

Biological Effects of Extracorporeal Shock Waves on Fibroblasts. A Review

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Summary

Tissue homeostasis is influenced by mechanical forces which regulate the normal function of connective tissues. Mechanotransduction, the process that transforms mechanical stimuli in chemical signals, involves mechanosensory units integrated in cell membrane. The mechanosensory units are able to activate gene expression for growth factors or cytokines as well as to induce a biological event which results in cell proliferation and/or differentiation. In connective tissue the fibroblasts are the cells more represented and are considered as a model of mechanosensitive cells. They are ubiquitous but specific for each type of tissue. Their heterogeneity consists in different morphological features and activity; the common function is the mechano-sensitivity, the capacity to adhere to extracellular matrix (ECM) and to each other, the secretion of growth factors and ECM components. Extracorporeal shock waves (ESW) have been recently used to treat damaged osteotendinous tissues. Studies *in vitro* and *in vivo* confirmed that ESW treatment enhances fibroblast proliferation and differentiation by activation of gene expression for transforming growth factor $\beta 1$ (TGF- $\beta 1$) and Collagen Types I and III. In addition, an increase of nitric oxide (NO) release is even reported in early stage of the treatment and the subsequent activation of endothelial nitric oxide synthase (eNOS) and of vascular endothelial growth factor (VEGF) are related to TGF- $\beta 1$ rise. The data have been related to the increase of angiogenesis observed in ESW treated tendons, an additional factor in accelerating the repairing process. A suitable treatment condition, characterized by a proper energy/shot number ratio, is the basis of treatment efficacy. Further ESWT applications are suggested in regenerative medicine, in all cases where fibroblast activity and the interaction with connective tissue can be positively influenced.

Key words: fibroblasts, extracorporeal shock waves, shock-

wave treatment, mechanotransduction, connective tissue, tendon healing

Fibroblasts

Fibroblasts are spindle shaped cells found in the majority of tissues and organs of the body associated with extracellular matrix (ECM) molecules. They are identified as mesenchymal connective tissue cells; the term "fibroblast" is a general one used for a variety of connective cell types. Fibroblasts are cells found throughout connective tissue such as tendon, ligament, skin but even myocardium, liver, lung, nerve, uterus and kidney. Since a specific marker for identification of fibroblasts is still lacking, it is evident that there many functional differences between fibroblasts from each of these sites which reflect different functions for each organ (1, 2). Interestingly, there are also variations among the fibroblasts within each site. There is a growing body of evidence that within fibroblasts isolated from the skin or lung, some cells will have very different biochemical characteristics. They may vary in their morphology, proliferation rate, surface markers, protein synthesis or response to a given stimulus. This difference among fibroblasts, or fibroblast subpopulations, is referred to as fibroblast heterogeneity. Heterogeneity has been defined in skin, kidney, lung, synovial, gingival and corneal fibroblasts (3).

Fibroblasts are the major mechanoresponsive cell type and are highly heterogeneous

Fibroblasts play a crucial role in remodeling of the extracellular matrix by synthesizing and organizing connective tissue components. They respond to various microenvironmental signal including soluble Cytokines and growth factors as well as cell matrix or cell-cell interactions that control the balance between synthesis and degradation of ECM (4). The ECM consists of a variety of substances, of which collagen fibrils and proteoglycans are truly ubiquitous. In addition to the proteoglycans, the hydrophilic ECM includes a variety of other proteins such as noncollagen glycoproteins (5).

In response to inflammatory stimuli at sites of injury, the fibroblast undergoes a profound phenotypic transition, resulting in chemotactic migration from the wound margin into the zone of injury, accelerated degradation and provisional replacement of damaged extracellular matrix, and induction of additional autocrine and paracrine mediators including IL-1, IL-6, IL-8, TGF- β , prostaglandins, and nitric oxide (NO) (6). Since fibroblasts are heterogenous in proliferative capacity, in synthesis of collagen and other matrix proteins and in response to immune mediators and growth factors, selective increase in fibroblast subpopulations may explain the long-term effects of acute *in vivo* activation on fibroblast behaviour (3).

How do fibroblasts sense mechanical stress and how do they use this information to regulate ECM biosynthesis and turnover? Specific changes in ECM synthesis and degradation are an important part of cellular responses to mechanical stress.

Mechanical forces and tissue homeostasis

Mechanical forces play a regulatory role in tissue homeostasis by influencing physical properties as well as cell morphology-physiology (2). Mechanical signals affect cell morphology, cytoskeletal organization, cell survival, cell proliferation and differentiation, gene expression as well as regulate the expression of different proteins of ECM. Those functions have been described as indirect or direct mediators and sensors of mechanical stress (7). Along with soluble mediators they are able to modify the metabolism and phenotype of the cell (8).

Mechanotransduction

Mechanotransduction is the process by which physical forces are converted into biochemical signals (9). A physical force can be applied in a variety of ways such as through substrate stretching or through movement of fluid or air. Mechanosensory units are integrated in membrane proteins such as ion channels, integrins, associated cytoplasmic complexes; they are switched by physical stimuli which result in biological events depending on the type of mechanical load, as well as on the tissue and the context in which they are applied. The cells stimulated by physical forces interplay with their surrounding matrices: this is a crucial point in the following biological events. There is a transmission through protein-protein interactions that rely upon the dynamic assembly of physically coupled protein networks that link the ECM to cytoskeletal components, via transmembrane proteins (7).

Physical forces even influence conformational changes of membrane proteins making unmasked cryptic binding or phosphorylation sites, or regions that display enzymatic activity (10, 11).

The size of focal contact points and the following signaling activity is force dependent (12). The following intracellular signaling affects gene expression with alteration of binding properties and/or of enzymatic functions (13).

The integrins, the major transmembrane components, can trigger signals in response to pulling forces applied to their ECM ligands (13-15). They are mechanoreceptors and mediate mechanotransduction by transferring forces to specific adhesion proteins into focal adhesions which are sensitive to tension and activate intracellular signals (16).

Thus, integrins may function as mechanotransducers by aggregating in the focal adhesion sites to transduce the mechanical stress into chemical signals. Force probably accelerates integrin activation, both by extracellular and intracellular rearrangements, and induces protein recruitment through protein stretching (17).

Cell signaling pathway, of consequence, is switched on or off by multiple molecule changes, in a critical puzzle where physical characteristics of the external forces play a crucial role together with the type of tissue where they are applied.

Mechanoresponse can be ubiquitous but is specific

There is a vast difference in cellular mechanobiological response depending on cell structure and cell mechanosensitivity. Connective tissue cells are able to distinguish between various modes of mechanical stress: compressive [e.g. in cartilage (18)], tensile [e.g. in tendons (19)] and shear [e.g. in blood vessel wall (20)]. Integrins have been implicated as mechanoreceptors in a wide range of cells including myocytes, fibroblasts, endothelial cells, chondrocytes, and bone cells (21).

