The ISMST and the ISMST Newsletter
International Society for Shockwave Therapy (ISST)

As 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 inerently past I have the impression that my soul, growing older and older, has become more sensitive to reality. 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 pseudarthrosis of the tibial scaphoid for more than two years and who, after only two shock wave applications through an old urological lithotripter, 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 congress 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 Vinnica with whom and together with other European scholars such as Prof. Wolfgang Schaden, Dr. Richard Thiele and others we met in Vinnica, 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 and orthopaedic needs, we achieved in few years the spreading of the method and of the therapeutic directions. The positive clinical responses of thousand of cases around the world bear witness to it, together with the scientific interest that the 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 Musculoskeletal Shock Waves Therapy (ISMST) in 2000, which counts among its members scholars coming from all over the world. As it was expected this new therapeutic 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 mechanical effect into the biological one. Nowadays we are able firmly point out that the main chemical mediator which causes no angiogenesis in 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 Vennesia chaired by Prof. Hristorii Sasuri.

According to what I have written and to what I have not reported for the sake of brevity, I must solemnly reaffirm that the last 15 years of research on shock waves have been the harbinger of really promising results in the medical branches we are considering. I am going to conclude this editorial even mentioning the success of the International Society for Shock Wave Therapy (ISMST) which has recorded a great participation and interest beyond the greatest expectations giving further value to this new therapeutic system.

In the end I would like to thank the founder and publisher Paulo Roberto Dias dos Santos for his careful direction of the Newsletter and because he allowed me to write this 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 vessels associated with neuropathy and secondary infection. The treatments of diabetic foot ulcers 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 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 30 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, immunohistochemical 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 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 to 3-d). The results of ESWT results in tissue regeneration with improvements in blood perfusion and molecular changes in chronic diabetic foot ulcers.

Conclusions
ESWT is more effective than HBO in the treatment of chronic diabetic foot ulcers. It appears that application of ESWT in the bacteria colony counts after treatment. 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.

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.

Table 1. Blood Flow Perfusion Rate Before and After Treatment

<table>
<thead>
<tr>
<th>Laser Doppler</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>P-value-1</th>
</tr>
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<tbody>
<tr>
<td>ESWT</td>
<td>0.64±0.28 (0.19-1.23)</td>
<td>0.75±0.19 (0.46-1.28)</td>
<td>0.04</td>
</tr>
<tr>
<td>HBO</td>
<td>0.50±0.21 (0.18-0.66)</td>
<td>0.58±0.11 (0.51-0.66)</td>
<td>0.140</td>
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<tr>
<td>P-value-2</td>
<td>0.30</td>
<td>0.043</td>
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Table 2. The Results of Bacteriological Examination

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<tr>
<th>Bacteria growth</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>VI</th>
<th>P-value-1</th>
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<tr>
<td>ESWT group</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Before treatment</td>
<td>13</td>
<td>4</td>
<td>11</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>After treatment</td>
<td>5</td>
<td>3</td>
<td>9</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>HBO group</td>
<td>11</td>
<td>0</td>
<td>12</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Before treatment</td>
<td>4</td>
<td>5</td>
<td>9</td>
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<td>3</td>
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<tr>
<td>After treatment</td>
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<td>P-value-2</td>
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Table 3. The Results of Immunohistochemical Analysis

<table>
<thead>
<tr>
<th>eNOS</th>
<th>VEGF</th>
<th>PCNA</th>
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</thead>
<tbody>
<tr>
<td>ESWT</td>
<td>26.62±14.87 (4-57)</td>
<td>31.36±22.27 (8-90)</td>
</tr>
<tr>
<td>HBO</td>
<td>25.2±17.09 (6-53)</td>
<td>42.6±12.6 (28-55)</td>
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<tr>
<td>P-value-2</td>
<td>0.438</td>
<td>0.086</td>
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<tr>
<td>eNOS</td>
<td>46.7±18.82 (6-72)</td>
<td>63.69±21.06 (25-91)</td>
</tr>
<tr>
<td>HBO</td>
<td>20.8±9.73 (6-30)</td>
<td>44.0±11.24 (30-56)</td>
</tr>
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<td>P-value-2</td>
<td>&lt;0.001</td>
<td>0.042</td>
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<tr>
<td>eNOS</td>
<td>62.42±15.0 (39-82)</td>
<td>76.3±13.44 (14-56)</td>
</tr>
<tr>
<td>HBO</td>
<td>49.4±17.0 (22-65)</td>
<td>65.9±18.68 (25-91)</td>
</tr>
<tr>
<td>P-value-2</td>
<td>0.162</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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

