cells on the cavernous nerve and low-energy shockwaves on the corpus cavernosum in a rat model of post-prostatectomy erectile dysfunction. Urology 2016;88:226-e1—e9.

30. Muller A, Akin-Olugbade Y, Deveci S, et al. The impact of shock wave therapy at varied energy and dose levels on functional and structural changes in erectile tissue. Eur Urol 2008;53:635-642.

31. Mariotto S, Cavalieri E, Amelio E, et al. Extracorporeal shock waves: From lithotripsy to anti-inflammatory action by NO production. Nitric Oxide 2005;12:89-96.

32. Ciampa AR, de Prati AC, Amelio E, et al. Nitric oxide mediates anti-inflammatory action of extracorporeal shock waves. FEBS Lett 2005;579:6839-6845.

33. Zhang X, Yan X, Wang C, et al. The effect of autologous endothelial progenitor cell transplantation combined with extracorporeal shock-wave therapy on ischemic skin flaps in rats. Cytotherapy 2014;16:1098-1109.

34. Tao SM, Guo T, Wang Y, et al. Extracorporeal cardiac shock wave therapy improved myocardial micro-vascular circulation after acute myocardial infarction at early stage in pigs. Sichuan Da Xue Bao Yi Xue Ban 2011;42:222-226.

35. Kuo YR, Wang CT, Wang FS, et al. Extracorporeal shock-wave therapy enhanced wound healing via increasing topical blood perfusion and tissue regeneration in a rat model of STZ-induced diabetes. Wound Repair Regen 2009;17:522-530.

36. Yan X, Zeng B, Chai Y, et al. Improvement of blood flow, expression of nitric oxide, and vascular endothelial growth factor by low-energy shockwave therapy in random-pattern skin flap model. Ann Plast Surg 2008;61:646-653.

37. Zhang X, Yan X, Wang C, et al. The dose—effect relationship in extracorporeal shock wave therapy: The optimal parameter for extracorporeal shock wave therapy. J Surg Res 2014; 186:484-492.

38. Goertz O, Hauser J, Hirsch T, et al. Short-term effects of extracorporeal shock waves on microcirculation. J Surg Res 2015;194:304-311.

39. Krokowicz Ł, Mielniczuk M, Drews M, et al. Long-term follow up of the effects of extracorporeal shockwave therapy (ESWT) on microcirculation in a denervated muscle flap. Pol J Surg 2011;83:325-333.

40. Goertz O, Lauer H, Hirsch T, et al. Extracorporeal shock waves improve angiogenesis after full thickness burn. Burns 2012; 38:1010-1018.

41. Hatanaka K, Ito K, Shindo T, et al. Molecular mechanisms of the angiogenic effects of low-energy shock wave therapy: Roles of mechanotransduction. Am J Physiol Cell Physiol 2016;311:C378-C385.

42. Nacak U, Calis M, Atilla P, et al. Extracorporeal shock wave therapy as a delay procedure to improve viability of zone 4: An experimental study in a rat tram flap model. Ann Plast Surg 2016;77:e15-e20.

43. Yahata K, Kanno H, Ozawa H, et al. Low-energy extracorporeal shock wave therapy for promotion of vascular endothelial growth factor expression and angiogenesis and improvement of locomotor and sensory functions after spinal cord injury. J Neurosurg Spine 2016;25:745-755.

44. Caron J, Michel PA, Dussaule JC, et al. Extracorporeal shock wave therapy does not improve hypertensive nephropathy. Physiol Rep 2016;4:e12699.

45. Sheu JJ, Lee FY, Yuen CM, et al. Combined therapy with shock wave and autologous bone marrow-derived mesenchymal stem cells alleviates left ventricular dysfunction and remodeling through inhibiting inflammatory stimuli, oxidative stress & enhancing angiogenesis in a swine myocardial infarction model. Int J Cardiol 2015;193:69-83.

46. Holfeld J, Tepeköylü C, Blunder S, et al. Low energy shock wave therapy induces angiogenesis in acute hind-limb ischemia via VEGF receptor 2 phosphorylation. PLoS One 2014;9:e103982.

47. Tepeköylü C, Wang FS, Kozaryn R, et al. Shock wave treatment induces angiogenesis and mobilizes endogenous CD31/CD34-positive endothelial cells in a hindlimb ischemia model: Implications for angiogenesis and vasculogenesis. J Thorac Cardiovasc Surg 2013;146:971-978.

48. Hayashi D, Kawakami K, Ito K, et al. Low-energy extracorporeal shock wave therapy enhances skin wound healing in diabetic mice: A critical role of endothelial nitric oxide synthase. Wound Repair Regen 2012;20:887-895.

49. Serizawa F, Ito K, Matsubara M, Sato A, Shimokawa H, Satomi S. Extracorporeal shock wave therapy induces therapeutic lymphangiogenesis in a rat model of secondary lymphoedema. Eur J Vasc Endovasc Surg 2011;42:254-260.

