

The Biologic Effects of SoftWaves™ and Rationale for Spine and Brain Therapy

The Evolution of Acoustic Wave Technology and the Discovery of SoftWaves™ for Regenerative Medicine.

I. INTRODUCTION

Shock waves are defined as types of acoustic pressure waves that develop during sudden releases of energy. The best-known natural phenomenon is thunder following lightning. Another example is the “bang” an aircraft produces when it breaks the sound barrier. Both focused shock waves and unfocused sound waves are produced in these examples. Additionally, almost all other forms of energy are released during these natural discharges, including electrical, heat, light, and electromagnetic energy. Since the first medical application of acoustic waves was the disintegration of kidney stones in the early 1980’s, high energy, focused shock waves were the first to be studied and understood.

In medicine, shock waves have been used at high energy levels for more than 30 years for the disintegration of kidney stones in lithotripsy (1-2) with minimal risk and rapid recovery. Lithotripsy is one of the safest noninvasive procedures known to medicine despite the focused, high energy shockwaves that are applied to skin, muscle, organ and bones during every procedure. No known long lasting side effects or adverse events have been observed other than slight bruising of the entry and exit sites. The FDA’s own database of adverse events over these 30 years confirms this observation. This fact represents an enormous advantage for the new, low energy SoftWave treatment and its application for tissue regeneration (3). SoftWaves™ are distinct and different from shockwaves in the following ways:

1. **Shock waves used to disintegrate kidney stones are at least 10 times the maximum pressure of SoftWaves™.** Kidney lithotripters are FDA class II devices and considered NSR devices (Non Significant Risk). SoftWaves are unfocused in the targeted area and are generated with a parabolic reflector. Shock waves are generated with an ellipsoidal lens. Most shock waves are only developed in the focal area of a reflector or coil where acoustic waves converge and generate high local pressures and cavitation. Shock waves can be used to destroy kidney stones whereas SoftWaves would have no effect on kidney stones. SoftWaves™ are parallel waves that are not focused in the body and have the same regenerative effect throughout the entire wave front as compared to the tiny focal zone of most shock wave devices.
2. SoftWaves are exclusively generated with electrohydraulic devices whose unique wave form creates a tensile wave that creates sheering forces at the cellular level causing cell membranes to become permeable. These sheering forces fool the body into thinking that a fresh trauma has occurred and causes the body to initiate a natural healing cascade including the production of stem cell attractants. Other forms of so called “shockwaves”, with the exception of other focused, electrohydraulic devices, do not cause this biologic effect. Less than 10% of the devices sold worldwide use electrohydraulic technology.
3. Shock wave technology is much more painful as it is focused to a single small point and typically is performed at much higher energy levels within the focal volume. Other companies assumed a model of noninvasive cellular trauma when they developed shock wave technology for non urologic indications. It was thought that shock waves could cause trauma non-invasively thus triggering a neo vascular response. They were right and wrong. Shock waves do initiate a neo vascular response; however, SoftWaves™ prove that damaging cells

and causing pain and trauma is not a requirement for a neo vascular response. TRT learned that low energy SoftWaves generate a stronger cellular response as SoftWaves™ treat larger areas across the entire reflector diameter with much less pain. TRT is the only company who holds patents for unfocused SoftWave™ technology.

4. SoftWaves™ are generated at a level (one tenth that of a kidney lithotripter) that does not cause cavitation. Cavitation is what causes cellular injury and produces pain. SoftWaves do not cause damage to tissue. It is not the one step backward, two step forward approach used by shock wave device manufacturers.

In Summary, SoftWaves™ are not shock waves. Shock waves are only relevant as their previous publications are useful in directing us to the most promising applications for SoftWave™ therapy. Any shock wave research or clinical study can be replicated with better results without the risk or pain associated with shock waves (with the exception of long bone pathologies). SoftWave technology is performed much faster and with fewer retreatments as the entire pathologic area can be treated with the entire parallel wave front projected from the parabolic lens without the pain and anesthesia required by the older shock wave devices.

In contrast to other approaches in this field such as stem cell treatment or gene therapies, the side-effect profile is well studied for a long time in a huge number of patients. We therefore know that no malignancies occur for Softwave patients in contrast to stem cell or gene therapy. . Moreover, SoftWaves and shock waves are in daily clinical use for the treatment of numerous indications especially in orthopedics and traumatology. These include tendinopathies such as the so-called tennis elbow or bone non-unions (non-unions is the one pathology that has the best outcomes with high energy focused shockwaves as large bones require high levels of energy to develop a healing response) and wound healing disorders. Furthermore, clinical application has been studied for indications including erectile dysfunction and ischemic heart disease (4-7). The new application for spine injury and brain pathology patients is therefore deemed a promising approach, especially as these patients are lacking other feasible and effective treatment options.

