MTS Science White Paper©

# SoftWave™- Mesenchymal Stem Cell Therapy

## Research Assessment & Scientific Guide

Author: Sarah Engel (M.Sc.) (Jan, 2021)

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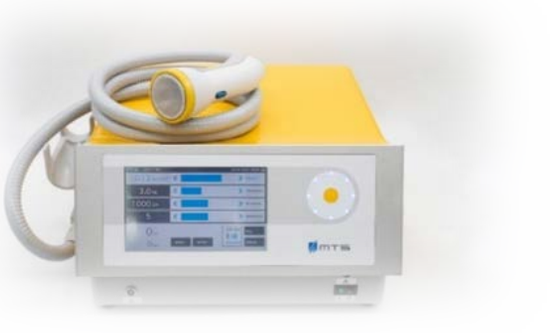

SPARK OF LIFE

# 1. Scientific Rationale – About Stem Cells and Extracorporeal Shock Wave Therapy

In order to maintain the natural body functions, new cells have to be generated permanently. **Stem cells** are the body's own reservoir to ensure this requirement, because many cells lose the ability to renew themselves once they have differentiated to take over a certain physiological function in the organism. Stem cells that are **unspecialized** have the ability to develop into many different cell types (called **differentiation**) of the body, and thus serve as an endogenous repair system to replace specialized cells that are used up by natual turn over or physical exertion or damaged by injury or disease.

Defining characteristics of stem cells:

- **Self-renewal:** They can divide and replicate themselves over a long time
- **Physiologically unspecialized:** They do not take over tissue-specific functions
- **Differentiation capacity:** They can divide and become specialized cells (e. g. muscle cell, blood cells, brain cells…)

The two main types are embryonic and adult stem cells. **Embryonic stem cells** (ESCs) exist only at the early stages of development and are **pluripotent**; meaning they have the unlimited capacity to differentiate into any cell type, which opens up an incredible potential for new therapeutic approaches. **Adult / tissue-specific / somatic stem cells** are **multipotent** and intended to maintain and repair tissue and organs throughout a lifetime. Multipotent means that these cells are type-specific for their source of origin. The multipotent cells are able to make a limited number of specialized cell types. Tissue-specific stem cells are not easy to isolate, and they do not renew themselves easily in culture between embryonic stem cells.

**Mesenchymal stem cells (MSCs)** are multipotent progenitor cells of different cell types that are derived from the mesenchymal *stroma* (= stromal cells); the connective tissue surrounding other organs and tissues. The first MSCs were isolated from bone marrow, but can be isolated from various tissue sources. The selection of the source is based on their logistical, practical, and in vitro characteristics. Currently, the main sources of MSCs are bone marrow, umbilical cord stroma, adipose tissue, and dental pulp. [1] They have a high proliferation and differentiation potential and can form bone and cartilage cells. These cells have a high regenerative and immunomodulatory potential.

In 2017, Caplan suggested that the name “mesenchymal stem cells” be changed to “medicinal signaling cells” to more accurately reflect the fact that, *in vivo*, these cells secrete bioactive factors that are immunomodulatory and atrophic, meaning these cells make medicinal drugs *in situ*. [2]

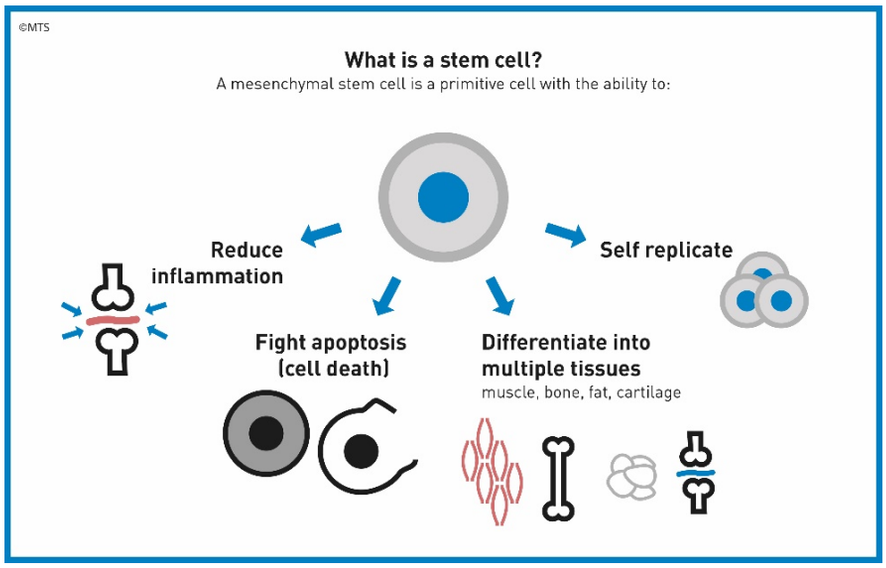


**Figure 1. Biological Function and Regenerative Potential of Stem Cells.**

**Extracorporeal Shock Wave Therapy (ESWT)** is activating endogenous tissue-resident MSCs. [3,4] Chemoattractant factors and a number of regeneration and growth-associated molecular signalling factors are released in the treatment area, which stimulates migration, proliferation and differentiation of MSCs, resulting in revascularisation, inhibition of inflammation, tissue regeneration and remodelling.[5–11] Due to this high self-regenerative potential of shock wave therapy, in the body, the spectrum of treatable diseases is constantly increasing: musculoskeletal disorders, wound healing, nerve regeneration, cardiovascular conditions, urogenital dysfunctions as well as many other clinical application areas. [12–15]

Although the clinical applications of autologous MSC therapy are broad and promising, they are limited by disadvantages regarding stem cell identity and potency of the isolated cell population. Furthermore, donor variability leads to a very heterogeneous cell composition and functionality, which may reduce reproducibility and efficacy and increase the risk of transplanting low-potency cells, with a low tissue integration rate, into the patient. Therefore, research is being conducted to identify methods that ensure a stably improved quality of MSCs for therapy. It was demonstrated that the stem cell activating effect of ESWT can be translated to application in classical regenerative stem cell therapy. ESWT improves the general constitution and viability of the cells and facilitates targeting, homing, and differentiation of the transplanted cells. Combination therapy of ESWT and preconditioning of stem cells *in vitro* and / or the donor area, *in vivo,* achieves a potentiated therapeutic effect. [16–21]

**Summary**

Because of their unique properties, stem cells have the potential to heal a wide range of conditions and hold out hope for a sustainable cure to the many devastating diseases which currently do not have one. Today, transplants are used to replace diseased or lost tissues / organs, but the demand far exceeds the availability. Stem cell therapies offer an alternative source of treatment for destroyed tissues, but there are significant technical hurdles which can only be overcome through intensive research and optimization of the methodology.