50. Mittermayr R, Hartinger J, Antonic V, et al. Extracorporeal shock wave therapy (ESWT) minimizes ischemic tissue necrosis irrespective of application time and promotes tissue revascularization by stimulating angiogenesis. Ann Surg 2011; 253:1024-1032.

51. Zins SR, Amare MF, Tadaki DK, et al. Comparative analysis of angiogenic gene expression in normal and impaired wound healing in diabetic mice: Effects of extracorporeal shock wave therapy. Angiogenesis 2010;13:293-304.

52. Kubo M, Li TS, Kamota T, et al. Extracorporeal shock wave therapy ameliorates secondary lymphedema by promoting lymphangiogenesis. J Vasc Surg 2010;52:429-434.

53. Meirer R, Brunner A, Deibl M, et al. Shock wave therapy reduces necrotic flap zones and induces VEGF expression in animal epigastric skin flap model. J Reconstr Microsurg 2007;23:231-236.

54. Kuo YR, Wu WS, Hsieh YL, et al. Extracorporeal shock wave enhanced extended skin flap tissue survival via increase of topical blood perfusion and associated with suppression of tissue pro-inflammation. J Surg Res 2007;143:385-392.

55. Kamelger F, Oehlbauer M, Piza-Katzer H, et al. Extracorporeal shock wave treatment in ischemic tissues: What is the appropriate number of shock wave impulses? J Reconstr Microsurg 2010;26:117-121.

56. Lee JY, Ha KY, Kim JW, et al. Does extracorporeal shock wave introduce alteration of microenvironment in cell therapy for chronic spinal cord injury? Spine 2014;39:E1553-E1559.

57. Aicher A, Heeschen C, Sasaki KI, et al. Low-energy shock wave for enhancing recruitment of endothelial progenitor cells. Circulation 2006;114:2823-2830.

58. Di Meglio F, Nurzynska D, Castaldo C, et al. Cardiac shock wave therapy: assessment of safety and new insights into mechanisms of tissue regeneration. J Cell Mol Med 2012;16:936-942.

59. Zhao Y, Wang J, Wang M, et al. Activation of bone marrow-derived mesenchymal stromal cells—A new mechanism of defocused low-energy shock wave in regenerative medicine. Cytotherapy 2013;15:1449-1457.

60. Yip HK, Chang LT, Sun CK, et al. Shock wave therapy applied to rat bone marrow-derived mononuclear cells enhances formation of cells stained positive for CD31 and vascular endothelial growth factor. Circ J 2008;72:150-156.

61. Chen YJ, Wang CJ, Yang KD, et al. Extracorporeal shock waves promote healing of collagenase-induced Achilles tendinitis and increase TGF-β1 and IGF-I expression. J Orthop Res 2004; 22:854-861.

62. Shao PL, Chiu CC, Yuen CM, et al. Shock wave therapy effectively attenuates inflammation in rat carotid artery following endothelial denudation by balloon catheter. Cardiology 2010;115:130-144.

63. Tepeköylü C, Lobenwein D, Blunder S, et al. Alteration of inflammatory response by shock wave therapy leads to reduced calcification of decellularized aortic xenografts in mice. Eur J Cardio Thorac Surg 2014;47:e80-e90.

64. Yang MY, Chiang YC, Huang YT, et al. Serum proteomic analysis of extracorporeal shock wave therapy—enhanced diabetic wound healing in a streptozotocin-induced diabetes model. Plast Reconstr Surg 2014;133:59-68.

65. Abe Y, Ito K, Hao K, et al. Extracorporeal low-energy shock-wave therapy exerts anti-inflammatory effects in a rat model of acute myocardial infarction. Circ J 2014;78:2915-2925.

66. Lobenwein D, Tepeköylü C, Kozaryn R, et al. Shock wave treatment protects from neuronal degeneration via a toll-like receptor 3 dependent mechanism: Implications of a first-ever

causal treatment for ischemic spinal cord injury. **J Am Heart Assoc** 2015;4:e002440.

67. Lee JH, Kim SG. Effects of extracorporeal shock wave therapy on functional recovery and neurotrophin-3 expression in the spinal cord after crushed sciatic nerve injury in rats. **Ultrasound Med Biol** 2015;41:790-796.

68. Yamaya S, Ozawa H, Kanno H, et al. Low-energy extracorporeal shock wave therapy promotes vascular endothelial growth factor expression and improves locomotor recovery after spinal cord injury. **J Neurosurg** 2014;121:1514-1525.

69. Kenmoku T, Ochiai N, Ohtori S, et al. Degeneration and recovery of the neuromuscular junction after application of extracorporeal shock wave therapy. **J Orthop Res** 2012; 30:1660-1665.

70. Hausner T, Pajer K, Halat G, et al. Improved rate of peripheral nerve regeneration induced by extracorporeal shock wave treatment in the rat. **Exp Neurol** 2012;236:363-370.

