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The ISMST and the ISMST Newsletter (International Society for Shockwave Therapy) An international platform for communication and knowledge transfer

The XII ISMST Congress will be held in Italy, in Sorrento, from the 1st to 4th April 2009. It has been organized by Sergio Russo of the University of Naples "Federico II", who generously appointed me honorary President of the Congress both for the affection and the esteem that join us, and mindful of our common and continuous activities in the last 15 years in order to assert in the medical world the value of shock waves in musculoskeletal pathologies and in other medical areas which step by step are singled out as possible therapeutic application fields of shock waves.

The place for the Congress, the Hilton Sorrento Palace, has been chosen to offer the participants the sight of a magic place well-known around the world for its natural beauties. And also to give the members the opportunity of a short immersion in Roman history, visiting the archaeological excavations of Ercolano which was brought to light in the last century after its destruction and burial following the catastrophic eruption of Vesuvius in 79 a.C. and the serious natural events.

Since years inexorably pass I have the impression that my soul, growing older and older, has become more sensitive to memories. While I am writing I can see, as regards the shock wave therapy, the last 15 years during which my closest partners, among whom I want to mention at least Sergio Russo, Sergio Gigliotti, Carlo De Durante, have been collaborating with me since the early experiments concerning shock waves in musculoskeletal pathologies and they supported me allowing us all to study a therapeutic field that has enriched our knowledge and spread in all directions.

I remember clearly 1993, when we carried out the first shock wave application on a patient affected by pseudoarthrosis of the carpal scaphoid for more than two years and who, after only two shock wave applications through an old urologic lithrotriptor, recovered almost by a miracle in a month. This thrilled us very much and made us believe more in the method. I remember the first congresses of Orthopaedics in Italy and in Europe where we introduced our early outcomes, causing positive interest and also much disbelief in our Italian and foreign colleagues.

I remember with affection Prof. Heinz Kuderna of Vienna with whom and together with other European scholars such as Prof. Wolfang Schaden, Dr. Richard Thiele and others we met in Vienna, in 1997, to establish the European Society of Shock Wave Therapy in the musculoskeletal pathologies (ESMST). Prof. Heinz Kuderna, eminent doctor and scholar, was the first president of the European Society and in 1999 I succeeded him during the Congress in London and in Naples in 2000, during the III Congress chaired by me, the European Society became the International Society (ISMST).

Since 1995 there has been a strong collaboration with scholars all over the world on the shock wave therapy and with the increase in experimental studies, in clinical experiences, as well as the adjustment of devices to new traumatological orthopaedic needs, we achieved in few years the spreading of the method and of the therapeutical directions. The positive clinical responses of thousand of cases around the world bears witness to it, together with the scientific interest that the new method caused, promoting the flourishing, especially in Europe, of many scientific Societies devoted to this field up to the foundation of the International Society for Muskoloscheletal Shock Waves Therapy (ISMST) in 2000, which counts among its members scholars coming from all over the world. As it was expected this new therapeutical system could not remain only confined to the orthopaedic traumatological and urological fields. Several
ongoing researches make us think that other medical branches will benefit from the shock wave therapy. We have been the first scholars to monitor the system and to experiment it on man as well as to document angiogenesis processes in the tissues hit by shock waves. We also wished to find out the chemical mediators able to turn the mechanic effect into the biological one. Nowadays we are able to firmly point out that the main chemical mediator which causes neoangiogenesis is nitrogen monoxide (NO) which originates in the tissues hit by shock waves in peculiar circumstances. This fundamental outcome has also been achieved thanks to the collaboration between our research group and a similar research group of the University of Verona chaired by Prof. Hisanori Suzuki.

According to what I have written and to what I have not reported for the sake of brevity, I must firmly reaffirm that the past 15 years of research on shock waves have been the harbinger of really satisfactory outcomes.

I am going to conclude this editorial even mentioning the success of the International Society for shock wave therapy (ISMST) which has recorded participation and interest beyond the rosiest expectations giving further value to this new therapeutical system.

In the end I would like to thank the founder and publisher Paolo Roberto Dias dos Santos for his careful direction of the Newsletter and because he allowed me to write my sincere editorial.

Extracorporeal Shockwave Treatment for Chronic Diabetic Foot Ulcers

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Background and Purpose

Diabetic foot ulcer is caused by ischemia/hypoxia due to occlusion of small vessel associated with neuropathy and secondary infection. The treatments of diabetic foot ulceres require a multidisciplinary approach including the control of diabetes, antibiotic, shoe wear, wound care and surgery in selected cases. The results of surgical and non-surgical treatments are inconsistent and most are unsatisfactory. Many adjunctive therapies are designed with the intention to cure the diabetic foot ulcers. Some showed limited success, but none showed universal results. Extracorporeal shockwave treatment (ESWT) was to shown to induce the ingrowth of neovascularization associated with increased angiogenic growth factors such as eNOS, VEGF and PCNA. Recent studies reported the effectiveness of ESWT in acute and chronic wounds. Others demonstrated the antibacterial effect of ESWT in experimental studies. It is reasonable to speculate that ESWT may be effective in chronic diabetic foot ulcers. The purpose of this prospective study was to evaluate the efficacy of ESWT in chronic diabetic foot ulcers, and to compare the results with that of hyperbaric oxygen therapy (HBO), and to investigate the regeneration effects with focus on blood perfusion and molecular changes after treatment.

Methods

Seventy patients with 72 chronic diabetic foot ulcers were randomly divided into two groups. The ESWT group consisted of 34 patients with 36 ulcers, and 36 patients with 36 ulcers in the HBO group. Both groups showed similar demographic characteristics. Patients in ESWT group received 300 + 100 impulses of shockwaves at 0.11 mJ energy flux density /cm2 of treatment area once every two weeks for 6 weeks. Patients in HBO group received HBO therapy in a sealed chamber at the pressure of 2.5 ATA once a day, 5 days a week for a total of 20 treatments. Local blood flow perfusion, bacterial culture, and biopsy were performed before and after treatment. The evaluations included clinical assessment on the healing status of the ulcer with photo-documentation, blood flow perfusion scan, bacteriological study, histomorphological examination and immunohistochemical analysis.

Results

The overall results showed completely healed in 31%, improved in 58% and unchanged in 11% for the ESWT group; and 22% completely healed, 50% improved and 28% unchanged for HBO group in favor of ESWT group (P = 0.001). ESWT group showed significantly better local blood flow perfusion rate (**Table 1, Fig. 1-a and Fig. 1-b**) and considerably higher cell concentration and more active proliferation and than HBO (**Fig. 2-a and Fig. 2-b**). The results of bacteria culture revealed significant decreases in the bacteria colony counts after treatment (**Table 2**). On immunohistochemical analysis, ESWT group showed significant increases in eNOS, VEGF and PCNA expressions and a decrease in TUNEL expression than the HBO group (**Table 3, Fig. 3-a-1, 3-a-2, Fig.3-b-1, 3-b-2, Fig. 3-c-1, 3-c-2, Fig. 3-d-1 and 3-d-2**).

Discussion

The causes of diabetic foot ulcer are multi-factorial including ischemia/hypoxia, neuropathy, and infection, and they often coexist. Management of chronic diabetic skin ulcers requires multidisciplinary approach including the control of diabetes, antibiotic, shoe wear, wound care and surgery in selected cases. The results of the customary standard treatments are inconsistent and most are unsatisfactory. Therefore, many adjunctive therapies are designed with the intention to cure the diabetic foot ulcers including hyperbaric oxygen therapy, ultrasound, recombinant platelet-derived growth factor-BB, vacuum assisted wound closure and acellular matrix. Among them, HBO is the most commonly employed modality at our institution. Some studies showed beneficial effects, however, none showed universal success. The results of the current study showed that ESWT is more effective than HBO in chronic diabetic foot ulcers.

The exact mechanism of ESWT remains unclear. The results of this study demonstrated that clinical improvement of the ulcers after ESWT were associated with increases in angiogenesis and improvement in local blood flow perfusion, and decreases in cell apoptosis and bacteria growth.

Conclusions

ESWT is more effective than HBO in the treatment of chronic diabetic foot ulcers. It appears that application of ESWT results in tissue regeneration with improvements in blood perfusion and molecular changes in chronic diabetic foot ulcers.

This paper was selected as the First Place Winner in Classification: Tumor/Metabolic Disease at the 75th Annual Meeting of the American Academy of Orthopedic Surgeons (AAOS) in San Francisco, CA.

This paper is also accepted for publication by Journal of Surgical Research.

Tables:

Table 1. Blood Flow Perfusion Rate Before and After Treatment

Laser Doppler	Before treatment	After treatment	P-value-1
ESWT			
Mean±SD	0.64±0.28	0.75±0.19	0.04
(Range)	(0.19-1.23)	(0.46-1.28)	
HBO			
Mean±SD	0.50±0.21	0.58±0.11	0.140
(Range)	(0.18-0.6)	(0.51-0.66)	
P-value-2	0.30	0.043	

P-value-1: Comparison of data before and after treatment in the same group.
P-value-2: Comparison of data between ESWT and HBO

Table 2. The Results of Bacteriological Examination

Bacteria growth*	0	I	II	III	VI	P-value-1
ESWT group						
Before treatment	4	3	9	17	3	
After treatment	13	4	11	8	0	0.002
HBO group						
Before treatment	5	3	9	15	4	
After treatment	11	0	12	12	1	0.042
P-value-2						0.984
P-value-3						0.198

P-value-1: Comparison of data before and after treatment within the same group
P-value-2: Comparison of data between the two groups before treatment
P-value-3: Comparison of data between the two groups after treatment
0: No growth; I: Rare growth; II: Light growth; III: Moderate growth; VI: Heavy growth

Table 3. The Results of Immunohistochemical Analysis

Mean±SD (Range)	Before treatment	After treatment	P-value-1
eNOS			
ESWT	26.62±14.87 (4-57)	48.67±18.82 (6-72)	< 0.001
HBO	25.2±17.09 (6-53)	20.08±9.73 (6-30)	0.317
P-value-2	0.438	<0.001	
VEGF			
ESWT	31.36±22.27 (8-90)	63.69±21.06 (25-91)	<0.001
HBO	42.6±12.6 (28-55)	44.40±11.24 (30-56)	0.409
P-value-2	0.086	0.042	
PCNA			
ESWT	27.0±15.15 (7-53)	55.9±27.86 (8-95)	0.005
HBO	23.0±2.83 (20-26)	26.20±3.11 (23-30)	0.064
P-value-2	0.188	0.004	
TUNEL			
ESWT	62.42±15.0 (39-82)	31.58±13.44 (14-56)	< 0.001
HBO	64.0±25.58 (23-86)	49.4±17.0 (22-65)	0.162
P-value-2	0.451	0.04	

eNOS: Endothelial nitric oxide synthase; VEGF: Vessel endothelial growth factor;
PCNA: proliferation cell nuclear antigen; TUNEL: Transference-mediated digoxigenin-deoxy-UTP nick end labeling
P-value-1: Comparison of data before and after treatment within the same group.
P-value-2: Comparison of data between ESWT and HBO.

Figures:

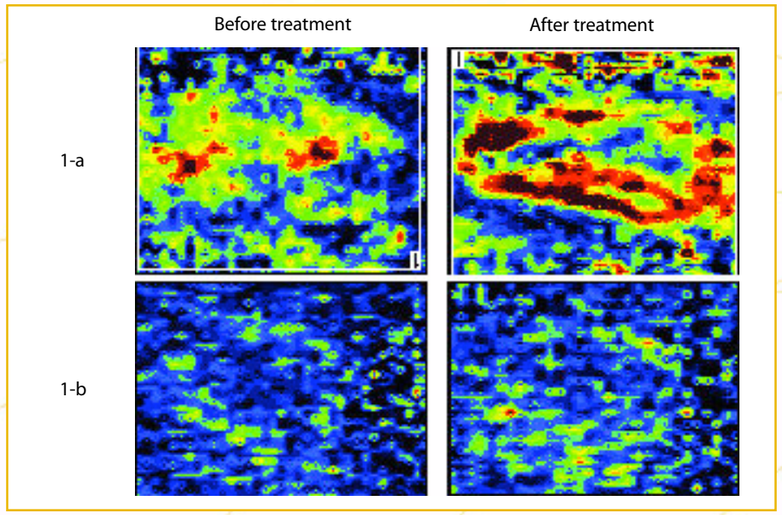

Figure 1. Laser Doppler scan showed significant increases in blood flow perfusion rate after ESWT (Fig. 1-a), whereas the changes were not significant after HBO (Fig. 1-b).

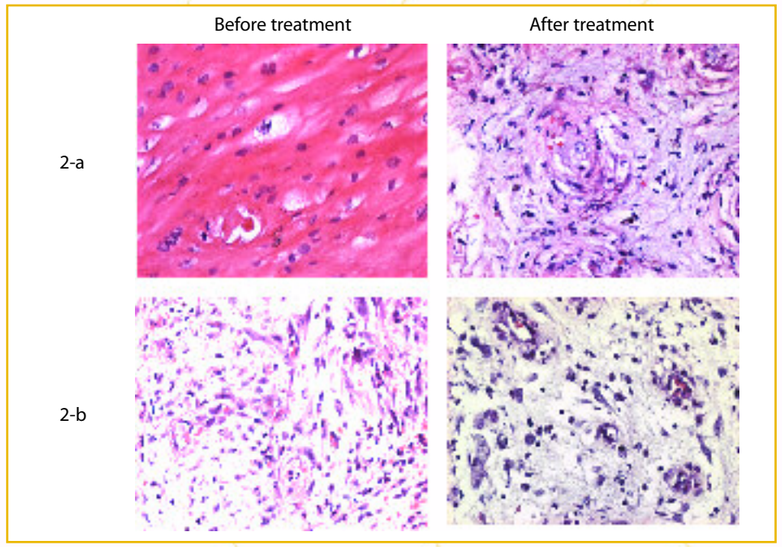

Figure 2. Microscopic features of the biopsy specimen showed higher cell concentration and more active cell proliferation after ESWT (Fig. 2-a), and less cell concentration and proliferation after HBO (Fig. 2-b) (H-E stain x 40).

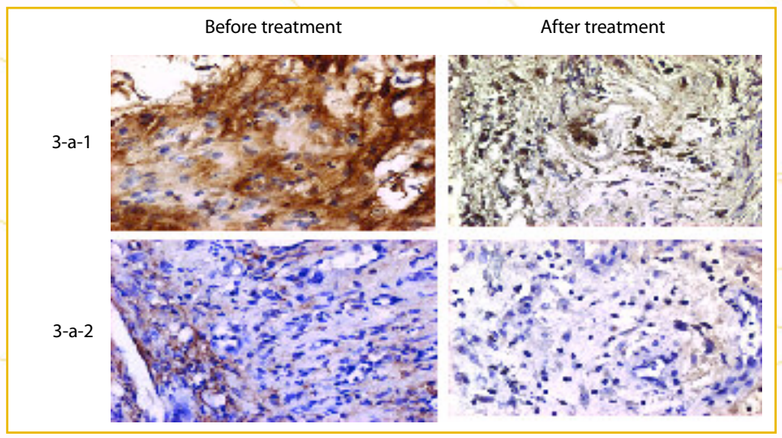

Figure 3a. Immunohistochemical stains showed significant increases in eNOS expression after ESWT (Fig. 3-a-1), whereas the changes were not significant after HBO (Fig. 3-a-2).

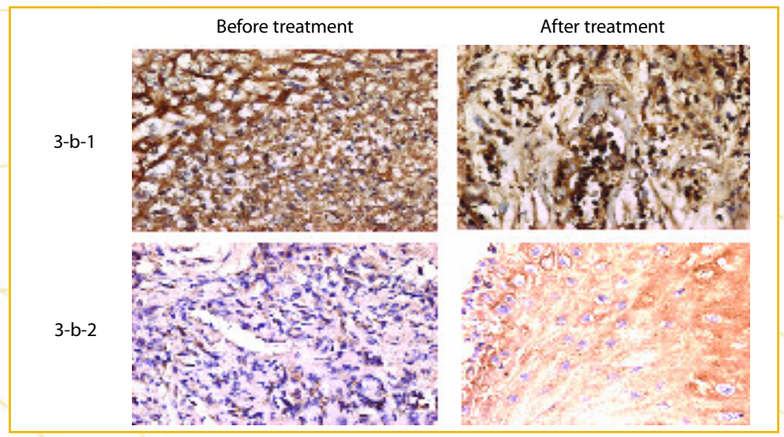

Figure 3b. Immunohistochemical stain showed significant increases in VEGF expression after ESWT (Fig. 3-b-1), whereas the changes were not significant after HBO (Fig. 3-b-2).

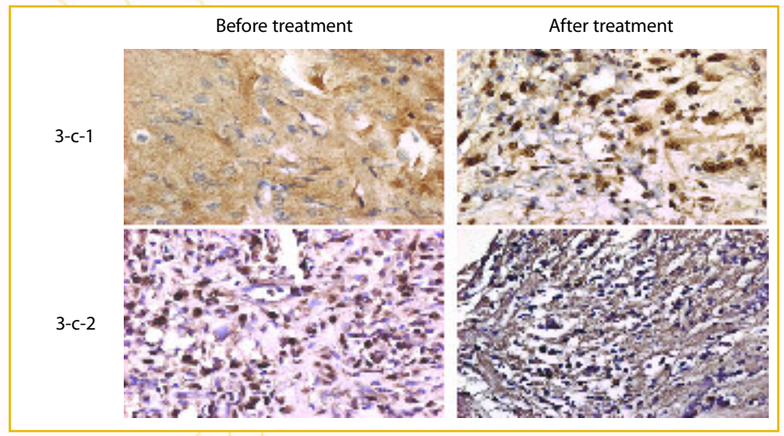

Figure 3c. Immunohistochemical stains showed significant increases in PCNA expression after ESWT (Fig. 3-c-1), whereas the changes were not significant after HBO (Fig. 3-c-2).

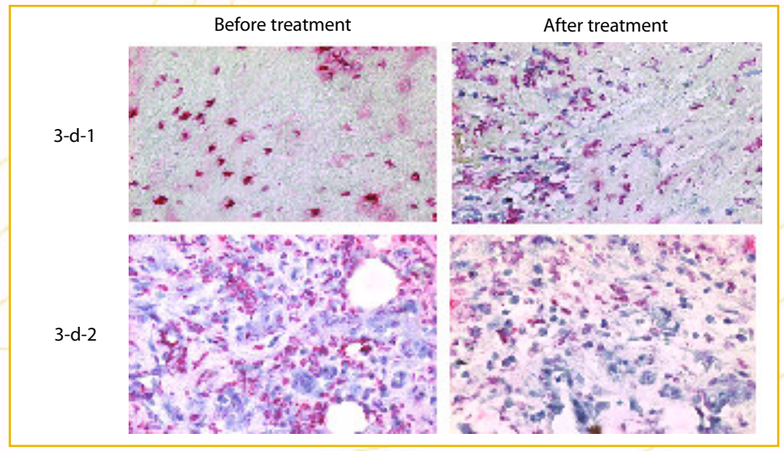

Figure 3d. Immunohistochemical stains revealed significant decreases in TUNEL expression after ESWT (Fig. 3-d-1), whereas the changes were not significant after HBO (Fig. 3-d-2).

Should we expect similar effects of extracorporeal shockwave therapy on wounds of different species?

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Equine distal limb wounds are common and often heal slowly by second intention. Primary closure of wounds of the distal portion of the limb is often prevented by the lack of soft tissue and immobility of the surrounding skin.[1,2] Wounds of the distal portion of the limb often heal by second intention and healing is often inhibited by the formation of exuberant granulation tissue.[3-5] Compared to wounds of the trunk, lacerations of the distal portion of the limb retract more, epithelialize more slowly, and cease to contract sooner.[6,7] Even within the equine species, there are differences in wound healing.

Second-intention healing of wounds occurs faster in ponies than horses.[7] This is the result of a greater and faster contraction of the wound in the ponies. Wounds in horses fill with granulation tissue faster, however in ponies, the granulation tissue is more regular with a smooth surface. Horses often develop exuberant granulation tissue, however this is less common in ponies.[5] (**Figure 1**) This may be explained by differences in the inflammatory response between horses and ponies.[8] Ponies have a greater initial inflammatory response that decreases rapidly after 3 weeks. Horses have less inflammation and fewer neutrophils initially, but the response remains for a longer period of time. During the longer inflammatory period, myofibroblasts are less organized in the horse than the pony. Overall, ponies have a more controlled inflammatory stage and greater organization of myofibroblasts resulting in the more rapid and greater wound contraction than the horse.[8] *In-vitro* studies have shown that there are no inherent differences in fibroblasts and myofibroblasts of horses and ponies therefore, environmental factors such as cytokines and the inflammatory response likely account for the differences.[9]

This is where shock wave therapy may be important to help direct the healing response. Numerous studies have shown an upregulation of multiple cytokines following ESWT. The consistent findings in multiple tissues are an increase in growth factors including VEGF, TGF-B1, and IGF.[10,11,12] TGF-B1 has been documented as important in stimulating wound contraction. The horse has lower production of TGF-B2 than ponies which may be on of the reasons for the differences in wound contraction rates between horses and ponies.[13,14] Another possibility could be oxygen derived free radicals including superoxide and nitric oxide which have been identified in other tissues following ESWT.[15,16] Increased endothelial nitric oxide synthase has been demonstrated by immunohistochemistry in tendon and bone following shockwave therapy.[10] A nitric oxide releasing gel was shown to increase the rate of epithelialization of burn wounds in rats[17] therefore, nitric oxide could be another potential mechanism for stimulation of wound healing. Associated with the increased growth factors is a resultant increase in neovascularization which should result in faster wound healing.[10,11,12,18]

Undoubtedly, in all species it would be desireable for wounds to heal quickly with a return to normal function. How this is accomplished in each species may be by different mechanisms. In the horse, a mechanism to stimulate the rate and amount of contraction would be beneficial. Contraction is usually beneficial for return to function of limb wounds in horses. It usually occurs faster than epithelialization, and results in a better cosmetic outcome. Therefore, maximal contraction is usually desireable in the horse. Disfiguring and disabling contracture does not occur on the lower limbs of the horse. Excessive contracture can occur with injuries to the lips, muzzle or eyelids, however, even this is not common. (**Figure 2**) Consequently, there is a large difference between horses and other species including humans and dogs where excessive wound contracture is more problematic.

Re-epithelialization is an important step in wound healing in the horse, but not important as contraction. Until a wound on the distal limb of a horse is completely re-epithelialized there is a risk of exuberant granulation tissue formation. Thin, nonpigmented epithelialized scars can result in repetitive injuries to the re-epithelialized tissue. Epithelialization is slow and frequently the most prolonged phase of the process with a maximum rate of 1mm/10 days. In horses, epithelialization is limited until contraction has subsided, therefore, wounds with greater contraction have less epithelialization.

Many drugs and devices to stimulate wound healing have been evaluated on distal limb wounds of horses [3,5,6,19,20] but few controlled studies document the benefits of these products. Schumacher, *et. al.* found no benefit of island grafting on the rates of epithelialization and contraction of surgically created wounds on the distal portion of limbs of horses.[6] Equine-derived amnion applied as a dressing to full-thickness wounds on the distal limb of horses significantly sped epithelialization in one study,[20] but this finding could not be repeated in another.[21] Topical medications, including antimicrobial drugs,[3] corticosteroids,[5] and various dressings,[19,20,21] have shown little benefit to wound healing. One study did demonstrate that application of a 1% silver sulfadiazine cream resulted in a faster rate of epithelialization.[3]

Recent studies, in which EWST was applied to skin grafts and epidermal burns in people, demonstrated a decreased time to healing.[22] When the effects of ESWT on the healing of partial-thickness wounds of pigs were evaluated, researchers found that the effect of ESWT on the speed of epithelialization was dose related and that the maximum effect on epithelialization occurred at 10 pulses at 14kV.[13] Recently, the survival of epigastric skin flaps of rats was shown to be enhanced by the application of ESWT.[11,23] Similarly, in a skin flap model, ESWT stimulated healing as much as did gene therapy using transforming growth factor-ß1 (TGF-ß1) or vascular endothelial growth factor (VEGF).[24,25] ESWT-treated flaps developed an area of necrosis of only 2.5%, whereas 17% of the area of flaps that did not receive ESWT was necrotic.[24] In another study, ESWT was shown to induce production of VEGF and perhaps to modulate expression of other growth factors.[25] ESWT was also shown to decrease time to re-epithelialization of deep, partial-thickness burns of human beings.[22]

Frequently equine distal limb wounds are expected to heal by second intention. It was unknown how ESWT would affect the wound healing in the horse.

Study of second intention healing of distal limb wounds in horses

In a recent study by the authors of this paper[26], the effect of ESWT on second intention healing of distal limbs wounds of the horse was evaluated. For this study, a 5-cm diameter circle was tattooed on the dorsomedial aspect of the mid-metacarpal region of each fore limb of each of 6 horses 4 weeks before the study began. This allowed for the measurement of wound expansion and contraction. On day 0, a 4-cm diameter, circular defect that included skin, subcutis, and periosteum was created in the center of each tattoo. At the same time, two similar 3-cm diameter wounds were made on the dorsomedial aspect of each metatarsus, one 4 cm proximal and one 4 cm distal to the middle of the metatarsus. These wounds were created to obtain biopies for immunohistochemical evaluation. (**Figure 3**)

On day 1, the wound on one randomly selected MC was covered with ultrasound coupling gel and treated with ESWT[a] using 500 pulses administered at of 0.11 mJ/mm^2. Both MT wounds on one randomly selected MT were treated with 280 pulses which provided an equal number of pulses per square centimeter of wound as the larger forelimb wounds. Treatments were repeatedly weekly until the wounds were healed. During treatments, the untreated control wounds on the contralateral MC and MT were also covered with ultrasound coupling gel. At each bandage change, the wounds were digitally photographed with a ruler positioned vertically and horizontally close to the wound as reference for the photographs. The wounds were maintained under a non-adherent dressing and bandage until healed.

At day 14, full-thickness, full-width of the wound, rectangular excisional biopsies were taken from the distal wound on each metatarsus, and at day 28, full-thickness biopsies were taken from each proximal wound on each metatarsus. Each biopsy was approximately 6 mm wide, oriented in a transverse plane across the center of the wound, and encompassed adjacent unwounded tissue on both the medial and lateral aspects of the wound. Each biopsy sample was placed into a 10% solution of neutral-buffered formalin and then into 70% isopropyl alcohol. The biopsy was embedded in paraffin using an automated system. Samples were cut into 5-μm thick sections.

Epithelialization and Contraction - The digital photographs were analyzed using computer software[b] to determine the area within the tattoo, the area of epithelialization, and the area of the non-epithelialized portion of the wound. The percentage of contraction was calculated using the following formula:

$$\%\ \text{contraction at } D_x = \frac{(tatD_y - tatD_x) \times 100\%}{wndD_y},$$

where $wndD_y$ denoted the maximum area of the wound, and $tatD_y$ denoted the maximum area of the tattoo, each of which was determined after the wound enlarged to its maximum extent after its creation, before contraction and epithelialization began. Wound contraction was expressed as a percentage of the wound's area on day D_y. D_x represented any specific day after D_y.

Granulation tissue - The quantity of granulation tissue was scored as: 0 = none; 1 = ≤ 5 mm depth and ≤ 1 cm^2 in area; 2 = ≤ 5 mm depth the entire area of the wound; 3 = ≥ 5 mm depth.

Immunohistochemical staining for IGF-I, VEGF and TGF-ß1

For immunohistochemical staining the IGF-1 antibodies[c] were diluted 1:10, and the VEGF and TGF-ß1 antibodies[c] were diluted 1:50 in Tris/PBS/BSA solution. Sections were bathed in the primary antibody solutions for 2 hours at room temperature. Endogenous peroxidase activity was inhibited by applying 3% H_2O_2 for 10 minutes. For IGF-1 and VEGF, a multilink, goat, anti-immunoglobulin secondary antibody was used at a dilution of 1:80 and were incubated for 15 minutes at room temperature. For TGF-ß1, a goat anti-rabbit secondary antibody was used at a 1:500 dilution. Samples were incubated for 15 minutes at room temperature. All slides were then exposed to a 1:200 dilution of Horse-radish Peroxidase-Streptavadin for 15 minutes then stained with Nova Red for 5 minutes and counter-stained with one-quarter strength Shandon's hematoxolin for 2 minutes. Negative controls were incubated in PBS instead of with the primary antibodies. Normal equine pancreas was used as the positive control for IGF-1. A section of skin with an extensive focus of granulation tissue was used for both the VEGF and TGF-ß1 controls.

For each time frame and treatment group, five fields of immature, loose, granulating fibrous connective tissue were randomly chosen for examination at 600X. The connective tissue was subjectively evaluated for intensity of cytoplasmic staining and objectively evaluated for the density of cells with positive staining. A field of view was assigned a score of 1 if the uptake of stain was low in intensity. This corresponded to <15 cells with stain uptake /600X field. The field of view was assigned a score of 2 if the uptake of stain was moderate in intensity. This corresponded to 15 -40 cells stained /600X field. The field of view was assigned a score of 3 if the uptake of stain was high in intensity. This score corresponded to >40 cells stained /600X field.

Results

Epithelialization and Contraction -The mean length of time for wound healing was 76 days for wounds treated with ESWT, and 90 days for the untreated control wounds (P = 0.051). The healed treated and control wounds had similar areas of epithelialization (T = 4.5 cm^2; C = 3.9 cm^2; P = 0.48) and percentages of contraction (T = 61.3%; C = 61.0%; P = 0.96). (**Figures 4 & 5**)

Granulation tissue - The mean sum of the granulation tissue scores did not differ significantly throughout the study period (P = 0.52). When the wounds were healed, the sum of the granulation tissue scores

throughout the study period was 10 for the treated wounds and 8 for the control wounds.

Immunohistochemical staining - In study tissues, IGF-1 staining was observed in the cytoplasm of macrophages, fibroblasts, neutrophils, and plump, immature endothelial cells. Positive staining for VEGF in control and study tissues was identified in the cytoplasm of fibroblasts, endothelial cells, macrophages, and smooth muscle cells. Staining for TGF-ß1 was identified in the fibrinous exudate, and the cytoplasm of macrophages, fibroblasts, and endothelial cells.

The density of staining of growth factors varied between regions of the section examined. Density of staining was greatest in areas of wound repair that were composed of immature, loose, granulating, fibrous connective tissue. There were no statistical differences in the score for cells with positive staining for VEGF (P = 1.0 and P = 0.37), IGF-1 (P =1.0 and P = 0.31), and TGF-ß1 (P = 1.0 and P = 0.37) between treatment and control wounds at day 14, or day 28, respectively. There was a statistically significant decrease in density score for cells staining positive for VEGF (P = 0.03) in control wounds from day 14 (mean 3) to day 28 (mean 2.17). A similar trend in VEGF score was noted in the treatment wounds (d 14 = 3; d 28 = 2.33; P = 0.06). There was a significant decrease in the IGF-1 density score between the initial and second biopsies among the control wounds (d 14 = 2.83; d 28 = 1.67; P = 0.015), but not among the treatment wounds (d 14 = 2.83; d 28 = 2.33; P = 0.25). TGF-ß1 scores decreased significantly during the time from the first to second biopsy in both the treatment (d 14 = 3.0; d 28 = 1.5; P = 0.015) and control (d 14 = 3.0; d 28 = 1.67; P = 0.015) wounds.

Discussion

It is very apparent that we must look at each study individually Not only do we need to account for EFD, pulse numbers, waveform, and device, we need to be aware of species differences. The extrapolation between species is only viable as starting points for research. The wound type must also be considered. Most burn wounds and skin flaps have some degree of dermis present. In our study we evaluated the rate of healing of full-thickness, cutaneous defects. These wounds included all skin, subcutaneoius tissue and periosteum. To our knowledge, similar wounds have not been evaluated in other species.

While the results of this study indicate that ESWT may speed the rate of healing of wounds on the distal portion of the fore limbs of horses, it only resulted in a 14 day improvement. When healed, the treated and control limbs had similar percentages of contraction and epithelialization. Our results show that the increased rate of healing was primarily due to an accelerated rate of epithelialization and to a lesser degree contraction during the early wound healing period. Clearly there are some benefits for ESWT in wounds that must heal by second intention in the distal limb of the horse. However, from this study and previous studies, there may be additional clinical indications.

Chronic non-healing wounds with exuberant granulation tissue are common in the horse. In these wounds, any mechanism to stimulate healing after debriding the exuberant granulation tissue is needed. In the study presented here, the effect of ESWT was seen predomiantly in the first 3-5 weeks after wounding. If ESWT could "restart" the early phases of wound healing it could be beneficial to these chronic wounds.

Limb wounds in the horse frequently have flaps of tissue, which are often lost due to avascular necrosis. The benefit of ESWT on the epigastric flaps in rats resulted in an 14.5% decrease in flap loss. This could be important in these wounds where there is limited soft tissue covering. (**Figure 6**)

The increase in epithelializaiton was the primary contributor to the differences seen in this study. Distal limb wounds in the horse frequently require skin grafting to achieve healing. The stimulation of epithelialization from skin grafts would greatly speed the healing of these grafted wounds. (**Figure 7**)

Additionally, ESWT in conjunction with other wound therapies may provide a way to enhance the response and further maximize the rate of wound healing. The topical application of platelet-rich plasma has been shown to accelerate epithelial differentiation,[38] which potentially could be synergistic with the ESWT. In equine lower limb injuries where contraction is limited and skin grafts are often required, the value of ESWT on graft take and epithelialization should be investigated. There were no complications seen with the treatment in this study and no contraindications were found involving 208 human patients.[37] The effects appear to be most predominant early in the healing process, so treatment may best be concentrated early after injury.

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[a] Equitron, Sanuwave Inc., Marietta, GA.
[b] Image J 1.37v, National Institutes of Health
[c] Antibody source: IGF-I (H-70)-sc-9013, TGFB1 (V)-sc-146, VEGF (A-20)-sc-152: Santa Cruz Biotechnology, Inc., 2145 Delaware Avenue, Santa Cruz, CA 95060

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Figures:

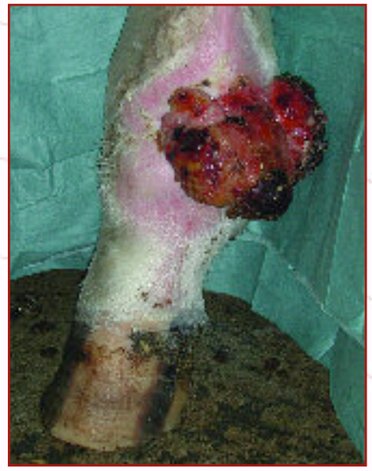

Figure 1. Exuberant granulation tissue on the lateral aspect of a chronic nonhealing wound.

Figure 2. Contracture of the lower lip of a young horse that was exposed to a caustic material that created the wounds. The contracture of the wound on the ventral lower lip resulted in the lip curling downward.

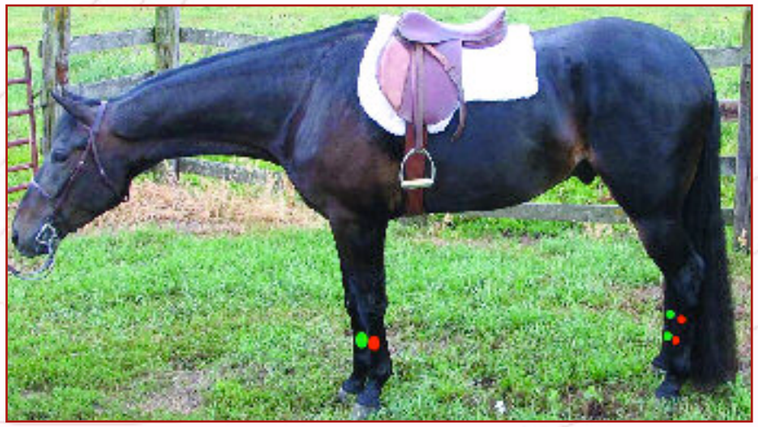

Figure 3. One 4 cm diameter wound on the metacarpus was randomly assigned to be treated (green) and the contralateral metacarpus wound served as the untreated control (red). Two 3-cm wounds were created on each metatarsus and they were assigned to treatment or control the same as the ipsilateral metacarpus.

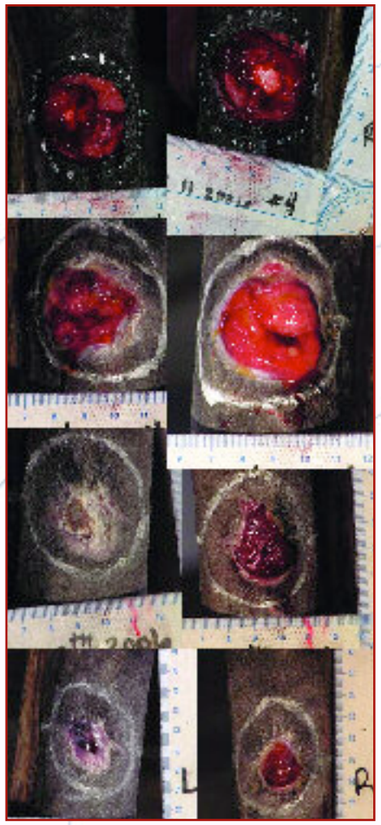

Figure 4. From top to bottom pictures taken on day 0, 30,62 and 90 days after the creation of the wounds. For this horse the left limb (on the left) was treated and the right was the untreated control. The dark skin in this horse made it difficult to see the tattoo so the tattoo was marked over with a white marker for pictures.

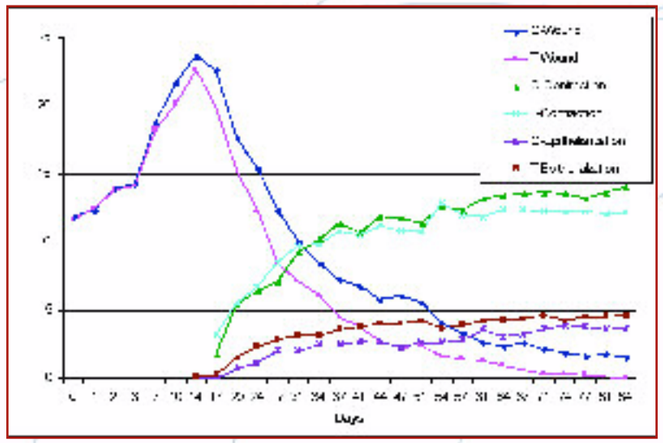

Figure 5. The total area of the control (C) and treated (T) wounds increased in size from day 0 to day 14 when they began to decrease in size from contraction and epithelialization. There was a greater area of contraction in the control wounds because they took longer to heal. The majority of the difference between treated and control wounds was the difference in the area of epithelialization from 17 through 34 days.

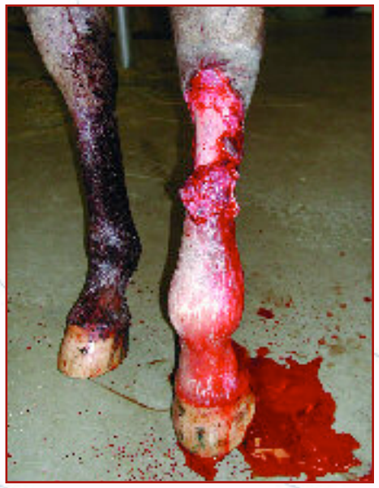

Figure 6. Lower limb injuries in the horse commonly result in the formation of tissue flaps with poor perfusion that become avascular. Decreasing the area of tissue lost from avascular necrosis would notably improve healing of these wounds.

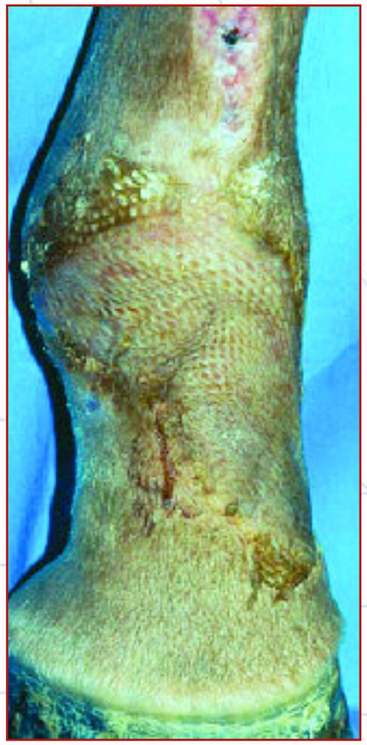

Figure 7. This mesh graft was applied 24 hours previously to a laceration over the pastern region. Shock wave therapy could potentially improving the rate of epithelialization from grafts and shorten healing times.

Shoulder Rotator Cuff Tendinopathy. Histological, Immunohistochemical and Vibrational Spectroscopy Analysis

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Introduction

Increasing numbers of adult patients with shoulder tendinopathies are presenting worldwide and latest advances in imaging techniques afford a better characterization of these patients, however, understanding of the pathophysiology of rotator cuff diseases remains incomplete. Recent reports have been focused on the biology of rotator cuff responses to selective treatment with cytokines [1,2,3], while others analyze the pathological findings with better study protocols [4,5,6].

In recent years Extracorporeal Shockwave treatment for this condition has been applied with increasingly improved results [7,8,9,10]. Our aim is to gain more knowledge of the biological response of the shoulder rotator cuff to this new therapy, including vibrational spectroscopy analysis [11,12].

Patients and Methods

From January 2004 to August 2008, we attended 40 patients (symptomatic rotator cuff tears (38 patients) and calcified tendinopathy (2 patients)) that underwent open surgical treatment. Over the same time period, shockwave therapy was applied for Calcified Shoulder Tendinosis (electro-hydraulic device, 4000 pulses, 0.33mJ/mm^2, single session, without anesthesia, outpatient procedure) and the same treatment protocol was offered to patients with rotator tears who had not previous surgery. Ten such patients accepted the treatment. Patients received full disclosure concerning the different medical and surgical treatment options available to them and informed consent about technical procedures and biopsy treatments. Fifty-three biopsies (Group A, all open surgery, 40 initial surgical patients, 10 patients with SW pre-op, 3 patients who underwent surgical resolution after SW-failed treatment for Calcified Tendinopathy) were collected, undergoing standard laboratory procedures for preservation and staining (haematoxylin-eosin technique), and examined under light microscope (Nikon-Eclipse E200). We used Riley's histopathological classification and semi-quantitative analyses for all 53 H-E stains, examining and photographing 3 microscopic fields (x10-objective); interesting findings were reviewed with x40 and x100-objectives and also were photographed.

Twelve biopsies (non-SW treated, Group B) and 13 SW-treated (Group C) underwent immunohistochemical procedures (monoclonal antibodies and techniques for PCNA, cd34+, cd14+, D2-40, Col I, Col III, Tenascin-C) and semi-quantitative analyses were done for countable stain on formed structures in cases of PCNA, D2-40 (lymphatic marker), cd34+ and cd14+ (endothelial cell marker). We reviewed 5 photographed microscopic fields (x10-objective) for each antibody, applying a grille 10x10 (100 chambers), and obtaining a total number and percentage (absent: 0%, low: up to 20%, regular: up to 70%, intense: 80% to 100%).

In the case of Col I, Col III and Tenascin-C the photographed fields received a grille 5x5 (25 chambers), characterizing the stain distribution comparing intensities over the analyzed area (low: up to 20% (5 chambers); regular: up to 40% (ten chambers); intense: all the rest). For both groups (B and C), interesting histological findings were reviewed with x40 and x100-objectives and were photographed.

Biopsies of Groups B and C received spectroscopic protocols for this kind of analysis. The tools selected for our studies are Raman spectroscopy and the ultrasensitive analytical technique of Surface-enhanced Raman scattering (SERS). Here we report structural information obtained from 1016 SERS spectra of 52 biopsies of tendon tissues on Ag nanoparticles.

Results

Histopathological analysis

Macroscopic features of biopsies include: (1) the edge of the torn rotator cuff with 2 to 3 mms of medial portions in a single piece; (2) sample of bone with a cartilage border (4-5mms wide, 6-7mms long), corresponding to the area of normal insertion of supraspinatus muscle.

For 53 histological observations (Group A, H-E sections), the distribution according to Riley Classification indicates 4 cases grading type II, 37 cases grading type III and 12 cases grading type IV , examined in order from medial to lateral edge. For Group C (SW) the Riley distribution was 7 cases type III and 6 cases type IV.

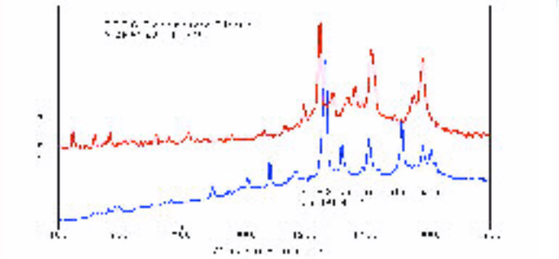
A careful examination of the vascular aspect in tendinopathic non SW-treated population showed that many vascular beds comprise damage of pericyte cells that envelope endothelial cells in nascent neo-angiogenesis and this area tends to develop micro-haemorrhagic instances (**fig.1a, b**). Chondroid metaplasia was seen much more in cases of Riley type IV, which occupies zones related to the torn edge. These areas evidence profound vascular metaplasia where non proper morphological features of vessels could be identified and also shows many acellular fields (**fig.1c**).

Biopsies of patients treated with SW demonstrates areas of fibroblastic repairs that tend to appears in clusters (more over the medial portions of specimens) and include definite signs of active neo-blood vessels with hypertrophic areas corresponding to

pericytes envelope (increased in number) ("hypermuscularized neo-vessels", **fig. 2a,b,c**). These "nodes" show areas close to vessels, stain like disorganized new collagen and resemble native collagen (**fig. 3a,b**), but disappear in areas of chondroid transformation of the tissue (chondroid metaplasia). Biopsies of patients with failed-SW treatment in Calcified Tendinosis also demonstrated development of lymphatic channels along which granular portions of calcium were being removed (**fig. 3c,d,e**).

Vibrational Spectroscopy analysis

The SERS spectra are dominated by signals corresponding to the collagen molecular system in the 1300-1200 cm^{-1} spectral region. Bands corresponding to the amide III mode shift in frequency and intensity in the tissues before and after shockwave treatment. This result is interpreted in terms of a conformational change in the collagen induced by shockwaves. However the increased intensity of the band at 1246 cm^{-1} could be also related to an increasing of the amount of the shockwave induced new conformation. This is supported by the spectral behaviour of the most abundant amino acid proline; in fact two of the most intense proline bands at about
840, 1080 and 1410 cm^{-1} drastically change in intensity. The collagen new conformation imposes a different analyte substrate interaction. The disappearance by shockwave effect of the type III collagen SERS band at 1609 cm^{-1} could be related to the relative decreasing of such species by shockwave treatment.



Discussion

It has been described that 2 to 3% of the volume area of a normal tendon should be occupied by blood-vessels [13,14]. In our histological review for tendinosis, we found that the volume area was enlarged up to 15% in tendinosis grade II and III. This spontaneous reparative effort includes vessels with damage on the pericytes envelope-sheath with probabilities for micro-hemorrhages, a significant percentage of which appear to be inactive, non-containing red blood cells. The total number of vessels and volume area percentage lessen significantly in tendinosis grade IV, where chondroid metaplasia predominates in many areas along the torn edge. Also on those type IV tendinosis SW-treated we did not find neo-blood vessels close to chondroid metaplasia.

In Group C we identified specific features: clusters of active neo-vessels occupying up to 23-28% of volume areas, their hypermuscularized aspect correspond to hyperplasia of pericytes surrounding endothelial cells (probably due to imbalance for more active than inactive forms of PDGF that exists in tendinopathic matrix), metabolic activity in these areas shows a precise augmented stain for Col I, Col III and Tenascin-C, suggestive of repair matrix behavior [15].

Recovery of PCNA and Tenascin-C from "low" to "regular or intense" suggests an improvement of repair capabilities in the SW-treated population. Also the recruitment for cd14+/34+ from "low/regular" to "intense/intense" is indicative of activation of blood-supply on those areas of neo-blood vessels[16].

In summary, according to our results we suggest that SW treatment induces an improvement of metabolic and intrinsic repair capabilities on tendinopathic rotator cuff of the shoulder. A multi-center investigative effort will ascertain the real meaning of these findings.

Figures:

Fig. 1

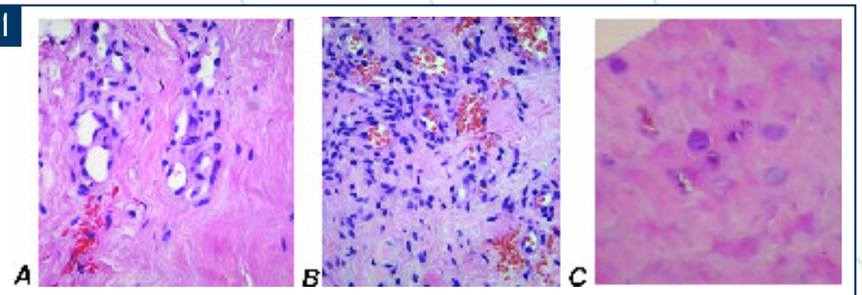


Fig. 1A, tendinosis type II, zone of initial and spontaneous angiofibroblastic response shows damage of pericyte envelop of nascent vessels (arrows) and hemorrhagic zone (bottom left). 1B, tendinosis type III, similar areas depict hemorrhagic aspect. 1C, tendinosis type IV with chondroid areas showing vascular metaplasic areas, not possible to recognize vascular endothelium (arrows).(H-E stain).

Fig. 2

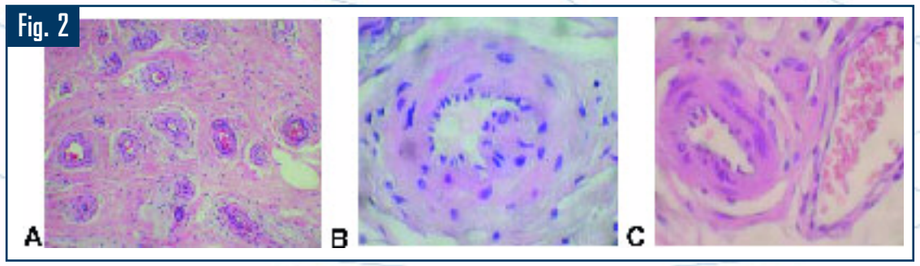


Fig. 2A, SW-treated case , showing increased number of induced neo-blood vessels (x40 objective). 2B and 2C, both Group C patients showing hypermuscularized aspect depending on the development of pericytes (2B and 2C , magnification x100-objective, H-E stain).

Fig. 3

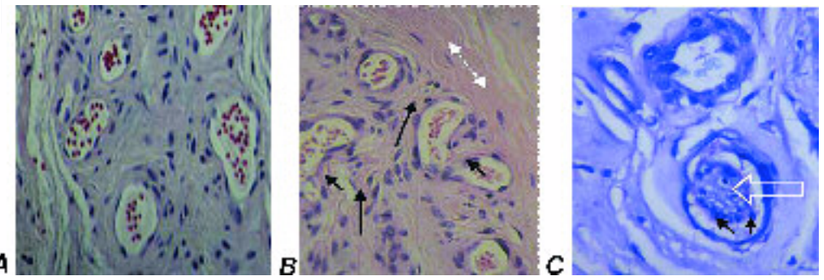


Fig. 3A, SW-treated case in tendinosis type III showing a node of angiofibroblastic response to SW (compare to fig.1A and 1B).; in 3B , same patient , depict relationship between the neo-angiogenesis area depositing collagen to stabilize the sprout (black arrows) and the native structural collagen on the tendon (white arrow). 3C, calcified tendinosis SW-treated showing a lymphatic vessel with a macropha ge inside (open arrow) containing microgranular calcium salts (black arrows) (Toluidine Blue Stain).

Fig. 3

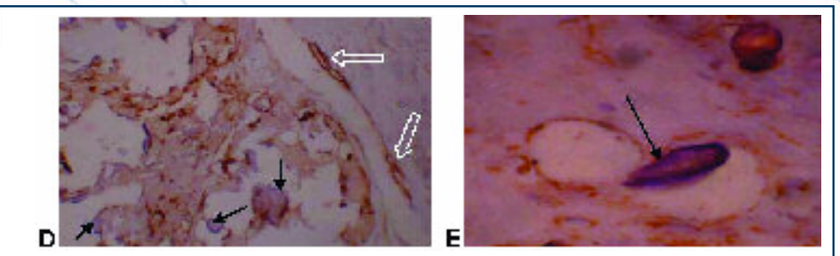


Fig. 3D, calcified tendinosis SW-treated and immunostaining for D2-40 (lymphatic marker) showing a calcium granuloma under resorption (black arrows) with surrounding lymphatic channels (open arrows) developed in the entire process of resorption. 3E, microgranular calcium inside of lymphatic channel (arrow , D2-40 immunosatining,x100-objective).

Fig. 4

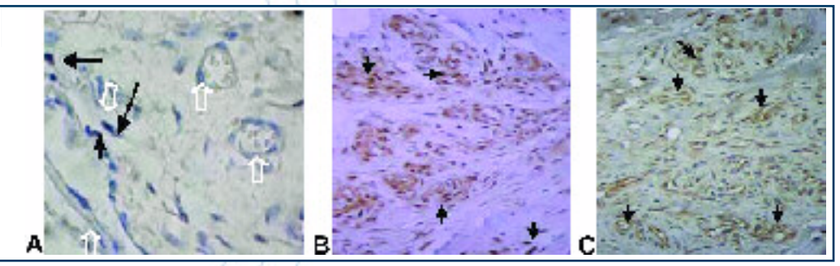


Fig.4A, tendinosis type III (Group B patient) with immunostaining for PCNA, showing almost absent staining (black arrows) upon 4 areas with vessels (open arrows). 4B, example of Group C immunostaining for PCNA, showing intense staining in angiofibroblastic nodes SW-induced (black arrows). 4C, Group C patient with immunostaining for collagen I, showing disposition of the marker in areas associated to neo-blood vessels SW-induced (black arrows).

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IVSWT: How can in-vitro shock wave therapy be performed best? - Preliminary results from cardiac cells

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Introduction

Transthoracic application of shock waves is recently known to augment myocardial vascularization in a porcine model of myocardial infarction [**1, 2**], besides it is shown to effect relief of angina symptoms in patients with severe coronary artery disease [**3**]. Nevertheless, pulmonary contusion causing life-threatening hypoxemia and haemoptysis is described as an adverse event of shock waves when hitting lung tissue [**4, 5**]. Therefore transthoracic cardiac shock wave application is limited by lungs partly covering the heart [**1-3**]. Direct epicardial shock wave therapy (DESWT) may be more safe, thereby enabling the treatment of larger myocardial areas and even the posterior wall of the heart.

We hypothesized that DESWT during open heart surgery may serve as an adjunct to surgical revascularisation (Coronary artery bypass surgery). Therefore we established animal models of ischemic heart failure to show that DESWT induces myocardial regeneration and improves ventricular function.

In June 2008 our Myocardial Regeneration Research Group from the Department of Cardiothoracic Surgery under the direction of Prof. Dr. Michael Grimm presented first results from these animal trials at the 11th International Congress of the ISMST in Juan les Pins, France. Therein DESWT showed very promising effects [**6**], although the mechanism remains largely unknown. Therefore we started an in-vitro shock wave trial (IVSWT) to learn more about the molecular and cellular mechanisms of shock waves.

Background

By reviewing literature we found very diverse methods of applying shock waves onto cell cultures [**7-10**]. While most research groups have in common that they use ultrasound transmission gel as a contact medium between the shock wave applicator and the target tubes, they all use different methods of applying shock waves onto the cells. Some of them are associated with distinct limitations, especially distracting physical effects. Due to this we tried to develop an experimental setup that would perfectly imitate in-vivo conditions without severe distractions. This resulted in our below-described water bath. However, since results of equal cells treated in different ways are not comparable, establishing a standardized model for future in-vitro trials was also deemed useful. A proper in-vitro model may be an important step for intergroup communication, which could help all of us working on IVSWT to learn more about the shock waves' mechanism by being able to compare our results.

Model

Basically our in-vitro model exists of a plexiglass built water bath with an adapter for the shock wave applicator (CP-155, DermaGold® from Tissue Regeneration Technologies LLC, Woodstock, USA manufactured by MTS Europe GmbH, Konstanz, Germany) [**Figure 1**]. This adapter can be customized for all kind of shock wave devices. The water bath is filled with degassed water to avoid cavitation, a heater at the bottom with a temperature sensor connected to a control unit enables to regulate temperature for imitation of in-vivo conditions. A holder for our cell samples filled in common cell culture flasks also serves as a distance control bar. Its fixation mechanism allows to change culture flasks easily and quickly [**Figure 2**].

One of the major reasons to design the water bath for IVSWT was to avoid reflections caused by the distinct difference in the impedance between culture medium and the ambient air. Due to this shock waves would be reflected, thereby causing negative pressure onto the cells and also disturbing upcoming waves. The water bath enables propagation of shock waves far beyond the cell culture flasks, thereby not causing any kind of distraction directly at the cell layer.

Materials & Methods

Primary cell cultures of endothelial cells and fibroblasts were established from native rat hearts. Additionally H9C2-cardiomyocytes (American Type Culture Collection) were used. All cell types were cultured using DMEM medium supplement with common nutrients and growth factors. Adherent cells in common cell culture flasks filled with culture medium were dunked into the water bath. (In contrast to cell suspensions adherent cultured cells give the possibility of analysing cell communication, e.g. gap junctions.)

Various energy flux densities of unfocused SWT were applied in different distances to the cells. Non-treated cells were used as a control group. Number of cells and their vitality then were analysed over a period of 7 days.

After the results of several pilot trials we focused on an energy flux density of 0.15mJ/mm² and a frequency of 5Hz, since these are the commonly used parameters in vivo.

Preliminary Results

Counting of cells and proving their vitality are the basic analysis of cell cultures. Vitality was proved using trypan blue staining. Trypan blue is not absorbed in vital cells, just dead cells become blue. This so called Dye Exclusion Method showed hardly any blue cells in the treatment as well as in the control group. Vitality of all cell samples was about 99%.

Cell counting revealed different results in each cell type, especially in comparison to the untreated control group. Shock wave treated cells obviously proliferated faster. Growth curves of cells are shown in [**Figure 3 A-C**].

As a very important parameter for proliferation we calculated the cell duplication time every 24 hours with the commonly used formula $T_c=0.3T/\log(A/A_0)$ [T_c...duplication time, T...24 hours, A...cell number after 24 hrs., A_0...initial cell number]. The very simple diagram in [**Figure 4**] shows that the mean value of duplication in treatment groups is decreased compared to controls. Especially in a distance of 5cm between the shock wave applicator and the sample the duration of cell duplication is much lower. In conclusion, each cardiac cell type needs less time for proliferation after shock wave treatment compared to its untreated controls. The distance between the applicator and the sample has a major impact on the cells' behaviour.

Detailed data interpretation is not yet possible since several analysis, especially concerning immunohistochemistry and molecular biology, are still in progress. In this pilot study we only used healthy cells from unharmed myocardium. From our previous mentioned in-vivo trials we already know that healthy cells do not respond that much to SWT than pathologic cells do [**6**]. Future trials with cells from ischemic harmed myocardium will show the potential effect of DESWT in-vitro.

Discussion

Besides the cost-effectiveness and the reduction of animal experiments, the biggest advantage of IVSWT is the possibility of studying the specific behaviour of a certain cell type. In shock wave mediated tissue regeneration most likely all cells of the treated tissue are involved, even systemic effects are discussed. Nevertheless, each cell type plays a specific role and has its own intrinsic function. These we are able to detect by doing IVSWT.

To the best of our knowledge all in-vitro models in literature make effort to elaborate application methods, but do not consider the propagation of waves after passing the cell culture.

In our model cell culture flasks are mounted directly into the degassed water bath, which is connected with the shock wave applicator through a circular opening. As cell culture flasks are filled with culture medium and no other coupling membranes are needed in this system, there is hardly any difference in acoustic impedance between the applicator an the cells. This leads to an undisturbed propagation of shock waves and avoids reflection as well as negative pressure and interference with upcoming waves.

Another advantage in doing IVSWT with this model is the possibility of varying the distance between the applicator and the culture flasks with the distance control bar fixing the sample.

Although SWT is used for several clinical indications, its exact molecular mechanism is still not exactly understood. IVSWT can help us to learn more about the molecular and cellular mechanisms of shock waves. By understanding them new indications could be established and moreover our today approved indications could be improved by knowing more about the influence of the different application parameters like pressure distribution, energy flux density, number of impulses and the specific impact of different shock wave technologies. To address this issue we will have to compare focused with defocused shock waves and electro-hydraulic with electro-magnetic and piezoelectric waves in future in-vitro trials.

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Figures:

Fig. 1

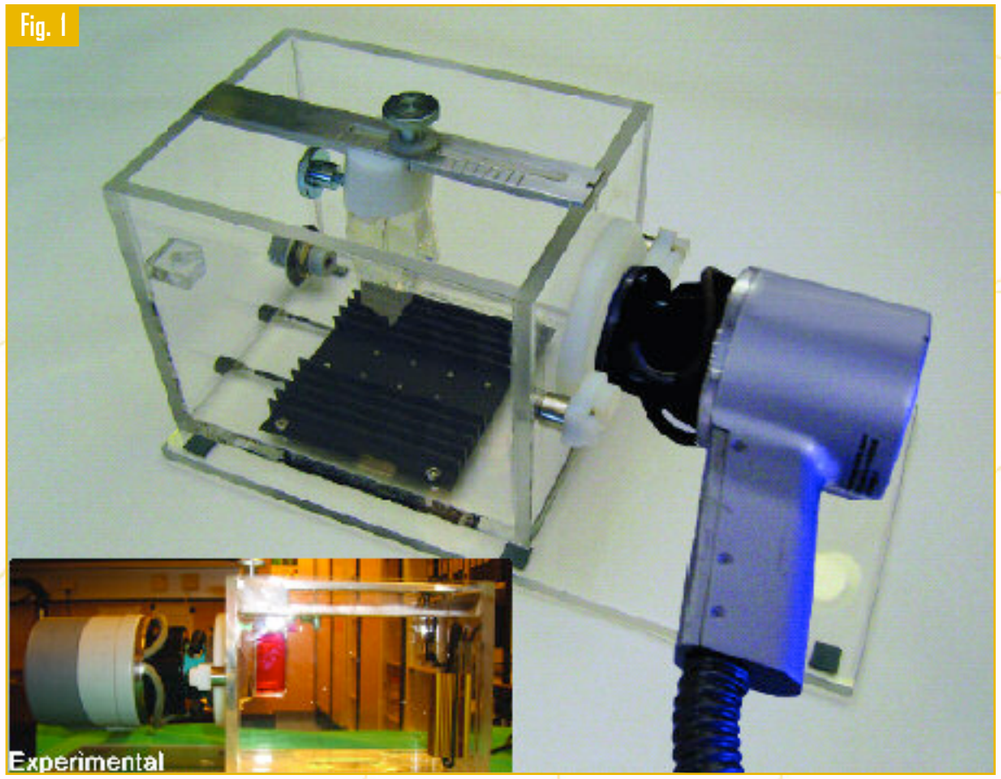


Fig. 2

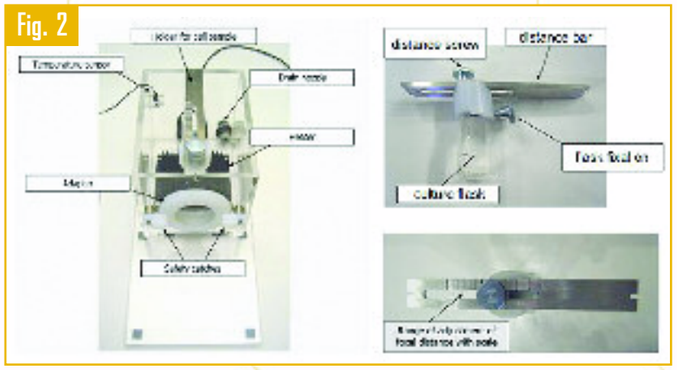


Fig. 3

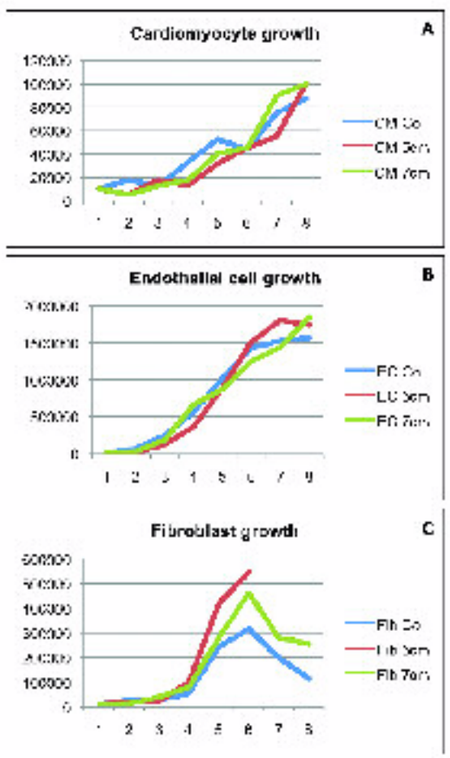
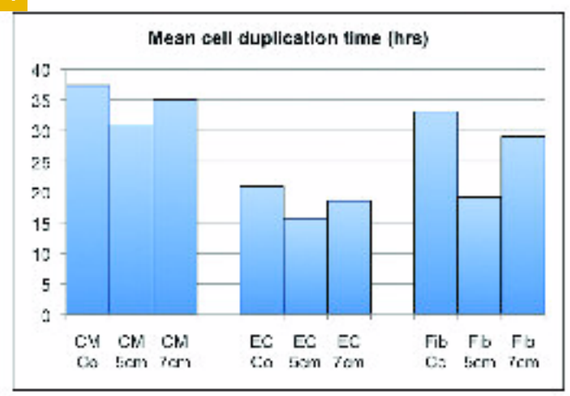


Fig. 4



Myofascial Pain Syndrome (ICD 10 – 79.1) –
An Excellent Indication for Low Energy Focussed ESWT

During the last years patient studies and clinical trials have revealed new indications for the use of focussed Shockwave Therapy (ESWT). Pain conditions of different types caused by diverse lesions of the musculo-skeletal system have been often in the centre of the attention. Whereas bony and tendinous structures have been since the beginning of ESWT in orthopaedic diseases literally in the focus of the treatment, muscle tissue has not been considered equally. Recently, along with new scientific studies about the understanding of muscle pain, which is totally different to the nociceptive system of the skin, putting the focus of ESWT on painful spots in the muscular tissue, the so called Myofascial Trigger Points (MTrP's), a new chapter of understanding and treating pain conditions has been opened.

According to Wheeler (2004) 44 million Americans are estimated to have Myofascial Pain Syndrome (MPS) so it is seen to be one of the most common causes of acute and chronic pain of the musculoskeletal system. It often imitates other pain conditions e.g. nerval root lesion. MPS is characterized by Myofascial Trigger Points (MTrPs), which are hyperirritable spots in a palpable tense band of skeletal muscle. MTrPs are caused by a dysfunction from involved motor endplates, which is followed by a segmental shortening of groups of sarcomeres. Diagnostic approach is based on the criteria defined by J.Travell and D.Simons: while palpating an active MTrP a referred and familiar (recognition) pain is elicited. Effective diagnosis and treatment requires clinical experience and diagnostic skills, especially palpation ability. Exact pressure or impulse with minimum irritation or even damage of the collateral tissue is needed to identify and release MTrPs.

Focussed ESWT is able to apply an exact mechanical impulse on a small spot to find MTrP's in the muscle, even in the deeper layers, and while eliciting the patient's typical pain (recognition and referred pain), it can much likely identify MTrP's as a major source of the patients complaints.

MPS can be treated successfully with focussed ESWT while putting MTrP's exactly in the focus and releasing these painful spots.

The use of focussed ESWT is a good method for the diagnosis and treatment of musculo-skeletal pain that is due to MPS.

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BRIEF COMMUNICATION

Raman and Surface Enhanced Raman Scattering Applications in Shock Wave Therapy Related Research

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The biological applications of Raman scattering (RS) in its different forms continues to grow exponentially, and the literature is so extensive that in a short communication references will not even attempt to do justice to the field. The fingerprint for molecular structure provided by vibrational spectroscopy, and their relation to functionality in biochemical systems can be used for the development of a quantitative technique for biomarkers. These vibrational fingerprints in the spectra are used to track and characterize species such as small low-molecular-weight metabolites and also follow molecular species in large living organisms. Today, researchers are making great progress applying RS to unravel the structure/function issues in proteins, nucleic acids, and lipids. Recently, the efforts are devoted to bioanalytical and medical diagnostic applications. In our group, for biomedical applications, we integrate a full range of Raman experimental methodologies, including Raman microscopy, resonance Raman scattering microscopy, near-infrared Raman, and the ultrasensitive analytical technique surface-enhanced Raman scattering.[1] This molecular approach is then integrated with the biomedical research in an attempt to understand the biological processes.[2] Here, we present the first steps towards the molecular understanding of the important improvements of rotator cuff supraspinatus tendons diseases that have seen after shockwave treatment. Neo-angiogenesis stimulation and hypercelularization are the result of short time periods of treatment. The beginning of this work, necessarily, requires an extensive background research dedicated to the creation of the appropriate database for fingerprint characterization of the biomolecules present in the tissue. This is an enormous task that involves a large group of multidisciplinary researchers with a top-down approach of the medical team (the real samples) and a bottom-up approach of the spectroscopist, all helped by the statistical analysis and modelling of the physics group.

The preliminary results have been selected from our Raman scattering and plasmonic driven technique of Surface-enhanced Raman scattering.[3] The background information included the studies of the basic amino acids forming collagen, two different types of collagen and 52 biopsies of tendon tissues. Briefly, the inelastic Raman scattering was collected using a micro-Raman system with a spatial resolution of 1 micron squared and the sample is illuminated with laser lines at 442 nm, 514.5 nm, 632.8 nm, or 785 nm, depending on the optimization of the experimental conditions. SERS was attained using overlayers of silver and also colloidal silver nanoparticles. Typical Raman spectra of the amino acids most commonly found in collagen are shown in **Figure 1**.

It can be seen, that each molecule of the amino acid has its own characteristic spectral pattern, and characteristic wavenumbers can be identified for each one of them. The extensive and complete analysis and computational work for each molecule will be published separately.

The collagen detection and characterization was demonstrated using to commercially available collagens; the rabbit skin (TR in the spectra), and ox bone (CB in the spectra). The experimental SERS was obtained by depositing 6 nm mass thickness of silver by vacuum evaporation onto the collagen sample. Micro-Raman was recorded using point-by-point-mapping. The mapping shows that a typical pattern repeat itself on the silver coated collagen for both collagens. These typical spectral patterns are shown in **Figure 2**.

It can be said that there are four characteristic wavenumbers in both collagen samples. Characteristic here means that these Raman bands have similar relative intensities and are observed at approximately the same wavenumber maxima: 799, 1003, 1353 and 1637 cm^{-1}. The band at 736 cm^{-1}, clearly marks the difference between the two forms of collagen. Notably, the vibrational spectra of collagen has also been studied using a pulsed source neutron spectrometer.[4]

The final and more challenging part of the work is prove of concept that good SERS spectra can be obtained form the tissue (biopsies) provided by the medical team. The SERS spectra obtained for several of these samples are shown in **Figure 3**. The technique is the same applied to obtain the spectra of collagen. It can be seen that the spectra are of excellent quality and we have found the experimental conditions that avoid sample burning and sample degradation. We are in the process of recording now a substantial amount of new data on these and other samples (thousands of spectra) that should give us the statistical validation for characterization of the molecular species. We are also beginning to work on the identification of other molecular components in the tissue.

The preliminary data are encouraging. It can be seen that there are characteristic wavenumbers that can be assigned to collagen, marked in Figure 3 in all five samples. The experimental work will continue as to enhance our database, and then we will used multicomponent analysis, specially adapted to our needs, to extract the information from the spectral maps obtained by SERS of tissue samples. The aim of the work is to provide a spectral characterization of the tissues before and after shockwave treatment.

Fig. 1

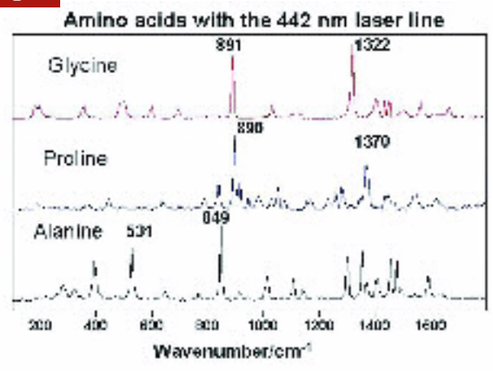


Fig. 2

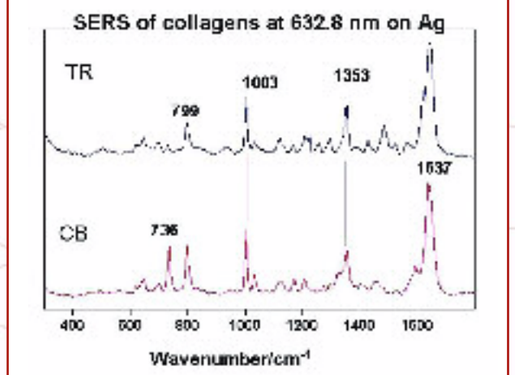
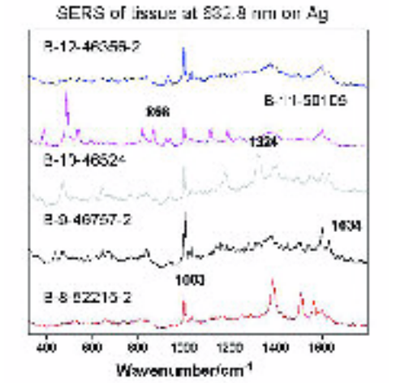


Fig. 3



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New Guidelines for ESWT

Thiele, Richard, MD

Since 1994 the ESWT is applied in the field of orthopedic and surgery and there were no concrete treatment guidelines ever published.

According to the consensus statement of international board of experts from 2008 (see Newsletter 2008) the experts worked out guidelines in that way the AWMF working group of scientific approved societies in Germany is using.

The expertgroup: (Dr.Auersperg, Dr. Buch, Dr. Gerdesmeyer, Dr. Gleitz, Prof. Maier, Dr. Neuland, Dr. Rädel, Prof. Rompe, Dr. Schaden, Dr. Thiele, Dr. Wille)

There are now guidelines for the approved standard indications for

- chronic tendinopathies as plantar fasciitis with or without heelspur
- Achilles tendon
- epicondylopathie (tennis elbow)
- rotator cuff with or without calcification
- patella tendon
- greater trochanteric pain syndrome

For the impact bone healing function:

- non unions and delayed bone healing
- stress fractures
- early stage of avasculaer bone necrosis (native X-ray without pathology)
- early stage osteochondritis dissecans (OD postsceletal maturity)

These guidelines will be published soon.

On the same meeting this board of international experts defined special guidelines for the ESWT of skeletal muscles.

ESWT of skeletal muscles

Preamble: Maofascial pain syndrome
Classification M62.8 ICD 10

Synonyms

Myogelosis, muscle hardenings, myofascial pain syndrome, pseudo-radicular pain syndrome, trigger points, RSI Syndrome

Etiology

Mainly a subsequent state of an extra muscular pathology e.g. by

- static disorders
- muscular dysbalance
- arthrogenic irritations
- visceral irritations
- internal diseases
- radiculopathies
- chronic overload / incorrect weight bearing
- acute and chronic injuries of the skeletal muscles

Symptoms

local pressure pain, stretching and tension pain, muscle hardening, muscle shortening, strength reduction, motoric dysfunction

Instrument-based diagnostics

ultrasonography
laboratory (inflammation parameter, muscle enzymes)

Differential diagnosis

primary myopathies, neurological diseases, neurogenic dysfunction, rheumatic pains, psychological diseases, neurovegetative syndrome, hormonal disorders (e.g. hyperparathyroidism, hypothyroidism), cardiac diseases, adverse reactions

Conservative therapy in alphabetic order

aupuncture, electrotherapy, immobilization, infiltration of local anaesthetics and (or cortisone, needling, neural therapy, non-steroidal antirheumatics, orthesis, strain relief, stretching, thermotherapy, ultrasound

Surgical interventions

denervation
subcutaneous tenotomy

Shockwave therapy

Indication: diagnosis by the MD (physician)

Contraindications: malignant tumor in the focal area, open epiphysis in the focal area, pregnancy

Spatial requirements: requirements for the certification of a medical practice e.g. hygiene plan, emergency plan according to ISO 9001:200 available.

Patient preparation: patient positioning in a position with relaxed muscles to be treated, orientating ultrsonography of the therapeutic area for local diagnostics and selection of focal depth, patient information about shockwave therapy and explicit information about potential hematoma.

MD and assistants: medical treatment, written documentation of the therapy

Therapy procedure:

- no local anaesthetics
- designation of the shockwave source
- designation of the muscles to be treated
- 1 - 6 treatments
- total energy flux density EFD: 0.05 - 0.35 mJ/mm_
- interval 1 week
- frequency: focused shockwave therapy FSW: 4 - 8 Hz, radial shockwave therapy RSW 10 - 30 Hz
- maximum of 2000 pulses per muscle per session
- ultrasound coupling gel, castor oil or Vaseline when indicated
- localization: patient oriented focusing

Post-therapeutic care: circulatory function monitoring when indicated

Complications: hematoma, pain increase, nerve irritation

Follow up-care: abstention from sports for 4 weeks (individual adjustment of the sports program)
continuation of stretching
clinical evaluation 8 -12 weeks post therapy

Conclusion

Based on this guideline the ESWT of skeletal muscles is a treatment only done by physicians. The part of the so called triggerpoint-treatment with radioshockwaves could be delegated to physiotherapists and non-physician-healers.