- Stem cells generate healthy cells to replace devastated cells, as therapy of many diseases (= regenerative medicine)

Low-energy SWT reduces tissue inflammation, apoptosis and anti-oxidative stress. It also increases angiogenesis, cell proliferation, and tissue repair.
ESWT activates endogenous MSCs and enhances recruitment and homing, as well as, improves their stemness and viability.
ESWT can enhance the efficacy of cell engraftment after intravenous transplantation of MSCs.
Combined treatment, of MCS therapy and ESWT, is superior to either one applied individually.

The fact that the therapeutic mechanisms of stem cell therapy as well as extracorporeal shock wave therapy are in direct biological interaction with each other, strongly implies that both therapies should be combined to **increase therapeutic potency** and achieve **maximum synergy**.

# 2. Preclinical and Preclinical Background

***Note: References written in dark blue text were performed with TRT / MTS SoftWave™ Technology.***

## 2.1. Evidence - Activating Effect of SWT on Endogenous Tissue-Resident MSCs *in situ*

❖ **Shock waves promote spinal cord repair via TLR3** [14]

*Medical University of Innsbruck, Innsbruck, Austria.*

Spinal cord injury (SCI) remains a devastating condition with poor prognosis and very limited treatment options. Affected patients are severely restricted in their daily activities. Shock wave therapy (SWT) has shown potent regenerative properties in bone fractures, wounds, and ischemic myocardium via activation of the innate immune receptor TLR3. Here, we report on the efficacy of SWT for regeneration of SCI. **SWT improved motor function and decreased lesion size in WT but not Tlr3-/- mice via inhibition of neuronal degeneration and IL6-dependent recruitment and differentiation of neuronal progenitor cells.** Both SWT and TLR3 stimulation enhanced neuronal sprouting and improved neuronal survival, even in human spinal cord cultures. We identified tlr3 as crucial enhancer of spinal cord regeneration in zebrafish. Our findings indicate that TLR3 signaling is involved in neuroprotection and spinal cord repair and suggest that TLR3 stimulation via SWT could become a potent regenerative treatment option.

❖ **Delayed Treatment with Low-Intensity Extracorporeal Shock Wave Therapy in an Irreversible Rat Model of Stress Urinary Incontinence** [22]

*Department of Urology, Knuppe Molecular Urology Laboratory, School of Medicine, University of California, San Francisco, CA.*

**Objective:** To determine the outcomes and mechanisms of delayed low-intensity extracorporeal shock wave therapy (Li-ESWT) in a rat model of irreversible stress urinary incontinence (SUI).
**Materials and methods:** Twenty-four female Sprague-Dawley rats were randomly assigned into 3 groups: sham control, vaginal balloon dilation + β-aminopropionitrile (BAPN; SUI group), and vaginal balloon dilation + BAPN + treatment with Li-ESWT (SUI-Li-ESWT group). An irreversible SUI model was developed by inhibiting the urethral structural recovery with BAPN daily for 5 weeks. Thereafter, in the SUI-Li-ESWT group, Li-ESWT was administered twice per week for 2 weeks. After a 1-week washout, all 24 rats were evaluated with functional and histologic studies at 17 weeks of age. Endogenous progenitor cells were detected via the EdU-labeling method.
**Results:** Functional analysis with leak point pressure testing showed that the SUI-Li-ESWT group had significantly higher leak point pressures compared with untreated rats. Increased urethral and vaginal smooth and striated muscle content and increased thickness of the vaginal wall were noted in the SUI-Li-ESWT group. The SUI group had significantly decreased neuronal nitric oxide /tyrosine hydroxylase positive nerves ratio in the smooth muscle layers of the urethra, while the SUI-Li-ESWT group had neuronal nitric oxide/tyrosine hydroxylase+ nerves ratio similar to that of the control group. The continuality of urothelial cell lining was also improved in the SUI-Li-ESWT group. **In addition, there were significantly increased EdU-positive cells in the SUI-Li-ESWT group.**
**Conclusion:** Li-ESWT appears to increase smooth muscle content in the urethra and the vagina, increase the thickness of urethral wall, improve striated muscle content and neuromuscular junctions, restore the integrity of the urothelium, and **increase the number of EdU-retaining progenitor cells in the urethral wall**.