71. Yu W, Shen T, Liu B, et al. Cardiac shock wave therapy attenuates H9c2 myoblast apoptosis by activating the AKT signal pathway. **Cell Physiol Biochem** 2014;33:1293-1303.

72. Lei PP, Tao SM, Shuai Q, et al. Extracorporeal cardiac shock wave therapy ameliorates myocardial fibrosis by decreasing the amount of fibrocytes after acute myocardial infarction in pigs. **Coron Artery Dis** 2013;24:509-515.

73. Yang G, Luo C, Yan X, et al. Extracorporeal shock wave treatment improves incisional wound healing in diabetic rats. **Tohoku J Exp Med** 2011;225:285-292.

74. Cui HS, Hong AR, Kim JB, et al. Extracorporeal shock wave therapy alters the expression of fibrosis-related molecules in fibroblast derived from human hypertrophic scar. **Int J Mol Sci** 2018;19:e124.

75. Ha CH, Kim S, Chung J, et al. Extracorporeal shock wave stimulates expression of the angiogenic genes via mechano-sensory complex in endothelial cells: Mimetic effect of fluid shear stress in endothelial cells. **Int J Cardiol** 2013;168:4168-4177.

76. Traub O, Berk BC. Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. **Arterioscler Thromb Vasc Biol** 1998;18:677-685.

77. Lu D, Kassab GS. Role of shear stress and stretch in vascular mechanobiology. **J R Soc Interface** 2011;8:1379-1385.

78. Zanetta L, Marcus SG, Vasile J, et al. Expression of von Willebrand factor, an endothelial cell marker, is up-regulated by angiogenesis factors: A potential method for objective assessment of tumor angiogenesis. **Int J Canc** 2000;85:281-288.

79. Förstermann U, Sessa WC. Nitric oxide synthases: Regulation and function. **Eur Heart J** 2011;33:829-837.

80. Wautier JL, Wautier MP, Schmidt AM, et al. Advanced glycation end products (AGEs) on the surface of diabetic erythrocytes bind to the vessel wall via a specific receptor inducing oxidant stress in the vasculature: A link between surface-associated AGEs and diabetic complications. **Proc Natl Acad Sci Unit States Am** 1994;91:7742-7746.

81. Schmidt AM, Hofmann M, Taguchi A, et al. RAGE: A multi-ligand receptor contributing to the cellular response in diabetic vasculopathy and inflammation. **Semin Thromb Hemost** 2000;26:485-493.

82. Yan SF, Ramasamy R, Schmidt AM. Receptor for AGE (RAGE) and its ligands—Cast into leading roles in diabetes and the inflammatory response. **J Mol Med** 2009;87:235-247.

83. Lattouf R, Younes R, Lutomski D, et al. Picrosirius red staining: A useful tool to appraise collagen networks in normal and pathological tissues. **J Histochem Cytochem** 2014;62:751-758.

84. Traish AM, Toselli P, Jeong SJ, et al. Adipocyte accumulation in penile corpus cavernosum of the orchiectomized rabbit: A potential mechanism for veno-occlusive dysfunction in androgen deficiency. **J Androl** 2005;26:242-248.

85. Alwaal A, Wang L, Zaid UB, et al. Case series of lipid accumulation in the human corpus cavernosum. **Medicine (Baltimore)** 2015;94:e550.

86. Lee HJ, Jang YJ. Recent understandings of biology, prophylaxis and treatment strategies for hypertrophic scars and keloids. **Int J Mol Sci** 2018;19:711.

87. Hausdorf J, Sievers B, Schmitt-Sody M, et al. Stimulation of bone growth factor synthesis in human osteoblasts and fibroblasts after extracorporeal shock wave application. **Arch Orthop Trauma Surg** 2011;131:303-309.

88. Cleveland RO, McAteer JA. The physics of shock wave lithotripsy. In: Smith AD, Badlani GH, Preminger GM, et al., eds. Smith's textbook of endourology. 2nd ed. Hamilton, Ontario, Canada: BC Decker; 2007. p. 529-558.

89. Hatzichristou D. Low-intensity extracorporeal shock waves therapy (LI-ESWT) for the treatment of erectile dysfunction: Where do we stand? **Eur Urol** 2017;71:234-236.

90. Giuliano F, Bernabe J, Jardin A, et al. Antierectile role of the sympathetic nervous system in rats. **J Urol** 1993;150:519-524.

91. Silva FH, Lanaro C, Leiria LO, et al. Oxidative stress associated with middle aging leads to sympathetic hyperactivity and downregulation of soluble guanylyl cyclase in corpus cavernosum. **Am J Physiol Heart Circ Physiol** 2014;307:H1393-H1400.

92. Toda N. Age-related changes in endothelial function and blood flow regulation. **Pharmacol Ther** 2012;133:159-176.