II. PRIMARY SHOCK WAVE AND SOFTWAVE EFFECTS

1. Shock waves and SoftWaves™ reduce apoptosis and suppress acute inflammation

In experimental wound healing models, defined acoustic waves were described to reduce apoptosis (Programmed cell death) and acute inflammatory reactions (8, 9). In a murine model of severe cutaneous burn wounds the application of shock waves caused decreased infiltration of the wound bed with inflammatory cells. Treated tissue exhibited lower expression of pro-inflammatory chemokines compared to untreated control wounds resulting in smaller scar sizes in the treatment group (8). Similar observations could be made in a skin-flap model in rats: treated flaps showed decreased leucocyte infiltration leading to enhanced skin flap survival. Interestingly, leucocytes in the peripheral blood of treated animals exhibited lower expression of hydrogen peroxide (H₂O₂). Analysis of tissue sections revealed decreased numbers undergoing programmed cell death after treatment (9).

2. Shock waves and SoftWaves modulate inflammation

Inflammation plays a major role in most pathologies. A well-orchestrated inflammatory response to pathologic stimuli is therefore very important. A very good example is the remodeling process after myocardial infarction. Adequate healing after deterioration of a large amount of cardiomyocytes requires a balanced response between inflammatory and reparative functions. Pro-inflammatory response is needed to replace the chemically harmed necrotic myocardium with scar tissue. Anti-inflammatory processes are required to create a milieu of regeneration and enable angiogenesis. Therefore, the common idea to state that inflammation is “bad” is wrong (10) (**Fig. 1**).

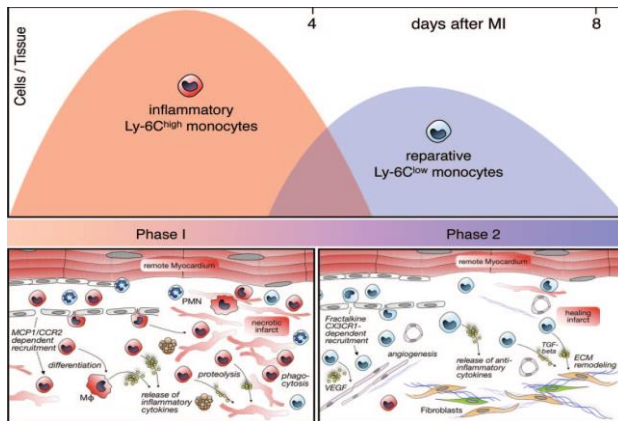


Fig. 1: Biphasic inflammatory response in myocardial infarction. Myocardial tissue remodeling after infarction (MI) is characterized by an early proinflammatory phase which leads to phagocytosis of necrotic muscle and debris. In the second phase reparative monocytes lead to tissue regeneration, angiogenesis and remodeling of the extracellular matrix (ECM). (10)

In a recent work, we found that Softwaves™ cause a very distinct modulation of the inflammatory response in human endothelial cells via the stimulation of Toll-like receptor 3. Toll-like receptor 3 is part of the innate immune system and is involved in the recognition of double-stranded RNA and fragmented DNA. Its stimulation by shock wave therapy resulted in an early pro-inflammatory initiation phase mediated by IL-6. Subsequently, a middle phase showing suppression of inflammation can be seen before the late anti-inflammatory limitation phase of IL-10 results. This modulation of the inflammatory response is prerequisite for angiogenesis and repair in injured tissue (11) (**Fig. 2**). An in vivo study in Toll-like receptor 3 knockout mice is currently underway to prove that the stimulation of this innate immune receptor is the pivotal effect of shock wave treatment and mediates all other effects.

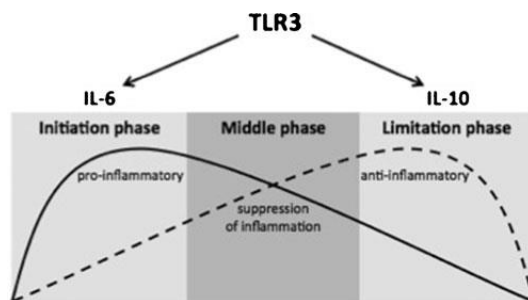


Fig. 2 Modulation of inflammation. The complex interaction between the two main cytokines IL-6 and IL-10 in Toll-like receptor 3 stimulation by shock wave treatment can be schematically seen as a three-phase regulation over time. After an early pro-inflammatory initiation phase mediated by IL-6, a middle phase showing suppression of inflammation can be seen before the late anti-inflammatory limitation phase of IL-10 results. This modulation of the inflammatory response is prerequisite for angiogenesis and repair in ischemic tissue (11).

3. Shock waves and SoftWaves induce angiogenesis

The angiogenic effect of acoustic wave treatment is probably the most extensively described issue in acoustic wave science. The induction of vessel sprouting has been reported in wounds, bone, muscle, heart and skin (12-16) (**Fig. 3**). Shock waves cause release of vascular endothelial growth factor (VEGF), which disrupts endothelial cell adhesions and enables migration of endothelial cells to form capillary structures. The additional up-regulation of placental growth factor (PIGF) expression in treated tissue leads to maturation of the vessels via pericyte and smooth muscle cell recruitment (15). Moreover, shock waves cause the release of nitric oxide, another crucial angiogenic player (13). Nitric

oxide as an endothelial survival factor has numerous effects on endothelial cells: enhancement of proliferation and migration, inhibition of apoptosis and further vascular endothelial growth factor and fibroblast growth factor (FGF) release (17).

Higher numbers of vessels after shock wave treatment have been shown in small animals, large animals as well as in clinical studies (15, 18). Patients with ischemic heart disease for instance were described to benefit highly from shock wave treatment resulting in relief of angina symptoms and improvement of heart function (4).

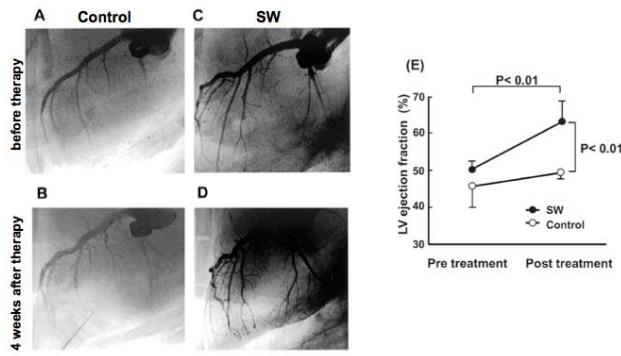
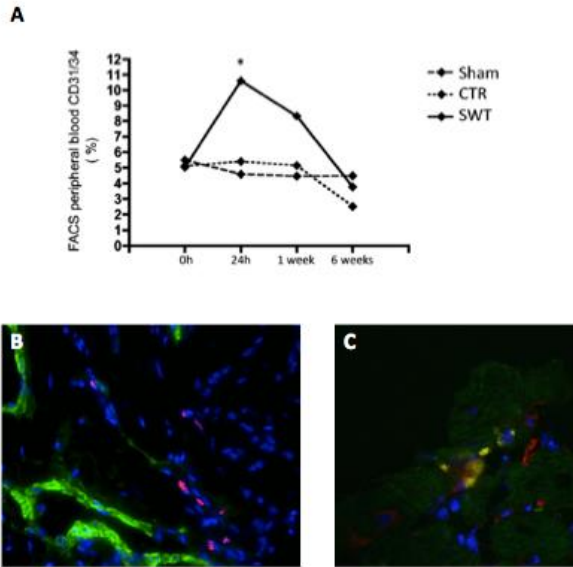


Fig. 3 Coronary artery constriction in pigs. A-D Cardiac shock wave therapy (SW) resulted in higher vessel density after four weeks (D) compared to untreated control animals (B). **E** Cardiac function was significantly improved in treated animals (18).

4. Shock waves and SoftWaves™ induce stem cell recruitment

Damaged tissue “cries for help” by expression of chemoattractants responsible for progenitor cell recruitment to the site of injury. However, this ability is lost in chronically harmed tissue. The very well respected research group around Stefanie Dimmeler and Andreas Zeiher in Frankfurt showed that this ability can be regained by the application of shock waves. In a model of chronic hind limb ischemia in rats they showed that shock wave treatment 24 hours prior to stem cell injection lead to significantly increased recruitment of the injected stem cells to the injured tissue (19). In a next step, they were able to translate these findings into a clinical setting: Patients with ischemic heart disease were treated with cardiac shock wave therapy prior to stem cell injection. Again, shock wave treatment resulted in significant improvement of symptoms and cardiac function compared to patients receiving only stem cell therapy without prior shock wave treatment (20). These results indicate in a very impressive manner that shock waves cause enhanced expression of stem cell recruiting chemokines. However, recent papers show that shock wave treatment not only causes enhanced recruitment of injected stem cells, but also the mobilization of body-own (endogenous) stem cells. In a chronic hind limb ischemia model in rats shock wave treatment of the ischemic muscle resulted in higher numbers of circulating stem cells in the peripheral blood of treated animals (14). Moreover, SoftWaves™ caused enhanced recruitment of stem cells to treated penises in a model of erectile dysfunction in rats (21). Our latest results show that shock wave treatment of ischemic cardiac muscle causes increased recruitment of stem cells to treated hearts (results not yet unpublished).



(Fig. 4).

Fig. 4 Shock waves recruit stem cells. A Mice with chronic hind limb ischemia showed increased numbers of circulating stem cells in the peripheral blood after shock wave treatment (SWT). (* $p < 0.05$) (14) **B** In a model of erectile dysfunction in diabetic rats shock waves induced enhanced recruitment of stem cells (pink) to treated tissue (21). **C** Cardiac shock wave therapy after myocardial infarction resulted in the recruitment of stem cells (yellow) to the injured hearts (authors' unpublished data).

5. SoftWaves™ and shock waves improve wound healing

Shock waves and SoftWaves™ promote wound healing in diabetic ulcers and non-healing wounds. This was shown in numerous animal experiments as in cutaneous burn injury models as well as skin flap models. The investigating authors describe reduced wound size and major healing improvement in treated animals. The molecular mechanism of this effect has been mainly linked to the angiogenic and anti-inflammatory properties of shock waves (22). These results have been translated into the clinic with very encouraging results. Reportedly, chronic soft tissue profits from shock wave treatment responding with improved healing rates (**Fig.5**) without any side effects (23). Interestingly, scar formation seems to be extensively reduced compared to untreated wounds. This effect is mainly linked to the recruitment of progenitor cells, which might result in functional tissue regeneration rather than replacement by scar tissue (22). A prospective, randomized phase II trial could show that shock waves accelerate re-epithelialization of second degree burn wounds (16).



Fig. 5 Shock waves promote wound healing. **A** Leg of a 57-year-old man with chronic arterial ulcer (duration >1 year) before therapy and **B** 6 weeks after first shock wave treatment. **C** Left lateral foot pressure ulcer of a 50-year-old male patient before treatment and **D** 2 weeks after single SW treatment (23).

6. Shock waves and SoftWaves cause pain reduction

Shock waves are described to induce long-term analgesia in chronic musculo-skeletal diseases such as calcifying tendonitis of the shoulder (24), tennis elbow (25) and chronic plantar fasciitis (26). Treated patients report of significant pain relief after treatment. These effects are not very well understood on a molecular level. Experimental studies describe a reduction of the number of neurons immunoreactive for substance P in dorsal root ganglia in rats (27). However, further research needs to be performed in this field.

7. SoftWaves™ induce neuronal regeneration

Shock waves have been described to induce regeneration of peripheral nerves after injury. Authors of the study dissected the sciatic nerve in rats, rotated it for 180 degrees, and re-attached it via epineural sutures. Shock wave treated animals showed improved regeneration and ameliorated functional performance of the treated limbs compared to untreated controls (28). The strong regenerative potential of shock waves encouraged us to try this technology in a field, where therapeutic options are very limited: spinal cord injury. In a pilot trial we performed aortic cross clamping in mice for 11 minutes thereby causing severe ischemic injury to the spinal cord. Animals showed paraplegic symptoms. Shock wave therapy to the spine was applied immediately while animals were still under anesthesia. Treated animals inhibited decreased neuronal degeneration, less microglial activation and increased expression of the angiogenic genes vascular endothelial growth factor and hypoxia inducible factor alpha (**Fig.6**). However, results are still preliminary and the experiments still in progress.

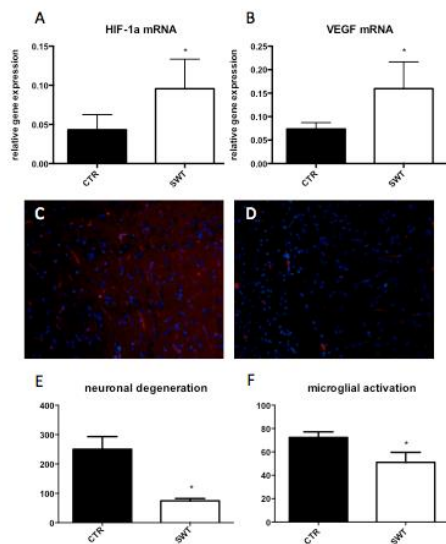


Fig. 6 Shock wave therapy for spinal cord ischemia. Spinal cord ischemia was induced by cross clamping of the aorta in rats. **A, B** Treated animals showed enhanced expression of angiogenic genes HIF-1a and VEGF. (* $p < 0, 05$) **C, D, F** Shock wave treatment resulted in decreased microglial activation, hence inflammation. (* $p < 0, 05$) **E** Staining for neuronal degeneration revealed lower rates of degenerating neurons in shock wave treated animals compared to untreated controls. (* $p < 0, 05$) These are results of a preliminary study, which need further confirmation. (authors' unpublished results)

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