- **Combined treatment with extracorporeal shockwaves therapy and an herbal formulation for activation of penile progenitor cells and antioxidant activity in diabetic erectile dysfunction** [23]
- **The effect of low-intensity extracorporeal shockwave therapy in an obesity-associated erectile dysfunction rat model** [24]
- **Treatment of stress urinary incontinence with low-intensity extracorporeal shock wave therapy in a vaginal balloon dilation induced rat model** [5]

*Knuppe Molecular Urology Laboratory, Department of Urology, School of Medicine, University of California, San Francisco, CA, USA.*

**Background:** To investigate the outcomes and mechanisms of low-intensity extracorporeal shock wave therapy (Li-ESWT) on stress urinary incontinence (SUI) in a vaginal balloon dilation (VBD) rat model.
**Methods:** Thirty Sprague-Dawley rats were randomly grouped into normal controls, VBD only, and VBD with Li-ESWT. Li-ESWT was administered twice per week for 3 weeks. Afterward, all 30 rats were assessed with functional and histological studies. To explore the acute effect of Li-ESWT, another 25 rats, given intraperitoneal 5-ethynyl-2-deoxyuridine (EdU) at birth, were treated with Li-ESWT followed by assessment of vascular endothelial growth factor (VEGF) expression and endogenous progenitor cells distribution at 24 hours or 1 week after the last Li-ESWT therapy. Additionally, rat myoblast L6 cells were used for myotube formation assay *in vitro*.
**Results:** Functional analysis with leak-point pressure (LPP) testing showed that rats treated with Li-ESWT following VBD had significantly higher LPP relative to those receiving VBD only (44.8±3.2 versus 27.0±2.9 $cmH_2O$, $P<0.01$). Histological examinations showed increased urethral sphincter regeneration in Li-ESWT group. The rats treated with Li-ESWT also had increased vascularity, which was confirmed by immunohistochemistry of rat endothelial cell antigen, while reverse-transcriptase polymerase chain reaction (RT-PCR) showed VEGF expression was significantly enhanced. Additionally, there were **significantly increased EdU+ cells in Li-ESWT treated rats** at 24 hours. *In vitro*, Li-ESWT promoted myotube formation from L6 cells.
**Conclusions: Li-ESWT ameliorated SUI by promoting angiogenesis, progenitor cell recruitment, and urethral sphincter regeneration in a rat model induced by VBD.** Li-ESWT represents a potential novel non-invasive therapy for SUI.

- **Shock Wave Therapy Improves Cardiac Function in a Model of Chronic Ischemic Heart Failure: Evidence for a Mechanism Involving VEGF Signaling and the Extracellular Matrix** [25]

*Cardiac Surgery Medical University of Innsbruck Austria.*

**Background** Mechanical stimulation of acute ischemic myocardium by shock wave therapy (SWT) is known to improve cardiac function by induction of angiogenesis. However, SWT in chronic heart failure is poorly understood. We aimed to study whether mechanical stimulation upon SWT improves heart function in chronic ischemic heart failure by induction of angiogenesis and postnatal vasculogenesis and to dissect underlying mechanisms.
**Methods and Results** SWT was applied in a mouse model of chronic myocardial ischemia. To study effects of SWT on postnatal vasculogenesis, wild-type mice received bone marrow transplantation from green fluorescence protein donor mice. Underlying mechanisms were elucidated in vitro in endothelial cells and murine aortic rings. Echocardiography and pressure/volume measurements revealed improved left ventricular ejection fraction, myocardial contractility, and diastolic function and decreased myocardial fibrosis after treatment. Concomitantly, numbers of capillaries and arterioles were increased. **SWT resulted in enhanced expression of the chemoattractant stromal**

**cell-derived factor 1 in ischemic myocardium and serum. Treatment induced recruitment of bone marrow-derived endothelial cells to the site of injury.** In vitro, SWT resulted in endothelial cell proliferation, enhanced survival, and capillary sprouting. The effects were vascular endothelial growth factor receptor 2 and heparan sulfate proteoglycan dependent.

**Conclusions** SWT positively affects heart function in chronic ischemic heart failure by induction of angiogenesis and postnatal vasculogenesis. SWT upregulated pivotal angiogenic and vasculogenic factors in the myocardium in vivo and induced proliferative and anti-apoptotic effects on endothelial cells in vitro. Mechanistically, these effects depend on vascular endothelial growth factor signaling and heparan sulfate proteoglycans. SWT is a promising treatment option for regeneration of ischemic myocardium.

- **Low-intensity extracorporeal shock wave therapy promotes myogenesis through PERK/ATF4 pathway** [26]
- **Induction of Endogenous Neural Stem Cells By Extracorporeal Shock Waves After Spinal Cord Injury** [27]
- **In Situ Activation of Penile Progenitor Cells With Low-Intensity Extracorporeal Shockwave Therapy** [28]
- **Endogenous Stem Cells Were Recruited by Defocused Low-Energy Shock Wave in Treating Diabetic Bladder Dysfunction** [3]
- **Extracorporeal Shock Wave Rebuilt Subchondral Bone In Vivo and Activated Wnt5a/Ca $^{2+}$ Signaling In Vitro** [29]
- **Extracorporeal Shock Wave Therapy Accelerates Regeneration After Acute Skeletal Muscle Injury** [30]
- **Low-energy Shock Wave Therapy Ameliorates Erectile Dysfunction in a Pelvic Neurovascular Injuries Rat Model** [31]

*University of California, San Francisco.*

**Introduction:** Erectile dysfunction (ED) caused by pelvic injuries is a common complication of civil and battlefield trauma with multiple neurovascular factors involved, and no effective therapeutic approach is available.

**Aims:** To test the effect and mechanisms of low-energy shock wave (LESW) therapy in a rat ED model induced by pelvic neurovascular injuries.

**Methods:** Thirty-two male Sprague-Dawley rats injected with 5-ethynyl-2'-deoxyuridine (EdU) at newborn were divided into 4 groups: sham surgery (Sham), pelvic neurovascular injury by bilateral cavernous nerve injury and internal pudendal bundle injury (PVNI), PVNI treated with LESW at low energy (Low), and PVNI treated with LESW at high energy (High). After LESW treatment, rats underwent erectile function measurement and the tissues were harvested for histologic and molecular study. To examine the effect of LESW on Schwann cells, in vitro studies were conducted.

**Main outcome measurements:** The intracavernous pressure (ICP) measurement, histological examination, and Western blot (WB) were conducted. Cell cycle, Schwann cell activation-related markers were examined in in vitro experiments.