Growth factors release after mechanical stimulation

Cells dynamically adapt to force by modifying their behaviour and remodeling their microenvironment: the release of growth factors after mechanical stimulation can be considered as initial step in the regeneration of new supporting connective tissue (21). Studies have indicated that mechanical loading increases the expression of several growth factors and Cytokines, such as Insulin-like Growth Factor 1 (IGF-1), Transforming Growth Factor- β 1 (TGF- β 1), and Interleukin-6 (IL-6) (22).

Cyclic stretching of fibroblasts, a well-known model of mechanical stress, was described to modulate secretion pattern of growth factors by Skutek et al. (23): an increased release of Fibroblast Growth Factor-2 (FGF-2), TGF- β 1, and Platelet Derived Growth Factor (PDGF) was recorded. In a previous report, Bishop et al. (24) showed that mechanical load enhanced the stimulatory effect of PDGF on procollagen synthesis of pulmonary artery fibroblasts, confirming their earlier report (25) on the enhanced stimulatory effect - induced by mechanical load - of serum growth factors on cardiac fibroblast procollagen synthesis.

In vitro as well as in vivo experiments have shown an increased expression of TGF- β in response to mechanic stimuli in a number of cell and tissue types. Moreover, mechanically induced type I collagen expression in ligament fibroblasts, in cardiac fibroblast, and human intestinal smooth muscle cells is directly dependent on TGF- β activity (22). Importantly, loading-induced type I and/or type III collagen expression appears to depend directly on TGF- β 1 activity in human ligament and patellar tendon fibroblasts (26). Several observations point to TGF- β 1 as an essential mediator of mechanically induced collagen synthesis in a variety of cell types. In addition to TGF- β 1, Connective Tissue Growth Factor (CTGF) could be implicated in a link between loading and collagen synthesis, possibly acting as a down-stream mediator of TGF- β 1 action (27, 28).

Fibroblasts firmly attach to their ECM substrate via matrix adhesion contacts on their cell surface. All matrix contacts contain integrin receptors as their major transmembrane proteins (29, 30). To establish new contacts with ECM, cells first have to activate integrins at their surface, mostly by signaling from within the cell (31).

Tendons and tendon fibroblasts

Mammalian tendons are composed of cells and almost exclusively of extracellular collagen fibrils embedded in a proteoglycan/water extracellular matrix or ground substance (32, 33). Collagen fibrils, the main structural components of

(tendon) collagen fibers, are formed by aggregated microfibrils composed of molecules of collagen (34). Tendon cells are mostly (90-95%) represented by tenoblasts (fibroblasts) along with endothelial cells and some chondrocytes located in the areas of compression (33). Chemical and mechanical stimuli may directly couple to functional responses such as changes in cell proliferation, growth factor release or gene expression. Growth factors may then act in an autocrine or paracrine fashion to "potentiate" the mechanical stimulus. (35)

Forces may be transmitted to and from cells through the extracellular matrix with changes in mechanical forces and cell shape which act as a biological regulator (36). Interactions between cells and extracellular matrix and exchanges between extracellular matrix and cells may be in a dynamic equilibrium: in vitro studies demonstrated that internal (cytoskeletal) and external (elastic) forces are related via integrins if the substrate of fibroblast cultures is stretched or compressed (29, 37).

Mechanoresponses of tendon fibroblasts

The tendon fibroblast is considered a key player in tendon maintenance, adaptation to changes in homeostasis and remodeling in case of minor or more severe disturbances to tendon tissue (38). Mechanoresponses of tendons, both anabolic and catabolic, are due to the activities of tendon cells in response to various mechanical loading conditions. As a dominant cell type in tendons, tendon fibroblast (or tenocyte) is the major mechanoresponsive cell in the tissue (28) and is certainly responsible for changes in tendons by altering ECM gene and protein expression. With the development of many in vitro cell-loading systems, a spectrum of mechanoresponses of tenocytes and their molecular mechanisms have been extensively investigated in the past two decades (see 39 for review).

Wang et al. (39) showed that human tendon fibroblasts increase in proliferation as well as gene expression and protein production of type I collagen in a stretching magnitude-dependent manner. In addition, depending on stretching magnitude, cyclic stretching increased gene expression and production of type I collagen (28).

Several cytokines and growth factors, e.g., insulin like growth factor I (IGF-I), transforming growth factor- β , and interleukin-6, have been implicated in mediating the effect of increased loading of the fibroblasts in the tendon to produce collagen (22, 23, 40). Moreover, TGF- β 1 is known as a potent inducer of collagen expression, and its induction in response to loading may well be important for mediating mechanically induced type I and/or type III collagen expression in tendon and muscle tissue (26-28).

While collagen type I is the main component of collagen fibers, collagen type III has been shown to be important in the regulation of initial fibril assembly and thus at the early stages of injury repair (41).

Tendon healing

Tendon healing is a complex process which involves many cellular, vascular, and extracellular matrix factors (42). According to Orhan et al. (43), the formation of adhesions,

an increase in fibroblasts, organization of collagen, an increase in capillaries and thickening of the epitenon may all be observed during the process of healing. Unfortunately, prolonged periods of immobilization of a limb or a joint may be complicated by atrophy of muscles and articular cartilage, osteoarthritis, skin necrosis, tendoncutaneous adhesions and thrombophlebitis. Any procedure which promotes tissue healing without triggering associated damage may decrease the incidence of complication after tendon injuries (see 44 for review). The response of fibroblasts to physical stimuli is, consequently, the rationale for taking into account physical modalities to treat damaged osteotendinous tissues.

Effects of mechanotransduction from different biophysical stimulations, shock waves included

Several studies have evaluated the effect of biophysical modalities on tendons and many physical modalities are used in the management of tendon disorders (see 45 for review).

Pulsed magnetic fields with a frequency of 17 Hz resulted in improved collagen fiber alignment in a rat Achilles tendinopathy model (46). In a rat Achilles tenotomy model, application of pulsed magnetic field therapy to the repair site resulted in an increase in tensile strength of up to 69% (47). The role of **laser phototherapy** in the management of tendon injuries has also been considered. In rabbits subjected to tenotomy and surgical repair of the Achilles tendon, laser phototherapy resulted in increased collagen production (48). In an experimental rat model of Achilles tendon injury, low level laser therapy reduced histological abnormalities and oxidative stress leading to a reduction of fibrosis (49).