PCNA: proliferation cell nuclear antigen; TUNEL: Transference-mediated digoxigenin-deoxy-UTP nick end labeling

This paper was accepted for presentation at the Annual Meeting of the American Tumor/Metabolic Disease at the 75th 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.
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).
Equine distal limb wounds are common and 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 distal limb and surrounding skin. Wounds of the distal portion of the limb often heal by second intention and heal slowly often inhibited by the formation of exuberant granulation tissue. Compared to wounds of the trunk, lacerations of the distal portion of the limb contract more and therefore epidermalize more slowly, and consequently, contract to a lesser extent. Even within the equine species, there are differences in wound healing.

Second-intention healing of wounds occurs faster in ponies than horses. 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 (Figure 1). This may be explained by differences in the inflammatory response between horses and ponies. Ponies have a greater initial recruitment, in all 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. Conversely, ponies may have a more controlled inflammatory stage and greater organization of myofibroblasts resulting in a more orderly, slower contraction than the horse. In vitro studies have shown that there are no inherent differences in fibroblasts and myofibroblasts in normal and scar tissue. Therefore, environmental factors such as cytokines and the inflammatory response likely account for the differences (Figure 2).

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-β, and PDGF. TGF-β1 has been documented as an important stimulant in stimulating wound contraction. The horse has a lower production of TGF-β2 than ponies which may be on of the reasons for the differences in wound contraction rates between horses and ponies. Another possibility could be oxygen derived free radicals including superoxide and nitric oxide which have been identified in other tissues following ESWT. Increased endothelial nitric oxide synthase has been demonstrated by immunohistochemistry in tendon and bone following shockwave therapy. A nitric oxide releasing gel was shown to increase the rate of epithelialization of burn wounds in rats 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. Increased nitric oxide release was shown to increase the rate of epithelialization of burn wounds in rats 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.
Significant decrease in density score respectively. There was a statistically positive staining for VEGF (P = 0.015), but not among the second biopsies among the control wounds. 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 facilitate healing after debridging the exuberant granulation tissue is needed. In the study presented here, the effect of ESWT was seen predominantly 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 15.0% decrease in flap loss. This could be important in these wounds where there is limited soft tissue covering. (Figure 6)

The increase in epithelialization was the primary contributor to the differences seen in this study. This study 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, which could potentially be synergistic with 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. The effects appear to be most predominant early in the healing process (Figure 8).

Equine Tissue Inc. 3800 Horse Farm Road, CA 95060

References

**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 disease remains incomplete. Recent reports have been focused on the biology of rotator cuff responses to selective surgical treatment with cytokines [1,2,3], while others analyze the pathological findings with better study protocols [4,5,6].

In recent years Extracorporeal Shockwave therapy 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 Calculated Shoulder Tendinosis (electro-hydraulic device, 4000 pulses, 0.33mJ/mm², single session) and the same treatment protocol was offered to patients with rotator cuff 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, 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 (hematoxylin-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, Coll I, Coll III, Tenascin-C) and semi-quantitative analyses were done for countable stain on formed structures in regions of PCNA, D2-40 (sympathetic 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 (albeit: 0%: low up to 20%; regular: up to 70%; intense: 80% to 100%).

In the case of Coll I, Coll III and Tenascin-C the photographed fields received a grille 5x5 (25 chambers) characterized 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 ultrasonic analysis 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 mm of medial portions in a single piece; (2) sample of horse with a cartilage border (4-5mm wide, 6-7mm long), corresponding to the area of normal insertion of subacromial muscles.

For SERS histological observations (Group A, H&E sections), the distribution according to Riley Classification indicates 4 cases grading type II, 37 grading type III and 6 cases grading type IV. 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 comprised of pericytes 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 profusional vascular metaplasia where non proper morphological features of vessels could be identified and also shown many acellular fields (fig.1c).

Biopsies of patients treated with SW demonstrate areas of fibroblastic repairs that tend to appear 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) (‘hypervascularized neo-vascular’, fig. 2a, b, c). These “nodes” show areas close to vessels, stain like disorganized new collagen and resemble native collagen (fig. 3a, b, c), 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 frequency and intensity in the tissues before and after shockwave treatment. Bands corresponding to the amide III mode shift in the shoulder. A multi-center investigative effort will indicate of activation of blood-supply on those areas of neo blood vessels with damage on the pericytes envelope-sheath with their hypermuscularized aspect correspond to hyperplasia of active neo-vessels occupying up to 23-28% of volume areas, 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 hypervascularized 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.