**Results:** LESW treatment improves erectile function in a rat model of pelvic neurovascular injury by leading to angiogenesis, tissue restoration, and nerve generation with **more endogenous EdU(+) progenitor cells recruited to the damaged area** and activation of Schwann cells. LESW facilitates more complete re-innervation of penile tissue with regeneration of neuronal nitric oxide synthase (nNOS)-positive nerves from the MPG to the penis. In vitro experiments demonstrated that LESW has a direct effect on Schwann cell proliferation. Schwann cell activation-related markers including p-Erk1/2 and p75 were upregulated after LESW treatment.

**Conclusion:** **LESW-induced endogenous progenitor cell recruitment** and Schwann cell activation coincides with angiogenesis, tissue, and nerve generation in a rat model of pelvic neurovascular injuries.

- **Effects of Focused Extracorporeal Shock Waves on Bone Marrow Mesenchymal Stem Cells in Patients with Avascular Necrosis of the Femoral Head** [32]
- **Shock wave treatment induces angiogenesis and mobilizes endogenous CD31/CD34-positive endothelial cells in a hindlimb ischemia model: implications for angiogenesis and vasculogenesis** [33]

*University Hospital for Cardiac Surgery, Innsbruck Medical University, Innsbruck, Austria.*

**Objectives:** Shock waves have been shown to induce recruitment of intravenously injected endothelial progenitor cells to ischemic hind limbs in rats. We hypothesized that shock wave treatment as sole therapy would induce angiogenesis in this ischemia model and would lead to mobilization of endogenous endothelial (progenitor) cells.
**Methods:** A total of 18 rats, aged 5 weeks old, were subdivided into 3 groups: sham (n = 6), ischemic muscle with shock wave treatment (shock wave treatment group, n = 6), and without shock wave treatment (control, n = 6). Hind limb ischemia was induced by ligation of the femoral artery. Three weeks later, shock wave treatment (300 impulses at 0.1 mJ/mm(2)) was applied to the adductor muscle; the controls were left untreated. Muscle samples were analyzed using real-time polymerase chain reaction for angiogenic factors and chemoattractants for endothelial progenitor cell mobilization. Fluorescence activated cell sorting analysis of the peripheral blood was performed for CD31/CD34-positive cells. Perfusion was measured using laser Doppler imaging. Functional improvement was evaluated by walking analysis.
**Results:** **Angiogenic factors/endothelial progenitor cell chemoattractants, stromal cell-derived factor-1 and vascular endothelial growth factor, were increased in the treatment group**, as shown by real-time polymerase chain reaction, indicating the mobilization of endothelial progenitor cells. Fluorescence activated cell sorting analysis of the peripheral blood revealed high numbers of CD31/CD34-positive cells in the treatment group. Greater numbers of capillaries were found in the treated muscles. Blood perfusion increased markedly in the treatment group and led to functional restoration, as shown by the results from the walking analysis.
**Conclusions:** Shock wave therapy therefore could develop into a feasible alternative to stem cell therapy in regenerative medicine, in particular for ischemic heart and limb disease.

- **Effects of Low-Energy Shockwave Therapy on the Erectile Function and Tissue of a Diabetic Rat Model** [34]

*Knuppe Molecular Urology Laboratory, Department of Urology, School of Medicine, University of California, San Francisco, CA, USA.*

**Introduction.** Low-energy shockwave therapy (LESWT) has been shown to improve erectile function in patients suffering from diabetes mellitus (DM)-associated erectile dysfunction (ED). However, the underlying mechanism remains unknown. **Aim**. The aim of this study is to investigate whether LESWT can ameliorate DM-associated ED in a rat model and examine the associated changes in the erectile tissues.
**Methods.** Newborn male rats were intraperitoneally injected with 5-ethynyl-2-deoxyuridine (EdU; 50 mg/kg) for the purpose of tracking endogenous mesenchymal stem cells (MSCs). Eight weeks later, eight of these rats were randomly chosen to serve as normal control (N group). The remaining rats were injected intraperitoneally with 60 mg/kg of streptozotocin (STZ) to induce DM. Eight of these rats were randomly chosen to serve as DM control (DM group), whereas another eight rats were

subject to shockwave (SW) treatment (DM+SW group). Each rat in the DM+SW group received 300 shocks at energy level of 0.1 mJ/mm(2) and frequency of 120/minute. This procedure was repeated three times a week for 2 weeks. Another 2 weeks later, all 24 rats were evaluated for erectile function by intracavernous pressure (ICP) measurement. Afterward, their penile tissues were examined by histology. **Main Outcome Measures**. Erectile function was measured by ICP. Neuronal nitric oxide synthase (nNOS)-positive nerves and the endothelium were examined by immunofluorescence staining. Smooth muscle and MSCs were examined by phalloidin and EdU staining, respectively.
**Results.** STZ treatment caused a significant decrease in erectile function and in the number of nNOS-positive nerves and in endothelial and smooth muscle contents. These DM-associated deficits were all partially but significantly reversed by LESWT. **MSCs (EdU-positive cells) were significantly more numerous in DM+SW than in DM rats.**
**Conclusion.** LESWT can partially ameliorate DM-associated ED by promoting regeneration of nNOS-positive nerves, endothelium, and smooth muscle in the penis. **These beneficial effects appear to be mediated by recruitment of endogenous MSCs.**

- **Shock Wave Treatment Induces Angiogenesis and Mobilizes Endogenous CD31/CD34-Positive Endothelial Cells in a Hindlimb Ischemia Model: Implications for Angiogenesis and Vasculogenesis** [33]

*University Hospital for Cardiac Surgery, Innsbruck Medical University, Innsbruck, Austria.*