Radiofrequency coablation is a new application of bipolar radiofrequency energy that creates a small, highly energized plasma at the tip of the active electrodes capable of breaking down molecular bonds of tissue. Radiofrequency coablation stimulates an angiogenic response in normal rabbit Achilles tendon (50). A prospective study performed on patients with recalcitrant plantar fasciitis that failed conservative care reported statistical improvement in outcome measures at 6 months and 1 year (51). The ability of bipolar radiofrequency to deliver energy to tendons, ligaments, fascia, and capsules transcutaneously (noninvasively) without arthroscopy or surgical exposure offers several advantages beyond the added safety (52).

Therapeutic ultrasounds have been taken into account as new tool to treat soft tissue ailments. There is strong supporting evidence from animal studies about the positive effects of ultrasound on tendon curing. Tendon fibroblasts (or more generally "tendon cells" or "tenocytes") have been used to investigate the underlying mechanism of ultrasound in tendon healing: in vitro studies have demonstrated that ultrasound can stimulate cell migration, proliferation, and collagen synthesis of tendon cells. Moreover, ultrasounds enhance tenocyte proliferation which correlates with increased gene and protein expressions of proliferating cell nuclear antigen (PCNA). It was shown that either continuous or pulsed mode ultrasound treatment enhanced gene and protein expressions of types I and III collagen in an intensity-dependent manner. The molecular mechanism

underlying the stimulation on migration, proliferation, and collagen synthesis of tendon cells is possibly caused by the up-regulation of TGF- β (53).

Low-intensity pulsed ultrasound (LIPUS), having removed the thermal component found at higher intensities, may be taken into account to improve bone and soft tissue healing. Some preclinical studies that support the positive effect of LIPUS therapy on soft-tissue healing could be translated into human use; however, powered human studies together with the standardization of intensities and dosages for each target tissue are needed to build a stronger clinical database for routine clinical use (54).

Extracorporeal shock waves. The application of shock wave therapy in certain musculoskeletal disorders has been around for approximately 20 years, and the success rate in non-union of long bone fracture, calcifying tendonitis of the shoulder, lateral epicondylitis of the elbow and proximal plantar fasciitis ranged from 65% to 91%. The complications are low and negligible (55, 56).

Physical principles of shock waves

Shock waves used in extracorporeal shock wave treatment (ESWT) are high amplitude sound waves from a transient pressure disturbance that propagate in three-dimension space with a sudden rise from ambient pressure to its maximum pressure at the wave front. The waves are transmitted to the patient through either water or a coupling gel. A shock wave is a sonic pulse that has certain physical characteristics. There is an initial rise of a high peak pressure, sometimes more than 100 MPa (1000 bar) within less than 10 ns (nanoseconds), followed with a low tensile amplitude (up to 10 MPa), a short life cycle of approximately 10 μ s and a broad frequency spectrum in the range of 16 to 20 MHz. Shock waves differ from ultrasound waves that are typically biphasic and have a peak pressure of 0.5 bar. In essence, the peak pressure of shock wave is approximately 1000 times that of ultrasound wave (55).

Within the last 20 years the understanding of shock waves has continuously improved. The physical principles as well as the tissue effects have been widely investigated. Regarding the action of shock waves on tissue, 4 phases have been postulated: 1) physical phase; extracellular cavitations, ionized molecules and an increase of membrane permeability are direct effects of the shock waves; 2) subsequent physical-chemical phase; diffusible radicals and interactions with biomolecules; 3) chemical phase; may be accompanied by intracellular reactions and molecular changes; and 4) biological phase. Anyway, many of the shock wave-tissue interactions are not yet completely understood and the exact mechanisms of shock wave therapy need to be fully identified (57).

Extracorporeal shockwave therapy

The initial therapeutic introduction of shock waves to the human body was to noninvasively treat kidney stones (lithotripsy), this technology has evolved to be considered the procedure of primary choice for urolithiasis (58). Since early nineties, many clinical studies on ESWT have been done

to treat orthopaedic disorders, such as epicondylitis, painful heel syndrome, calcific tendonitis of the shoulder, chronic plantar fasciitis, nonunions, pseudarthrosis and femoral head necrosis in adults (59). Despite the efficacy in clinical application, scientific evidence of shock wave therapeutic effects and biochemical mechanisms on tenocytes remain limited, and much remains to be learned about the etiology, pathophysiology and management of these tendinopathies (60).

Orhan et al. (61) created an experimental rat model to investigate the histopathological and biochemical effects of ESWT in the healing of Achilles tendon injury that may accompany fractures. Authors described that ESW treatment increased tendon healing rate, as indicated by increased collagen synthesis and the histological findings. The same Authors later adopted a rat model which differed slightly in the experimental design (partial tendon rupture instead of a full cut followed by suture). Histopathological analyses showed an increase of the number of capillaries in the group subjected to ESWT and a significantly greater force was required to rupture the tendon in the study group (43). Johannes et al. (62) were the first who explored the influence of the energy density and the number of applied shockwaves on the viability of cell suspension of normal fibroblasts. The Authors reported that shock waves have a dose-dependent destructive effect on cells in suspension: the number of applied shots had a statistically significant influence on the decrease in growth potential compared to the control cells, a higher number of shock waves leading to a more severe depression in the growth potential of the shocked cells. The same Authors, by analyzing the relation between the logarithm of the number of shots and the growth potential of the viable shockwave treated cells, supported that a very low number of applied shockwaves had a stimulating influence on the growth potential of the cells subjected to ESW treatment.

This observation was comparable to previous report by Haupt and Chvapil (63) who showed a dose-dependent influence of shock waves on the healing of partial-thickness skin lesions in pigs. Low-dose shockwave treatment stimulated the reepithelisation while intermediate-dose ESW treatment had no effect, and high-dose ESW treatment had an inhibiting effect.

Extracorporeal Shock Waves: mechanism of action on fibroblasts

In recent times, Berta et al. (64) treated normal fibroblasts in suspension with low- to medium- energy shock waves and evaluated fibroblast viability, the growth rate and pattern, and gene expression for TGF- β 1 and collagen types I and III - the main factors involved in the repair process. Low- to medium- energy shockwave treatment induced fewer immediate cytotoxic effects and there was a better subsequent stimulation of cell proliferation, in accordance with the work of Wang et al. (65, 66) and Martini et al. (67). Fibroblast viability was influenced by the number of shots much more than by energy level and there was evidence of a suitable energy/shot number ratio to have a minor cytotoxic

dal effect. Shock waves had a dose-dependent destructive effect on cells in suspension, as well as they had a dose-dependent stimulatory effect on cell proliferation. In addition, a significant increase in proliferation rate was observed with respect to the unshocked cells. A critical increase in cell growth was observed from the sixth to the twelfth day of the proliferation curve.