**References**

Introduction

Transcardiac application of shock waves in recently is known to augment myocardial vasculature in a porcine model of myocardial infarction [1,2], besides it is shown to effect relief of angina symptoms in patients with coronary artery disease [3]. Nevertheless, pulmonary contusion causing life-threatening hypoxemia and hypotension is described as an adverse event of shock waves when hitting lung tissue [4,5]. Therefore transcardiac cardiac shock wave application is limited by lungs partly covering the heart [1-3]. Direct epicardial shock wave therapy (DESWT) may be more feasible, 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 grafting). 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 ISSMT 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 [7,8]. 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 consists of a Plexiglas bulk water bath with an adapter for the shock wave applicator (CP-155, Dermaflex® from Tissue Regeneration Technologies LLC, Woodstock, USA manufactured by Medizinische Technik Europe GmbH, Konstanz, Germany). This water bath is filled with degassed water to avoid cavitation, a heater at the bottom with a temperature sensor connected to a read-out unit enables to regulate the 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 cell 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 air. Due to this shock waves would be reflected, thereby causing negative pressure onto the cells and also disturbing upcoming waves. The water bath is filled with culture medium 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 tissues. Additionally HBC2-cardiomyocytes (American Type Culture Collection) were used. All cell types were cultured using DMEM medium supplemented with common nutrients and growth factors. Adherent cells in common cell culture flasks were incubated with culture bath medium. The water bath was filled with degassed water to avoid cavitation, a heater at the bottom with a temperature sensor connected to a read-out unit enables to regulate the 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 cell culture flasks easily and quickly.

Preliminary Results

Counting of cells and proving their viability are the basic anlaysis of cell cultures. Viability was proved using trypan blue staining. Trypan blue is not transmembrane permeable, non-viable cells become blue. That so called Dye Exclusion Method showed hardly any blue cells in the treatment as well as in the control group. Viability 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 3A-C. As a very important parameter for proliferation we calculated the cell duplication time every 24 hours with the commonly used formula $T_{d} = \frac{\ln 2}{\ln y}$ ($T_{d}$: cell duplication time, $y$: proliferation, A: cell number after 24h, A: initial cell number). The very simple diagram in Figure 4 shows that the mean value of $\ln y$ in untreated and treated 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 analyses, e.g. concerning immunohistochemistry and molecular biology, are still in progress. In this pilot study we only used healthy cell cultures, which can be established and further. From our previous mentioned in-vitro trials we already know that healthy cells do not respond that much to SWT than cells from unharmed myocardium. From these in-vitro results we conclude that SWT could be established and moreover our previous mentioned in-vivo trials we focused on an energy flux density of 5Hz, since these are the commonly used parameters in vivo.

Discussion

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

This leads to an unimpaired 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 flask 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 further. From 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 adress this issue we will have to compare samples with different focused shock wave devices and electro-hydraulic with electromagnetic and piezoelectric waves in future in-vitro trials.

References

Figures:

Fig. 1

Fig. 2

Fig. 3

Fig. 4

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

During the last years patient studies and clinical trials have revealed new indications for the use of focused 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. neural root lesion. MPS is characterized by Myofascial Trigger Points (MTrP’s), 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.

FOCUSED 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 focused ESWT while putting MTrP’s exactly in the focus and releasing these painful spots.

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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 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 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, near-infrared Raman, and the plasmonic driven technique of analytical technique surface-enhanced Raman scattering. 

This molecular approach is then integrated with the biomedical research in an attempt to understand the biological processes. Here, we present the first steps towards the molecular understanding of the important improvements of rotator cuff supraspinatus tendons diseases that have been treated by shockwave therapy. Neooxigenation stimulation and hypercellularization 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. 

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 collagen; 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 1387 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 used using a pulsed source Raman spectrometer.

The final and more challenging part of the work is prove of concept that good SERS spectra can be obtained from 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 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, especially 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.

References


Fig. 1

Amino acids with the 424 nm laser line:

SERS of collagens at 632.9 nm on Ag

Fig. 2

SERS of tissue at 514.8 nm on Ag

Fig. 3
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. Auerkamp, Dr. Buch, Dr. Gerdesmeyer, Dr. Gleitz, Prof. Maier, Dr. Neuland, Dr. 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 heel spur
- 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 avascular 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: Myofascial 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**
ultrasoundography
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**
apuncture, electrotherapy, immobilization, infiltration of local anesthetics and (or cortisone, needling, neural therapy, non-steroidal antiinflammatories, orthosis, 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 ultrasound 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.