**Objectives:** Shock waves have been shown to induce recruitment of intravenously injected endothelial progenitor cells to ischemic hind limbs in rats. We hypothesized that shock wave treatment as sole therapy would induce angiogenesis in this ischemia model and would lead to mobilization of endogenous endothelial (progenitor) cells.
**Methods:** A total of 18 rats, aged 5 weeks old, were subdivided into 3 groups: sham (n = 6), ischemic muscle with shock wave treatment (shock wave treatment group, n = 6), and without shock wave treatment (control, n = 6). Hind limb ischemia was induced by ligation of the femoral artery. Three weeks later, shock wave treatment (300 impulses at 0.1 mJ/mm(2)) was applied to the adductor muscle; the controls were left untreated. Muscle samples were analyzed using real-time polymerase chain reaction for angiogenic factors and chemoattractants for endothelial progenitor cell mobilization. Fluorescence activated cell sorting analysis of the peripheral blood was performed for CD31/CD34-positive cells. Perfusion was measured using laser Doppler imaging. Functional improvement was evaluated by walking analysis.
**Results: Angiogenic factors/endothelial progenitor cell chemoattractants, stromal cell-derived factor-1 and vascular endothelial growth factor, were increased in the treatment group**, as shown by real-time polymerase chain reaction, indicating the mobilization of endothelial progenitor cells. Fluorescence activated cell sorting analysis of the peripheral blood revealed high numbers of CD31/CD34-positive cells in the treatment group. Greater numbers of capillaries were found in the treated muscles. Blood perfusion increased markedly in the treatment group and led to functional restoration, as shown by the results from the walking analysis.
**Conclusions: Shock wave therapy therefore could develop into a feasible alternative to stem cell therapy in regenerative medicine, in particular for ischemic heart and limb disease.**

- **The use of extracorporeal shock wave-stimulated periosteal cells for orthotopic bone generation** [35]
- **Unfocused extracorporeal shock waves induce anabolic effects in rat bone** [36]

*Department of Orthopaedics, Erasmus MC, University Medical Center Rotterdam, The Netherlands.*

**Background:** Extracorporeal shock waves are known to stimulate the differentiation of mesenchymal stem cells toward osteoprogenitors and induce the expression of osteogenic-related growth hormones. The aim of this study was to investigate if and how extracorporeal shock waves affected new bone formation, bone microarchitecture, and the mechanical properties of bone in a healthy rat model, in order to evaluate whether extracorporeal shock wave therapy might be a potential treatment for osteoporosis.
**Methods:** Thirteen rats received 1000 electrohydraulically generated unfocused extracorporeal shock waves to the right tibia. The contralateral, left tibia was not treated and served as a control. At two, seven, twenty-one, and forty-nine days after administration of the shock waves, in vivo single-photon-emission computed tomography (SPECT) scanning was performed to measure new bone formation on the basis of uptake of technetium-labeled methylene diphosphonate ((99m)Tc-MDP) (n = 6). Prior to and forty-nine days after the extracorporeal shock wave therapy, micro-computed tomography (micro-CT) scans were made to examine the architectural bone changes. In addition, mechanical testing, microcrack, and histological analyses were performed.
**Results:** Extracorporeal shock waves induced a strong increase in (99m)Tc-MDP uptake in the treated tibia compared with the uptake in the untreated, control tibia. Micro-CT analysis showed that extracorporeal shock waves stimulated increases in both trabecular and cortical volume, which resulted in higher bone stiffness compared with that of the control tibiae. Histological analysis showed intramedullary soft-tissue damage and **de novo bone with active osteoblasts and osteoid in the bone marrow of the legs treated with extracorporeal shock waves**. Microcrack analysis showed no differences between the treated and control legs.
**Conclusions:** This study shows that a single treatment with extracorporeal shock waves induces anabolic effects in both cancellous and cortical bone, leading to improved biomechanical properties. Furthermore, treatment with extracorporeal shock waves results in transient damage to the bone marrow, which might be related to the anabolic effects. After further examination and optimization, unfocused extracorporeal shock waves might enable local treatment of skeletal sites susceptible to fracture.

## 2.2. Evidence - Activating Effect of SWT on MSCs *in vitro*

- **The effects of shock wave stimulation of mesenchymal stem cells on proliferation, migration, and differentiation in an injectable gelatin matrix for osteogenic regeneration** [37]
- **Smooth Muscle Differentiation of Penile Stem/Progenitor Cells Induced by Microenergy Acoustic Pulses In Vitro** [16]
- **Extracorporeal shock waves trigger tenogenic differentiation of human adipose-derived stem cells** [38]
- **Radial shockwave treatment promotes human mesenchymal stem cell self-renewal and enhances cartilage healing** [39]
- **Improvement of adipose tissue–derived cells by low-energy extracorporeal shock wave therapy** [21]

*Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Austrian Workers' Compensation Board (AUVA) Research Center, Vienna, Austria; Austrian Cluster for Tissue Regeneration, Vienna, Austria.*

**Background:** Cell-based therapies with autologous adipose tissue-derived cells have shown great potential in several clinical studies in the last decades. The majority of these studies have been using the stromal vascular fraction (SVF), a heterogeneous mixture of fibroblasts, lymphocytes,

monocytes/macrophages, endothelial cells, endothelial progenitor cells, pericytes and adipose-derived stromal/stem cells (ASC) among others. Although possible clinical applications of autologous adipose tissue-derived cells are manifold, they are limited by insufficient uniformity in cell identity and regenerative potency.

**Methods:** In our experimental set-up, low-energy extracorporeal shock wave therapy (ESWT) was performed on freshly obtained human adipose tissue and isolated adipose tissue SVF cells aiming to equalize and enhance stem cell properties and functionality.

**Results:** After ESWT on adipose tissue we could achieve higher cellular adenosine triphosphate (ATP) levels compared with ESWT on the isolated SVF as well as the control. ESWT on adipose tissue resulted in a significantly higher expression of single mesenchymal and vascular marker compared with untreated control. Analysis of SVF protein secretome revealed a significant enhancement in insulin-like growth factor (IGF)-1 and placental growth factor (PLGF) after ESWT on adipose tissue.

**Discussion:** Summarizing we could show that **ESWT on adipose tissue enhanced the cellular ATP content and modified the expression of single mesenchymal and vascular marker, and thus potentially provides a more regenerative cell population.** Because the effectiveness of autologous cell therapy is dependent on the therapeutic potency of the patient's cells, this technology might raise the number of patients eligible for autologous cell transplantation.

- **Extracorporeal shock waves modulate myofibroblast differentiation of adipose-derived stem cells** [40]
- **Extracorporeal shockwave treatment: A novel tool to improve Schwann cell isolation and culture** (C.M.A.P. Schuh et al., 2016)

*AUVA Research Center, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria; Austrian Cluster for Tissue Regeneration, Vienna, Austria.*

**Background aims:** As new approaches for peripheral nerve regeneration are sought, there is an increasing demand for native Schwann cells for in vitro testing and/or reimplantation. Extracorporeal shockwave treatment (ESWT) is an emergent technology in the field of regenerative medicine that has also recently been shown to improve peripheral nerve regeneration.

**Methods:** In this study, we elucidate the effects of ESWT on Schwann cell isolation and culture. Rat sciatic nerves were dissected and treated with ESWT, and Schwann cells were isolated and cultured for 15 passages.

**Results:** Single treatment of the whole nerve ex vivo led to significantly increased extracellular adenosinetriphosphate as an immediate consequence, and subsequently a number of effects on the culture were observed, starting with a significantly increased Schwann cell yield after isolation. In the ESWT group, the quality of culture, reflected in consistently higher purity (S100b, morphology), proliferation rate (5-bromo-2-deoxyuridine, population doublings per passage) and expression of regenerative phenotype-associated markers (P75, glial fibrillary acidic protein, c-Jun), was significantly improved. In contrast, the control group exhibited progressively senescent behavior, reflected in a decrease of proliferation, loss of specific markers and increase in P16(INK4A) expression.

- **Extracorporeal Shock Wave Treatment (ESWT) enhances the in vitro-induced differentiation of human tendon-derived stem/progenitor cells (hTSPCs)** [41]
- **Shock Wave Treatment Enhances Cell Proliferation and Improves Wound Healing by ATP Release-coupled Extracellular Signal-regulated Kinase (ERK) Activation** [42]

*From the Department of Biochemical Engineering, University of Applied Sciences Technikum Wien, 1200 Vienna, Austria.*

Shock wave treatment accelerates impaired wound healing in diverse clinical situations. However, the mechanisms underlying the beneficial effects of shock waves have not yet been fully revealed. Because cell proliferation is a major requirement in the wound healing cascade, we used in vitro studies and an in vivo wound healing model to study whether shock wave treatment influences proliferation by altering major extracellular factors and signaling pathways involved in cell proliferation. We identified extracellular ATP, released in an energy- and pulse number-dependent manner, as a trigger of the biological effects of shock wave treatment. Shock wave treatment induced ATP release, increased Erk1/2 and p38 MAPK activation, and enhanced proliferation in three different cell types (C3H10T1/2 murine mesenchymal progenitor cells, primary human adipose tissue-derived stem cells, and a human Jurkat T cell line) in vitro. Purinergic signaling-induced Erk1/2 activation was found to be essential for this proliferative effect, which was further confirmed by in vivo studies in a rat wound healing model where shock wave treatment induced proliferation and increased wound healing in an Erk1/2-dependent fashion. In summary, this report demonstrates that **shock wave treatment triggers release of cellular ATP, which subsequently activates purinergic receptors and finally enhances proliferation in vitro and in vivo** via downstream Erk1/2 signaling. In conclusion, our findings shed further light on the molecular mechanisms by which shock wave treatment exerts its beneficial effects. These findings could help to improve the clinical use of shock wave treatment for wound healing.

- **The dose-effect relationship in extracorporeal shock wave therapy: the optimal parameter for extracorporeal shock wave therapy** [43]
- **In vitro extracorporeal shock wave treatment enhances stemness and preserves multipotency of rat and human adipose-derived stem cells** (C.M.A.P. Schuh et al., 2014)

*Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Vienna, Austria; Austrian Cluster for Tissue Regeneration, Vienna, Austria; University of Applied Sciences Technikum Wien-Department of Biochemical Engineering, Vienna, Austria.*

**Background aims:** Adipose-derived progenitor/stem cells (ASCs) are discussed as a promising candidate for various tissue engineering approaches. However, its applicability for the clinic is still difficult due to intra- and inter-donor heterogeneity and limited life span in vitro, influencing differentiation capacity as a consequence to decreased multipotency.
**Methods:** Extracorporeal shock wave treatment has been proven to be a suitable clinical tool to improve regeneration of a variety of tissues for several decades, whereas the mechanisms underlying these beneficial effects remain widely unknown.
**Results:** In this study we show that human and rat adipose derived stem cells respond strongly to repetitive shock wave treatment in vitro, resulting not only in maintenance and significant elevation of mesenchymal markers (CD73, CD90, CD105), but also in significantly increased differentiation capacity towards the osteogenic and adipogenic lineage as well as toward Schwann-cell like cells even after extended time in vitro, preserving multipotency of ASCs.
**Conclusions: ESWT might be a promising tool to improve ASC quality for cell therapy in various tissue engineering and regenerative medicine applications.**

- **Effect of extracorporeal shock wave on proliferation and differentiation of equine adipose tissue-derived mesenchymal stem cells in vitro** [45]

## 2.3. Evidence - Synergistic Regenerative Effect of SWT in Combination with MSC Therapy

- **Shockwave Therapy Combined with Autologous Adipose-Derived Mesenchymal Stem Cells Is Better than with Human Umbilical Cord Wharton's Jelly-Derived Mesenchymal Stem Cells on Knee Osteoarthritis** [46]
- **Adjunctive mesenchymal stem/stromal cells augment microvascular function in poststenotic kidneys treated with low-energy shockwave therapy** [47]
- **A promising therapeutic option for diabetic bladder dysfunction: Adipose tissue-derived stem cells pretreated by defocused low-energy shock wave** [17]
- **Combined Adipose-Derived Mesenchymal Stem Cells and Low-Energy Extracorporeal Shock Wave Therapy Protect the Brain From Brain Death-Induced Injury in Rat** [19]
- **Comparison efficacy of ESWT and Wharton's jelly mesenchymal stem cell in early osteoarthritis of rat knee** [48]
- **Smooth Muscle Differentiation of Penile Stem/Progenitor Cells Induced by Microenergy Acoustic Pulses In Vitro** [16]
- **Efficient Promotion of Autophagy and Angiogenesis Using Mesenchymal Stem Cell Therapy Enhanced by the Low-Energy Shock Waves in the Treatment of Erectile Dysfunction** [49]
- **Extracorporeal Shock Wave-Supported Adipose-Derived Fresh Stromal Vascular Fraction Preserved Left Ventricular (LV) Function and Inhibited LV Remodeling in Acute Myocardial Infarction in Rat** [50]
- **A novel bimodal approach for treating atrophic bone non-unions with extracorporeal shockwaves and autologous mesenchymal stem cell transplant** [51]
- **Combined Therapy with Extracorporeal Shock Wave and Adipose-Derived Mesenchymal Stem Cells Remarkably Improved Acute Ischemia-Reperfusion Injury of Quadriceps Muscle** [52]
- **Combined treatment with extracorporeal shock-wave therapy and bone marrow mesenchymal stem cell transplantation improves bone repair in a rabbit model of bone nonunion** [53]
- **Combination of low-energy shock-wave therapy and bone marrow mesenchymal stem cell transplantation to improve the erectile function of diabetic rats** [18]
- **Radial Extracorporeal Shock Wave Therapy Enhances the Proliferation and Differentiation of Neural Stem Cells by Notch, PI3K/AKT, and Wnt/β-catenin Signaling** [54]
- **An Innovative Approach for Enhancing Bone Defect Healing Using PLGA Scaffolds Seeded with Extracorporeal-shock-wave-treated Bone Marrow Mesenchymal Stem Cells (BMSCs)** [55]
- **Human autologous mesenchymal stem cells with extracorporeal shock wave therapy for non-union of long bones** [56]
- **Does extracorporeal shock wave introduce alteration of microenvironment in cell therapy for chronic spinal cord injury?** [57]
- **Low-energy shock wave for enhancing recruitment of endothelial progenitor cells: a new modality to increase efficacy of cell therapy in chronic hind limb ischemia** [58]

# 3. Conclusion

Numerous research studies have shown that non-invasive SoftWave Therapy, through stimulation of mechanosensitive cellular sensors, does the following: activates endogenous stem / progenitor cells, induces neoangiogenesis, improves microcirculation, reduces the inflammatory and cellular stress response, and results in sustainable tissue regeneration. So far, various underlying cellular signalling pathways involved have been identified which are modulated by low-intensity ESWT. [6,12,14,59–61]

## Effect of SoftWave™ Tissue Regeneration Therapy

- The underlying precise cellular mechanism of action about how SoftWave Therapy could improve the respiratory tissue of the affected patients is based on its unique capacity to stimulate the cells' own regenerative processes:
  - SWT enhances the expression and release of important growth factors. [25]
  - SWT induces endogenous stem cell activation and recruitment into the target tissue and stimulates proliferation and differentiation. [31,42,62]
  - SWT increases homing, uptake and survival rate of during cell-based therapies (e. g. mesenchymal stem cells, mitochondria) and can be used as an effective adjuvant therapy. [21,44]
  - SWT has a proangiogenic effect and induces angiogenesis and vasculogenesis thereby improving blood circulation and tissue supply. [63]
  - SWT exerts anti-inflammatory, antioxidative, immunomodulatory effects. [61,64,65]
  - SWT acts antifibrotic and reduces tissue necrosis and apoptosis. [66]
  - SWT acts antimicrobial and helps to relief the bacterial burden of a tissue. [67,68]

  **→ Resulting in significant functional and structural recovery and tissue regeneration.**

## Synergistic Effect SoftWave™ MSC Therapy

- SWs have the potential to modulate the patient's MCS and lead to improved efficiency of therapy. Different combination approaches can be used to increase the target organ engraftment:
  a) The affected tissue should be treated directly, with SoftWaves, to locally activate the endogenous MSCs and to optimally prepare the conditions, in the tissue, for the MSC graft. [51]
  b) In addition to the physical stimulus in the affected tissue by ESWT, the stem cell donor site should be treated *in vivo / in situ* to precondition the MSCs prior to aspiration. In this procedure, the resident MSCs are prepared by enlarging and conditioning their population. [9–11]
  c) The isolated MSCs can be stimulated *in vitro / ex vivo* prior to injection, which increases their stemness and viability. [21,44,69,70]

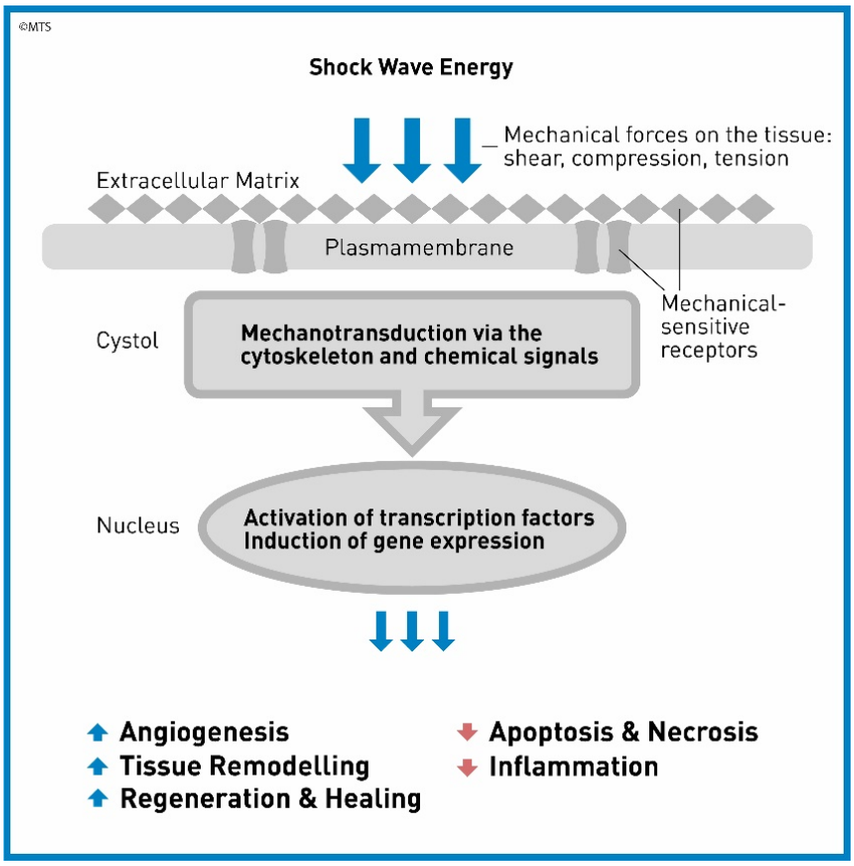


**Figure 2. Mechanotransduction Mechanism - Phases of Spark Wave Therapy. 1. Physical Phase:** Shock waves generate a positive pressure to generate absorption, reflection, scattering and transmission**. 2. Chemical Phase:** The mechanical stimulus leads to biochemical reaction. Biomolecules are released and cell signaling pathways are activated. **3. Biological Phase:** Modulation of angiogenesis, alteration of inflammatory response, bone and soft tissue healing.

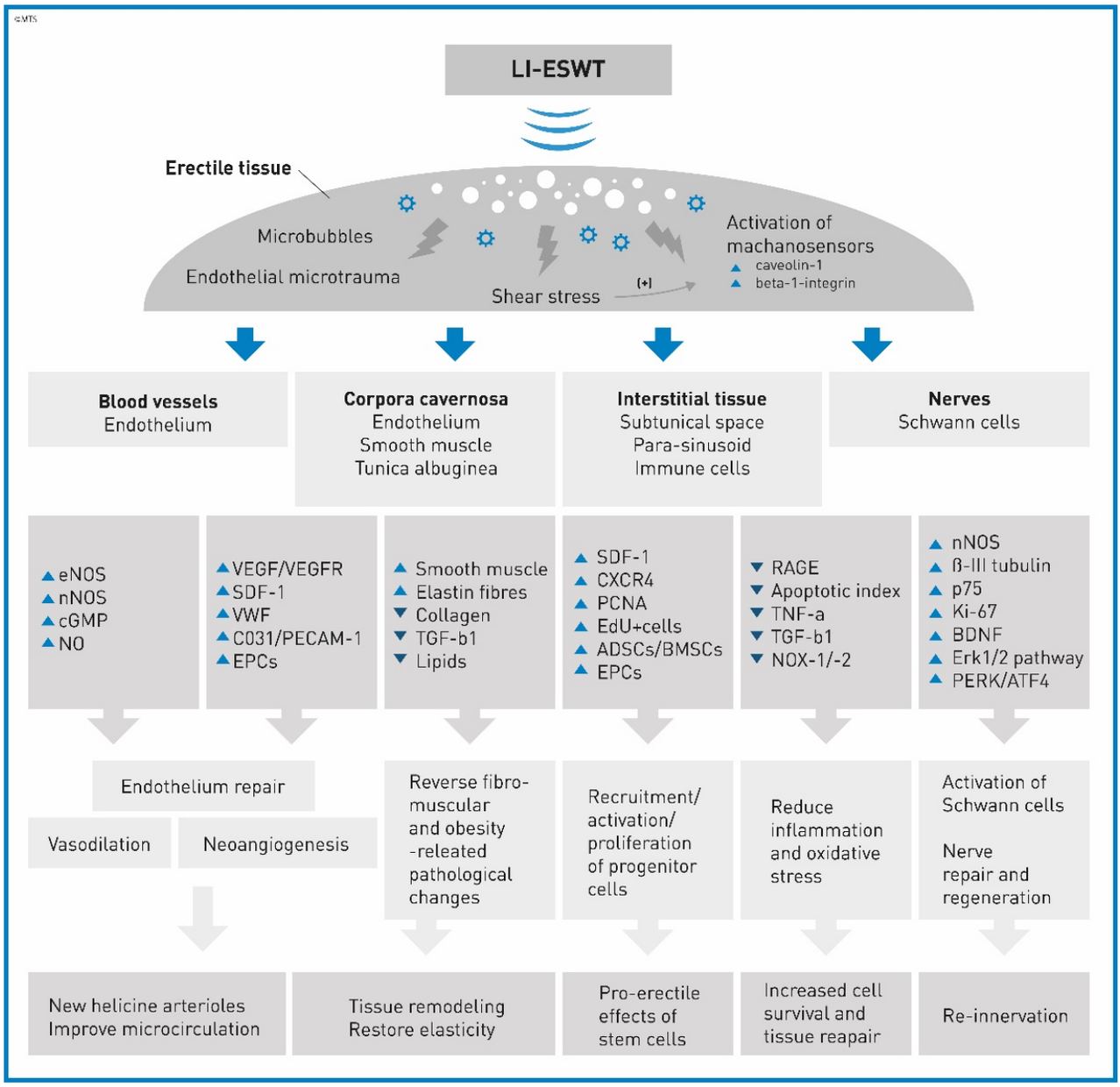


**Figure 3. Putative mechanism of action of Li-ESWT by example of ED.** ED = erectile dysfunction; Li-ESWT = low-intensity extracorporeal shockwave therapy. (Figure and caption adopted from [60])

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