Bearing in mind that the goal of ESW treatment of tendon lesions is to promote and improve the repair process, Authors concluded that treatment at the 0.22 mJ/mm² energy level with 1,000 impulses appears to be the condition in which fibroblast viability fits growth dynamics.

The pattern of expression of TGF- β 1 mRNA showed higher values in treated fibroblasts than in untreated fibroblasts for day 6 ($p = 0.02$) and day 9 ($p = 0.02$), respectively. Elevated expression of mRNA was observed for collagen types I and III, although with different timing: on the sixth day for collagen type I and on the ninth day for collagen type III. In both cases, ESW treatment enhanced expression of the genes encoding collagen types I and III.

The timing of increase of mRNA expression for TGF- β 1 and for collagen types I and III is in accordance with the role of collagen types I and III in repairing process and confirms that TGF- β 1 is involved in differentiation of fibroblasts, according to previous reports (60, 68).

Hausdorf et al. (69) evaluated the capability of shockwave therapy to evoke the release of bone growth factors (namely TGF- β 1 and FGF-2) from target tissue cells like fibroblasts and osteoblasts which are essential parts of the tissue of a nonunion treatment. While Transforming Growth Factor- β 1 (TGF- β 1) is responsible for multiple reactions in tissue growth and is not only produced in osteoblasts and fibroblasts (70), Fibroblast Growth Factor-2 (FGF-2) represents a more selective bone growth factor. Fibroblast and osteoblast production of TGF- β 1 and of FGF-2, predominant elements in the osteoneogenesis cascade, was shown to be increased after ESW treatment.

Chen et al (68) evaluated the effect of various ESW treatment schedules on the healing of collagenase-induced Achilles tendinitis, and investigated biochemical and biomechanical properties of healing tendons.

Rats with the collagenase-induced Achilles tendinitis were given a single ESW treatment at 0.16 mJ/mm² energy flux density, with different number of shots. Low number (200) of impulses restored biomechanical and biochemical characteristics of healing tendons 12 weeks after treatment, while 500 and 1000 shots elicited inhibitory effects on tendinitis repair. Within 1 week and 6 weeks after ESW, a large number of tenocytes displayed intensive proliferating cell nuclear antigen (PCNA) expression after ESW treatment, suggesting that physical ESW could increase the mitogenic responses of tendons. Moreover, the proliferation of tenocytes adjunct to hypertrophied cell aggregate and newly formed tendon tissue coincided with intensive TGF- β 1 expression observed in the early stage of tendon repair, while elevated IGF-I expression persisted throughout the healing period. The stimulatory effect on tendon cell proliferation through PCNA-related pathway was confirmed by Tsai et al. (71) in ultrasound treated rat tendon fibroblasts.

Shockwave induced Nitric Oxide production

Van Griensven et al. (72) observed that cyclic mechanical strain induced NO production in human patellar tendon fibroblasts. The levels of released NO by the tendon fibroblasts varied with the duration of strain application: short strain duration induced an immediate, transient occurrence of high levels of NO. Authors hypothesized that NO produced by tendon fibroblasts, as a response to alteration of their mechanical microenvironment, could modulate fibroblast function.

The association between increase in NO production and collagen synthesis still has to be fully explained. One explanation may be related to TGF- β 1. Vodovotz et al. (73) showed that NO can lead to the activation of latent TGF- β 1. Besides TGF- β 1, other cytokines such as basic Fibroblast Growth Factor (b-FGF), Nuclear Transcription Factor-kappaB (NF-kB) and Insulin-like Growth Factor (IGF) can be induced by NO in wound healing and these cytokines also play an important role in collagen synthesis (74-76).

Chao et al (60) observed that shockwave treatment on tenocytes harvested from rat Achilles tendons elicited up-regulation of proliferating cell nuclear antigen (PCNA) and collagen types I and III as well as TGF- β gene expression; these were followed by the increases in NO production, TGF- β 1 release and collagen synthesis.

A growing number of studies demonstrated that shockwave treatment rapidly induces elevation of systemic nitric oxide (NO) level and subsequent increases in systemic osteogenic factors in non-union of long bone (77, 78). Others reported NO as the mediator in callus formation in fracture healing after mechanical stimulation (79).

A rapid and non-enzymatic formation of NO was observed by Gotte et al. (80) by treating with SW a solution containing 1mM hydrogen peroxide and 10mM L-arginine and ESW-elicited production of nitrites increased in dependence on the number of shots.

Nitric oxide and VEGF had been demonstrated as important mediators of angiogenesis (81, 82). Wang et al. (83) showed that shock wave therapy induces the ingrowth of neo-vessels and tissue proliferation associated with the early release of angiogenesis-related factors including endothelial nitric oxide synthase (eNOS) and VEGF at the tendon-bone junction in rabbits. Therefore, the mechanism of shock wave therapy appears to involve the early release of angiogenic growth factors in one week, and induces cell proliferations and formation of neovessels in approximately four weeks at the tendon-bone junction. The neovascularization may lead to the improvement of blood supply and play a role in tissue regeneration at the tendon-bone junction.

It appears that the mechanism of shock wave therapy involves the early release of angiogenic growth factors (eNOS and VEGF) and subsequent induction of neovascularization and tissue proliferation. The neovascularization may play a role in pain relief of tendinitis and the repair of chronically inflamed tendon tissues.

In this context, it is of interest the recent report by Yin et al. (84) who showed that extracorporeal shockwave enhances angiogenesis and osteogenesis gene expression in bone

marrow stromal cells from hips with osteonecrosis through the NO-mediated pathway. The role of NO as a non negligible mediator factor of repairing process has been evidenced. Moreover, as suggested by Mariotto et al. (85), the clinically observed anti-inflammatory action of shock waves may, at least in part, be mediated by a shockwave-induced increase in NO production.

Shock waves, fibroblasts and wound healing

Wound healing proceeds through a complex series of events involving inflammatory, proliferative, and remodeling phases (86). The proliferative phase of wound healing consists of rapid fibroblast growth and increased synthesis of collagen/NCP in response to chemotactic factors released during the inflammatory phase. Wound healing is marked by angiogenesis through which ingrowth of capillaries accompanies fibroblast and osteoblast (in the case of bone) proliferation to form granulation tissue. Finally, fibroblasts maintain collagen production, which accumulates during the remodeling phase (87).

In early 2000, as it was definitely demonstrated that ESW treatment is effective in promoting the healing of fractures and injuries by stimulated expression of growth factors and eNOS, some authors started to investigate whether this approach was feasible and useful from the perspective of plastic surgery by dealing with the critical problem of ischemic tissues in reconstructive procedures (88, 89).

Prior animal studies (63) indicated positive influence of shock waves on the reepithelialization of partial-thickness wounds in Yorkshire piglets. Experimental results obtained in a dorsal skin flap rodent model indicated that ESW treatment substantially increased PCNA expression, especially that of fibroblasts in the basal layers of epidermis and subcutaneous layers, reduced leukocyte infiltration, and elicited suppression of tumor necrosis factor alpha expression in flap tissue ischemic zone. Immunohistochemical study evidenced angiogenesis as demonstrated by VEGF expressions in the flap tissue ischemic zone. VEGF expression was even significantly increased, particularly in fibroblasts and endothelial cells (90, 91). Moreover, a significant bactericidal effect of high energy shock waves - in an energy-dependent manner - was observed in vivo by Gerdsmeyer et al. (92). Later, Kuo et al. (93) observed, in a skin-flap rodent model, that ESW application reduced tissue necrosis by increasing cellular proliferation, especially by recruiting fibroblast proliferation and actively producing procollagen. Flap-tissue ischemic injury was thereby attenuated and tissue repair increased. Further, the eNOS expression level in the ischemic zone of the flap tissue after application of ESWs was significantly greater. Thus, flap survival might be promoted by ESW treatment, at least in part, by attenuating oxygen radicals and recruiting eNOS expression in the ischemic zone of the flap tissue. Moreover, adopting a rat model of STZ-induced diabetes, Authors showed that ESW treatment enhanced wound healing through an increase in the PCNA expression levels, especially in the fibroblasts in the basal epidermal and subcutaneous layers (94). The expression levels of VEGF were evidently up-regulated, particularly in the fibroblasts and endothelial cells. Further-

more, the rats in the ESW groups exhibited a marked decrease in the leukocyte-mediated inflammatory response.

Most recent studies, which addressed the clinical utility of this approach, confirmed positive responses of shockwave therapy for soft tissue indications in addition to enhanced neovascularization (95-97) and potential tissue regeneration (98-100). This modality has been investigated in a variety of traditionally problematic soft tissue wounds including diabetic foot ulcers (101), burns (102), and chronic decubitus ulcers (103).

Animal work and preliminary human experimental data point to a complex, multifactorial mechanism of therapeutic shock waves; however, that shockwave therapy has an effect on biological tissue based on the current level of scientific knowledge is incontrovertible.

The report released at the beginning of 2012 by Ottomann et al. (104) describes a clinically important effect of low energy shock waves in superficial second-degree burns: although the study is limited by modest sample size and lacks of long-term follow-up, the difference in time to complete burn site healing was highly significant in favour of the shock wave treated group.

The efficacy of ESWT for chronic ulcers warrants confirmation in further prospective randomized clinical trials. Shockwave therapy may prove to be a feasible, noninvasive, safe, and cost-effective method to enhance the healing of both acute and chronic soft tissue wounds.

Concluding remarks and perspectives

Fibroblasts are considered the primary source of most extracellular matrix (ECM) components: thus they result the key mediator between external and internal environment. As mechanoresponsive cells, they convert mechanical signals into biological events such as expression of numerous genes, including those responsible for ECM. In this context it is of interest their non negligible role both in tissue homeostasis and in repairing processes.

Fibroblast cells are ubiquitous but they are specific for each type of tissue. This difference among fibroblasts is referred to as fibroblast heterogeneity and fibroblast subpopulations have been identified in various tissue where they can have different morphological features and activity. Nevertheless, they share common functions: mechanosensitivity, adhesion capacity to ECM and to each other, secretion of collagen and growth factors..

Fibroblasts grown in tissue culture have been shown to react within minutes to a variety of mechanical stimuli (stretch, pressure, traction, shear forces) with cellular responses ranging from changes in intracellular ATP release to signaling pathway activation.

In this review we considered the effect of ESWT on fibroblasts as a model of therapeutic application of the mechanical source. Studies on fibroblasts in vitro and in vivo confirm that shockwaves stimulate fibroblast activity; it is now scientifically established that ESW activate fibroblasts proliferation rate, collagen synthesis and gene expression for Growth factors and/or Cytokines. Moreover, a relation-

ship was found between NO production and TGF- β 1 gene expression in the early stage of ESW treatment as well as an increased gene expression both for eNOS and VEGF has been demonstrated in ESW treated tendons. The data can be related to the increased blood flow observed in ESW treated tissues. Both effects of ESW treatment on fibroblast activity and increase of blood flow cooperate to accelerate repairing process.

Shockwaves have been used successfully in the treatment of several chronic tendon ailments; recently they have been applied in acute phlogosis of soft tissues as bursitis or traumatic tendonopathies. Their efficacy has been demonstrated even in accelerating the wound repairing. Of consequence, ESWT can be applied in regenerative medicine, in all cases where fibroblast activity and the interaction with connective tissue can be positively influenced.

This novel approach with an existing technology shows a comparable, if not greater, efficacy relative to current therapeutic approaches: noninvasiveness, highly favorable side-effect profile, no known drug interactions, time-efficient simplicity of use, and cost effectiveness.

References

1. Ross MH, Romrell LJ, Kaye GI. Histology: a text and atlas. 3rd ed. Philadelphia, London; Williams and Wilkins 1995.
2. Wang JHC, Thampatty BP, Lin JS, Im HJ. Mechanoregulation of gene expression in fibroblasts. *Gene* 2007; 391:1-15.
3. Jelaska A, Strehlow D, Korn JH. Fibroblast heterogeneity in physiological conditions and fibrotic disease. *Springer Semin Immunopathol* 2000; 21:385-395.
4. Atamas S P. Complex cytokine regulation of tissue fibrosis. *Life Sciences* 2002; 72:631-643.
5. Benjamin M, Ralphs J R. The cell and developmental biology of tendons and ligaments. *Int Rev Cytol* 2000; 196:85-130.
6. Long CS, Brown LD. The Cardiac Fibroblast, Another Therapeutic Target for Mending the Broken Heart? *J Mol Cell Cardiol* 2002; 34:1273-1278).
7. Vogel V. Mechanotransduction involving multimodular proteins: converting force into biochemical signals. *Annu Rev Biophys Biomol Struct* 2006; 35:459-488.
8. Eckes B, Krieg T. Regulation of connective tissue homeostasis in the skin by mechanical forces. *Clin Exp Rheumatol* 2004; 22 (suppl.33):S73-S76.
9. Huang H, Kamm RD, Lee RT. Cell mechanics and mechanotransduction: pathways, probes, and physiology. *Am J Physiol Cell Physiol* 2004; 287:C1-C11.
10. Bustamante C, Chemla YR, Forde NR, Izhaky D. Mechanical processes in biochemistry. *Annu Rev Biochem* 2004; 73:705-748.
11. Tamada M, Sheetz MP, Sawada Y. Activation of a signaling cascade by cytoskeleton stretch. *Dev Cell* 2004; 7:709-718.
12. Galbraith CG, Yamada KM, Sheetz MP. The relationship between force and focal complex development. *J Cell Biol* 2002; 159:695-705.
13. Shyy JY-J, Chien S. Role of integrins in cellular responses to mechanical stress and adhesion. *Curr Opin Cell Biol* 1997; 9:707-713.
14. Galbraith CG, Sheetz MP. Forces on adhesive contacts affect cell function. *Curr Opin Cell Biol* 1998; 10:566-571.
15. Zhou S, Schmelz A, Seufferlein T, Li Y, Zhao J, Bachem MG. Molecular mechanisms of low intensity pulsed ultrasound in human skin fibroblasts. *J Biol Chem* 2004; 279:54463-54469.
16. Régent M, Planus E, Bouin AP, et al. Specificities of β 1 integrin signaling in the control of cell adhesion and adhesive strength. *Eur J Cell Biol.* 2011; 90:261-269.
17. Puklin-Faucher E, Sheetz MP. The mechanical integrin cycle *J Cell Sci* 2009; 122:179-186.
18. Guiliak F, Alexopoulos LG, Upton ML, et al. The pericellular matrix as a transducer of biomechanical and biochemical signals in articular cartilage. *Ann NY Acad Sci* 2000; 1068:498-512.
19. MacKenna D, Summerour SR, Villarreal FJ. Role of mechanical factors in modulating cardiac fibroblast function and extracellular matrix synthesis. *Cardiovasc Res* 2000; 46:257-263.
20. Davies PF, Barbee KA, Volin MV, et al. Spatial relationships in early signaling events of flow-mediated endothelial mechanotransduction. *Annu Rev Physiol* 1997; 59:527-549.
21. Rubin J, Rubin C, Jacobs CR. Molecular pathways mediating mechanical signaling in bone. *Gene* 2006; 367:1-16.
22. Heinemeier K, Langberg H, Olesen JL, Kjaer M. Role of TGF- β 1 in relation to exercise-induced type I collagen synthesis in human tendinous tissue. *J Appl Physiol* 2003; 95:2390-2397.
23. Skutek M, van Griensven M, Zeichen J, et al. Cyclic mechanical stretching modulates secretion pattern of growth factors in human tendon fibroblasts. *Eur J Appl Physiol* 2001; 86:48-52.
24. Bishop JE, Butt R, Dawes K, Laurent G. Mechanical load enhances the stimulatory effect of PDGF on pulmonary artery fibroblast procollagen synthesis. *Chest* 1998; 114 (1 Suppl):25S.
25. Butt RP, Bishop JE. Mechanical load enhances the stimulatory effect of serum growth factors on cardiac fibroblast procollagen synthesis. *J Mol Cell Cardiol* 1997; 29:1141-1151.
26. Yang G, Crawford RC, Wang JH. Proliferation and collagen production of human patellar tendon fibroblasts in response to cyclic uniaxial stretching in serum-free conditions. *J Biomech* 2004; 37:1543-1550.
27. Heinemeier KM, Olesen JL, Haddad F, et al. Expression of collagen and related growth factors in rat tendon and skeletal muscle in response to specific contraction types. *J Physiol* 2007; 582:1303-1316.
28. Heinemeier KM, Olesen JL, Haddad F, Schjerling P, Baldwin KM, Kjaer M. Effect of unloading followed by reloading on expression of collagen and related growth factors in rat tendon and muscle. *J Appl Physiol* 2009;

- 106:178-186.
29. Chiquet M, Renedo AS, Huber F, Fluck M. How do fibroblasts translate mechanical signals into changes in extracellular matrix production? *Matrix Biol* 2003; 22:73–80.
 30. Chiquet M, Gelman L, Lutz R, Maier S. From mechanotransduction to extracellular matrix gene expression in fibroblasts. *Biochim Biophys Acta* 2009; 1793:911-920.
 31. Arnaout MA, Goodman SL, Xiong JP. Structure and mechanics of integrin based cell adhesion. *Curr Opin Cell Biol* 2007; 19:495-507.
 32. Elliott DH. Structure and function of mammalian tendon. *Biol Rev* 1965; 40:392-421.
 33. Kannus P. Structure of the tendon connective tissue. *Scand J Med Sci Sports* 2000; 10:312-320.
 34. Provenzano PP, Vanderby R, Jr. Collagen fibril morphology and organization: implications for force transmission in ligament and tendon. *Matrix Biol* 2006; 25:71-84.
 35. MacKenna D, Summerour SR, Villarreal FJ. Role of mechanical factors in modulating cardiac fibroblasts function and extracellular matrix synthesis. *Cardiovasc Res* 2000; 46:257-263.
 36. Ingber DE, Dike L, Hansen L, et al. Cellular tensegrity: exploring how mechanical changes in the cytoskeleton regulate cell growth, migration, and tissue pattern during morphogenesis. *Int Rev Cytol* 1994; 150:173-224.
 37. Franchi M, Trirè A, Quaranta M, Orsini E, Ottani V. Collagen structure of tendon relates to function. *Scientific-WorldJournal* 2007; 7:404-420.
 38. Kjaer M, Langberg H, Heinemeier K, et al. From mechanical loading to collagen synthesis, structural changes and function in human tendon. *Scand J Med Sci Sports* 2009; 19:500-510.
 39. Wang JH, Guo Q, Li B. Tendon Biomechanics and Mechanobiology-A Minireview of Basic Concepts and Recent Advancements. *J Hand Ther* 2011; Sep 17 [Epub ahead of print].
 40. Olesen JL, Heinemeier KM, Haddad F, et al. Expression of insulin-like growth factor I, insulin-like growth factor binding proteins, and collagen mRNA in mechanically loaded plantaris tendon. *J Appl Physiol* 2006; 101:183-188.
 41. Birk DE, Mayne R. Localization of collagen type I, III and V during tendon development. Changes in collagen types I and III are correlated with changes in fibril diameter. *Eur J Cell Biol* 1997; 72:352–361.
 42. Enwemeka CS. Inflammation, cellularity, and fibrillogenesis in regeneration tendon: implications for tendon rehabilitation. *Phys Ther* 1989; 69:816-825.
 43. Orhan Z, Ozturan K, Guven A, Cam K. The effect of extracorporeal shock waves on a rat model of injury to tendo Achillis. A histological and biomechanical study. *J Bone Joint Surg (Br)* 2004; 86:613-618.
 44. Sharma P, Maffulli N. Tendon injury and tendinopathy: healing and repair. *J Bone Joint Surg (Am)* 2005; 87:187-202.
 45. Sharma P, Maffulli N. Tendinopathy and tendon injury: the future. *Disabil Rehabil* 2008; 30:1733-1745.
 46. Lee EW, Maffulli N, Li CK, Chan KM. Pulsed magnetic and electromagnetic fields in experimental Achilles tendonitis in the rat: A prospective randomized study. *Arch Phys Med Rehab* 1997; 78:399-404.
 47. Strauch B, Patel MK, Rosen DJ, Mahadevia S, Brindzei N, Pilla AA. Pulsed magnetic field therapy increases tensile strength in a rat Achilles' tendon repair model. *J Hand Surg (Am)* 2006; 31:1131-1135.
 48. Reddy GK, Stehno-Bittel L, Enwemeka CS. Laser photostimulation of collagen production in healing rabbit Achilles tendons. *Lasers Surg Med* 1998; 22:281-287.
 49. Fillipin LI, Mauriz JL, Vedovelli K, et al. Low-level laser therapy (LLLT) prevents oxidative stress and reduces fibrosis in rat traumatized Achilles tendon. *Lasers Surg Med* 2005; 37:293-300.
 50. Tasto JP, Cummings J, Medlock V, Harwood F, Hardesty R, Amiel D. The tendon treatment center: New horizons in the treatment of tendinosis. *Arthroscopy* 2003; 19(Suppl 1):213-223.
 51. Weil L Jr, Glover JP, Weil LS Sr. A new minimally invasive technique for treating plantar fasciosis using bipolar radiofrequency: a prospective analysis. *Foot Ankle Spec* 2008; 1:13-18.
 52. Whipple T, Villegas D. Thermal and electric energy fields by noninvasive monopolar capacitive-coupled radiofrequency: temperatures achieved and histological outcomes in tendons and ligaments. *PM R* 2010; 2:599-606.
 53. Tsai WC, Tang ST, Liang FC. Effect of therapeutic ultrasound on tendons. *Am J Phys Med Rehabil* 2011; 90:1068-1073.
 54. Khanna A, Nelmes RT, Gougoulis N, Maffulli N, Gray J. The effects of LIPUS on soft-tissue healing: a review of literature. *Br Med Bull* 2009; 89:169-182.
 55. Wang CJ. Shock wave therapy in musculoskeletal disorders. *Chang Gung Med J* 2003; 26:220-232.
 56. Maffulli N, Longo UG, Denaro V. Novel approaches for the management of tendinopathy. *J Bone Joint Surg (Am)* 2010; 92:2604-2613.
 57. Haupt G. Use of extracorporeal shock waves in the treatment of pseudarthrosis, tendinopathy and other orthopedic diseases. *J Urol* 1997; 158:4-11.
 58. Lotan Y, Pearle MS. Economics of stone management. *Urol Clin North Am* 2007; 34:443– 453.
 59. Speed CA. Extracorporeal shock-wave therapy in the management of chronic soft-tissue conditions. *J Bone Joint Surg [Br]* 2004; 86-B: 165–171.
 60. Chao Y H, Tsuang Y H, Sun J S, et al. Effect of shock waves on tenocyte proliferation and extracellular matrix metabolism. *Ultrasound Med Biol* 2008; 34:841-852.
 61. Orhan Z, Alper M, Akman Y, Yavuz O, Yalçiner A. An experimental study on the application of extracorporeal shock waves in the treatment of tendon injuries: preliminary report. *J Orthop Sci* 2001; 6:566-570.
 62. Johannes EJ, Kaulesar Sukul DM, Matura E. High-energy shock waves for the treatment of nonunions: an experiment on dogs. *J Surg Res* 1994; 57:246-252.
 63. Haupt G, Chvapil M. Effect of shock waves on the healing of partial-thickness wounds in piglets. *J Surg Res*

- 1990; 49:45-48.
64. Berta L, Fazzari A, Ficco AM, Enrica PM, Catalano MG, Frairia R. Extracorporeal shock waves enhance normal fibroblast proliferation in vitro and activate mRNA expression for TGFbeta1 and for collagen types I and III. *Acta Orthop* 2009; 80:612-617.
 65. Wang F S, Wang C J, Huang H J, Ching H, Chen R F, Yang K D. Physical shock wave mediates membrane hyperpolarization and ras activation for osteogenesis in human bone marrow stromal cells. *Biochem Biophys Res Comm* 2001; 287: 648-655.
 66. Wang F S, Yang K D, Chen R F, Wang C J, Sheen-Chen S M. Extracorporeal shock wave promotes growth and differentiation of bone-marrow stromal cells towards osteoprogenitors associated with induction of TGF-β1. *J Bone Joint Surgery (Br)* 2002; 84: 457-461.
 67. Martini L, Fini M, Giavaresi G, et al. Primary osteoblasts response to shock wave therapy using different parameters. *Artif Cells Blood Substit Immobil Biotechnol* 2003; 31:449-466.
 68. Chen YJ, Wang CJ, Yang KD, et al. Extracorporeal shock waves promote healing of collagenase-induced Achilles tendinitis and increase TGF-beta1 and IGF-I expression. *J Orthop Res* 2004; 22:854-861.
 69. Hausdorf J, Sievers B, Schmitt-Sody M, Jansson V, Maier M, Mayer-Wagner S. Stimulation of bone growth factor synthesis in human osteoblasts and fibroblasts after extracorporeal shock wave application. *Arch Orthop Trauma Surg* 2011; 131: 303-309.
 70. Lieberman JR, Daluiski A, Einhorn TA. The role of growth factors in the repair of bone. Biology and clinical applications. *J Bone Joint Surg (Am)* 2002; 84:1032-1044.
 71. Tsai WC, Hsu CC, Tang FT, Chou SW, Chen YJ, Pang JHS. Ultrasound stimulation of tendon cell proliferation and upregulation of proliferating cell nuclear antigen. *J Orthop Res* 2005; 23:970-976.
 72. Van Griensven M, Zeichen J, Skutek M, Barkhausen T, Krettek C, Bosch U. Cyclic mechanical strain induces NO production in human patellar tendon fibroblasts - A possible role for remodelling and pathological transformation. *Exp Toxic Pathol* 2003; 54:335-338.
 73. Vodovotz Y, Chesler L, Chong H, et al. Regulation of transforming growth factor beta1 by nitric oxide. *Cancer Res* 1999; 59:2142-2149.
 74. Motani A, Forster L, Tull S, Anggård EE, Ferns GA. Insulin-like growth factor-I modulates monocyte adhesion to EAhy 926 endothelial cells. *Int J Exp Pathol* 1996; 77:31-35.
 75. Chang J, Most D, Thunder R, Mehrara B, Longaker MT, Lineaweaver WC. Molecular studies in flexor tendon wound healing: The role of basic fibroblast growth factor gene expression. *J Hand Surg [Am]* 1998;23(6):1052-1058.
 76. Takahashi S, Fujita T, Yamamoto A. Role of nuclear factor-kappaB in gastric ulcer healing in rats. *Am J Physiol Gastrointest Liver Physiol* 2001; 280: G1296-G1304.
 77. Maier M, Averbek B, Milz S, Refior HJ, Schmitz C. Substance P and prostaglandin E2 released after shock wave application to the rabbit femur. *Clin Orthop* 2003; 406:237-245.
 78. Wang CJ, Yang KD, Ko JY, Huang CC, Huang HY, Wang FS. The effects of shockwave on bone healing and systemic concentrations of nitric oxide (NO), TGF-β1, VEGF and BMP-2 in long bone non-unions. *Nitric Oxide* 2009; 20:298-303.
 79. Diwan AD, Wang MX, Jang D, Zhu W, Murrell GAC. Nitric oxide modulates fracture healing. *J Bone Miner Res* 2000; 15:342-351.
 80. Gotte G, Amelio E, Russo S, Marlinghaus E, Musci G, Suzuki H. Short-time non-enzymatic nitric oxide synthesis from L-arginine and hydrogen peroxide induced by shock waves treatment. *FEBS Lett.* 2002; 520:153-155.
 81. Babaei S, Stewart DJ. Overexpression of endothelial NO synthase induces angiogenesis in a co-culture model. *Cardiovascular Res* 2002; 55:190-200.
 82. Spyridopoulos I, Luedeman C, Chen D, et al. Divergence of angiogenesis and vascular permeability signaling by VEGF: inhibition of protein kinase C suppresses VEGF-induced angiogenesis, but promotes VEGF induced NO-dependent vascular permeability. *Arterioscl Throm Vas Biol* 2002; 22:901-906.
 83. Wang CJ, Wang FS, Yang KD, Huang CS, Hsu CC. Shockwave therapy induced neovascularization at the tendon- bone junction. A study in rabbits. *J Orthop Res*, 2003, 21:984-989.
 84. Yin TC, Wang CJ, Yang KD, Wang FS, Sun YC. Shockwaves enhance the osteogenetic gene expression in marrow stromal cells from hips with osteonecrosis. *Chang Gung Med J* 2011; 34:367-374.
 85. Mariotto S, de Prati AC, Cavalieri E, Amelio E, Marlinghaus E, Suzuki H. Extracorporeal shock wave therapy in inflammatory diseases: molecular mechanism that triggers anti-inflammatory action. *Curr Med Chem* 2009; 16:2366-2372.
 86. Clark RA. Basics of cutaneous wound repair. *J Dermatol Surg Oncol* 1993; 19:693-706.
 87. Doan N, Reher P, Meghji S, Harris M. In vitro effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibroblasts, osteoblasts, and monocytes. *J Oral Maxillofac Surg.* 1999; 57:409-419.
 88. Meirer R, Kamelger FS, Huemer GM, et al. Extracorporeal shock wave may enhance skin flap survival in an animal model. *Br J Plast Surg* 2005; 58:53-57.
 89. Huemer GM, Meirer R, Gurunluoglu R, et al. Comparison of the effectiveness of gene therapy with transforming growth factor-beta or extracorporeal shock wave therapy to reduce ischemic necrosis in an epigastric skin flap model in rats. *Wound Repair Regen* 2005; 13:262-268.
 90. Kuo YR, Wu WS, Hsieh YL, et al. Extracorporeal shock wave enhanced extended skin flap tissue survival via increase of topical blood perfusion and associated with suppression of tissue pro-inflammation. *J Surg Res* 2007; 143:385-392.
 91. Meirer R, Brunner A, Deibl M, Oehlbauer M, Piza-

- Katzer H, Kamelger FS. Shock wave therapy reduces necrotic flap zones and induces VEGF expression in animal epigastric skin flap model. *J Reconstr Microsurg* 2007; 23:231-236.
92. Gerdesmeyer L, von Eiff C, Horn C, Henne M, Roessner M, Diehl P, Gollwitzer H. Antibacterial effects of extracorporeal shock waves. *Ultrasound Med Biol* 2005; 31:115-119.
93. Kuo YR, Wang CT, Wang FS, et al. Extracorporeal shock wave treatment modulates skin fibroblast recruitment and leukocyte infiltration for enhancing extended skin-flap survival. *Wound Repair Regen* 2009; 17:80-87.
94. Kuo YR, Wang CT, Wang FS, et al. Extracorporeal shock wave therapy enhanced wound healing via increasing topical blood perfusion and tissue regeneration in a rat model of STZ-induced diabetes. *Wound Repair Regen* 2009; 17:522-530.
95. Stojadinovic A, Elster EA, Anam K, et al. Angiogenic response to extracorporeal shock wave treatment in murine skin isografts. *Angiogenesis*. 2008; 11:369-380.
96. Yan X, Zeng B, Chai Y, Luo C, Li X. Improvement of blood flow, expression of nitric oxide, and vascular endothelial growth factor by low-energy shockwave therapy in random-pattern skin flap model. *Ann Plast Surg* 2008, 61:646-653.
97. Mittermayr R, Hartinger J, Antonic V, et al. Extracorporeal shock wave therapy (ESWT) minimizes ischemic tissue necrosis irrespective of application time and promotes tissue revascularization by stimulating angiogenesis. *Ann Surg* 2011; 253:1024-1032.
98. Schaden W, Thiele R, Kolpl C, et al. Shock wave therapy for acute and chronic soft tissue wounds: a feasibility study. *J Surg Res* 2007; 143: 1-12.
99. Saggini R, Figus A, Troccola A, Cocco V, Saggini A, Scuderi N. Extracorporeal shock wave therapy for management of chronic ulcers in the lower extremities. *Ultrasound Med Biol*. 2008; 34:1261-1271.
100. Ottomann C, Hartmann B, Tyler J, et al. Prospective randomized trial of accelerated re-epithelization of skin graft donor sites using extracorporeal shock wave therapy. *J Am Coll Surg* 2010; 211:361-367.
101. Moretti B, Notarnicola A, Maggio G, et al. The management of neuropathic ulcers of the foot in diabetes by shock wave therapy. *BMC Musculoskelet Disord* 2009;10:54.
102. Arnò A, Garcia O, