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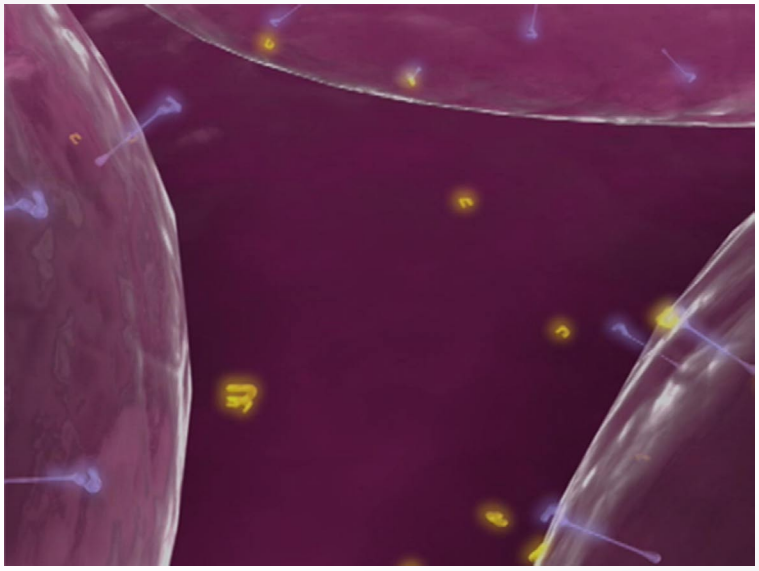
ESWT Mechanisms of Action in Veterinary Applications



VersaTron®

VersaTron® 4 Paws

Extracorporeal Shock Wave Technology (ESWT) is a high energy acoustic pressure wave technology that has been demonstrated to treat a variety of musculoskeletal and wound conditions in canine, equine, and human populations. The technology originated as lithotripsy which focuses energy on kidney stones in order to break them into small, passable pieces. Since then it has proven to be very useful for treating numerous other conditions. Recent studies have shown that ESWT decreases inflammation and regenerates tissue with improved quality of healing.[1] The mechanical forces of the pressure waves, including the resulting cavitation, cause various biological responses in the body including recruitment of stem cells, expression of angiogenic growth factors leading to neovascularization and increased release of PCNA leading to tissue proliferation.[1,18] ESWT has also been proven efficacious in alleviating pain associated with degenerative diseases and may protect osteoarthritic joint tissue.[2] More specific modes of action in different types of tissues are outlined below.

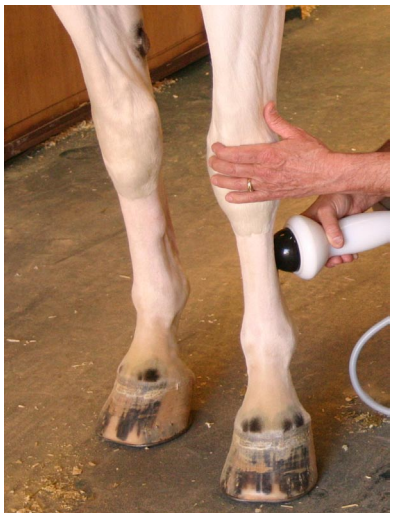
ESWT regenerates and protects tissue[1-18]
- Decreases inflammation and speeds healing process
- Increases release of angiogenic growth factors and PCNA leading to neovascularization and cellular proliferation
- Improves fiber alignment in tendon repair and thereby increases tensile strength
- Disintegrates calcifications
- Increases cellular release of BMP leading to faster bone healing and improved bone strength
- Protects chondrocytes by down regulating TNF-alpha and IL-10
- Recruits stem cells to ischemic tissue

Tendinitis:

ESWT has a long history of safety and efficacy in treating tendon and ligament injuries. The technology is FDA-approved for human use for chronic plantar fasciitis (heel pain) and chronic lateral epicondylitis (tennis elbow). Tendon and ligament injuries are the most common use reported for ESWT in equine patients. There have been numerous clinical publications about ESWT for tendon/ligament repair including studies that have investigated how ESWT helps to promote healing in connective tissue.

A study by Alves, et al. reported on the mechanism of ESWT in the treatment of superficial digital flexor tendinitis in horses and demonstrated that ESWT effectively speeds healing in addition to improving the quality of healing.[3] In the study, a tendon injury was induced by injection of type I collagenase to both front limbs of the patients. Thirty days following the injection the front right limbs were treated with 500 shocks over the lateral aspect with the 5mm Trode, 500 shocks with the 5mm Trode over the medial aspect and 500 shocks with the 35mm trode over the plantar aspect. The VersaTron® (SANUWAVE, Alpharetta, GA) device was used at its highest energy setting (E6). The front left limbs were not treated and acted as the control group. Both the treatment group and the control group were monitored using ultrasound throughout the study and the tendons were biopsied at the end of the study. The tissue was evaluated for the number and characteristics of the fibroblasts, vascularization in the injured area, presence of collagen fibers and the parallelism of these fibers. Upon evaluation, fiber alignment scores of the test group increased significantly faster than the control group and the lesions on the tendons in the test group decreased significantly with better ecogencity scores. It was observed that the process of collagen fiber remodeling was more efficient in the test group versus the control and therefore ESWT stimulated the alignment of collagen fibers during the repair process. The authors emphasized the

importance of a high degree of parallelism of collagen fibers as this contributed to increased resistance in tendon fibers. This is extremely important for the future health of the patient and preventing relapse upon return to normal activity.[3] In conclusion, ESWT caused significant improvement in the quality of tendon healing and a favorable prognosis for the treated animal due to the arrangement of collagen fibers.[3]



Chen, et al. also evaluated the mechanism of action of ESWT in Achilles tendinitis repair in rats and investigated the biochemical and biomechanical properties of healing tendons.[4] In this study, the treatment group was treated with ESWT at an energy setting of 0.16 mJ/mm^2 and with 200, 500, or 1000 shocks. It was observed that at one week after treatment, inflammation resolved and intensive blood capillaries and extracellular matrix production were observed at the lesion sites. Four weeks following the treatment, the sites showed fibrous bridges and the granulation and inflammation was completely improved. At this time, well-aligned fiber bundles were gradually forming in a parallel fashion. At six weeks, fibrous tissue was replaced by newly developed tendon tissue with a greater fiber density. The study also evaluated the effect of ESWT on tenocyte proliferation and demonstrated that ESWT significantly increased cell proliferation within 6 weeks of treatment compared to the control group. The treatment group showed significant increases in transforming growth factor-beta 1 (TGF-β1) and insulin-like growth factor-I (IFG-I) expression. The authors concluded that the findings indicated that ESWT raises mitogenic and morphogenic responses thereby stimulating tenocyte growth and the formation of healthy tendon microstructure and promoting tendon regeneration.[4] Finally, the cavitation effect of ESWT contributes to the technology's ability to fragment and disintegrate calcifications for calcific tendinitis.[5]

Bone:

VersaTron has been shown to heal bone fractures in canine and equine patients.[6,7] ESWT has been shown to have very positive effects on bone healing for delayed healing and non-union fractures. In human studies, ESWT has been demonstrated to improve nonunion and delayed healing fractures in 75% of patients and has been recommended as the first choice of treatment for these conditions due to its efficacy, safety, and non-invasive nature.[8] Wang, et al. studied high energy ESWT for the prevention of non-unions in acute fractures of the lower extremity in humans.[9] In this trial, the study group received open reduction and internal fixation followed by shock wave treatment immediately after surgery. The control group received open reduction and internal fixation only. Evaluation parameters included clinical assessments of pain score, weight bearing status and radiographs at 3, 6, and 12 months. The primary end point was the rate of non-union at 12 months. The ESWT group non-union rate (11%) was significantly lower than the control group (20%) ($P<0.001$), and there was a significantly better rate of healing in the study group at 3, 6, and 12 months ($P<0.001$). ESWT has also shown success in the treatment of avascular necrosis (AVN) of the femoral head and has been demonstrated as a viable and economically sound alternative to invasive methods for this indication. [10,11]

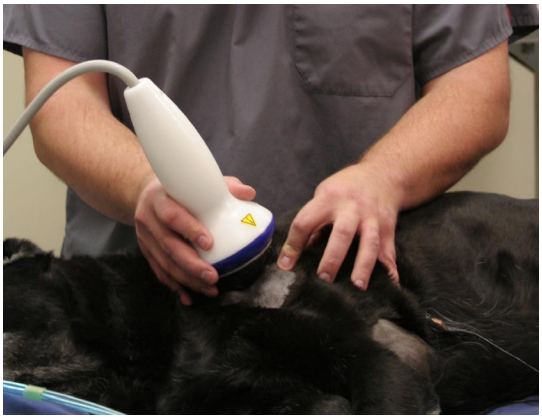
The mechanism in which ESWT promotes bone regeneration has initially been studied by Wang et al. in a rabbit model.[1] Closed fractures of the right femur were created and confirmed on radiographs and the rabbits were divided in three groups: sham, low-energy shock wave, and high-energy shock wave. Low energy shock wave was defined as 2000 shocks at 0.18 mJ/mm^2 and high energy as 4000 shocks at the same energy level. The animals were sacrificed at 12 weeks and bone was harvested for biomechanical testing including peak load, peak stress, and elasticity. Following the biomechanical testing, a histomorphological examination was conducted to distinguish fibrous tissues, cartilaginous and bone tissues. The biomechanical results showed that ESWT induced improved bone strength as measured by peak load, peak stress and elasticity versus low energy shock wave and the control groups. Positive eNOS, BMP, VEGF and PCNA immunostained cells and the number of neovessels were also significantly higher in the high energy group versus the low energy and control groups.

Osteoarthritis (OA):

In 2001, researchers at Colorado State University evaluated the mechanism of action of ESWT for induced osteoarthritis in equine patients. The study evaluated the efficacy of ESWT in reducing lameness associated with OA vs. no treatment (control group 1) and vs. intramuscular polysulfated glycosaminoglycans (PSGAG) (control group 2).[12] In the study, ESWT performed better than both control groups in reducing lameness. In addition to the significant efficacy over PSGAG, another key observation was that ESWT significantly reduced synovial fluid total protein, a parameter of synovitis.

More recently, a new publication by Moretti, et al. presents the mechanism of action of ESWT in more depth and unveiled a variety of ways in which ESWT modifies degenerative joint disease.[2] While there are many aspects of OA that are still under investigation, there are a variety of physical and chemical aspects proven to be accountable for the development and advancement of osteoarthritis. Three key attributes of OA chondrocytes are: 1. a decrease in beta 1 integrin, which seems to be an early event that begins the degradation process, 2. an increase in tumor necrosis factor-alpha (TNF-α), and 3. an increase in interleukin10 (IL-10).

TNF-α contributes to the advancement of OA by activating matrix metalloproteinase (MMP) production by chondrocytes which induce chondrocyte apoptosis and extracellular matrix (ECM) breakdown, and by working with other cytokines to degrade the cartilage matrix. Increased levels of IL-10 are also confirmed to be a key contributing factor to the acceleration of OA. This degradation process is cyclical: as chondrocytes breakdown, these chondrocytes, inflamed synovial membrane, and osteoblasts produce more TNF-α and IL-10. In summary, there are multiple pathways of degradation and once the cycle begins, the outcome is devastating.



Moretti, et al. revealed that ESWT can alter the process of OA breakdown as it mediates a variety of pathways.[2] Osteoarthritic chondrocytes express low beta 1 integrin and high TNF-α and IL-10 levels. In this study, ESWT was shown to down regulate levels of TNF-α and IL-10 in OA chondrocytes to normal

levels. It is therefore speculated that the reduction of TNF-α as a result of ESWT can be considered a protective effect as it may prevent MMP activation and cartilage breakdown. This discovery has huge implications for the potential of ESWT for the treatment of OA. As a disease modifying agent, using ESWT on patients earlier on in the disease timeline could protect the joint and slow the onset of OA symptoms, helping veterinary patients to lead a more active life and have a better quality of life. While ESWT is often reserved for the most difficult to treat and severe patients, using the treatment more proactively will provide benefits beyond pain management.

Antibacterial & Wound healing:

In vitro studies have demonstrated highly significant bactericidal effects of ESWT on Staphylococcus aureus and on different gram-positive and gram-negative pathogens such as Staphylococcus epidermidis, Enterococcus faecium and Pseudomonas aeruginosa and this efficacy has been confirmed in vitro.[13] Further study is warranted for infections such as osteomyelitis or endocarditis. ESWT appears to work by targeting the membrane systems of biofilms leading to increased permeability of membranes and cell walls. The thin bacterial cells layers are therefore damaged by the ESWT leading to leakage and death.[13] ESWT has great potential for wound healing and research is ongoing for this application in humans.

ESWT has been shown to significantly increase growth factors such as eNOS, VEGF and PCNA leading to neovascularization in chronic wounds.[1] ESWT has been shown to be effective in healing diabetic foot ulcers and burns in humans and wound studies in animals have been positive as well.[14-16] ESWT improves blood supply to ischemic tissue and is therefore also beneficial for skin flap survival as it can significantly decrease necrotic areas.[17] ESWT has been evaluated for treatment of distal limb lacerations in horses and it decreased healing time by 2 weeks vs. the control group.[16] Research has also been conducted showing the positive effects of shock wave in recruiting circulating endothelial progenitor cells into nonischemic and chronic ischemic tissue.[18] ESWT was shown to increase tissue expression of chemoattractant factors including stromal cell-derived factor 1 and VEGF which lead to recruitment of circulating progenitor cells. This research suggests that ESWT may improve efficacy of stem or progenitor cell therapy.

The electrohydraulic technology used in the VersaTron® and VersaTron 4 Paws® devices is the only type of ESWT that causes true shock waves at all energy settings.[19] The high energy VersaTron technology is relied on by more veterinary practitioners and the applications for use continue to expand via practical and clinical trials. SANUWAVE is committed to expanding published research on the mechanisms and efficacy of ESWT and research is ongoing for tendinitis, osteoarthritis, laminitis and wound indications.

A better understanding of the mechanisms in which ESWT causes biological effects can help veterinarians to truly maximize use of ESWT and reap the benefits of the technology. The technology has an excellent safety profile and decades of use have demonstrated its efficacy in a variety of conditions including tendinitis, bone fractures, and osteoarthritis. While there is still more to learn about the complexity of how this noninvasive technology helps the body to heal itself, current knowledge clearly demonstrates the positive impact of ESWT on so many difficult to treat conditions.

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SANUWAVE

11680 Great Oaks Way,

Suite 350

Alpharetta, GA 30022

www.sanuwave.com

1-800-245-4417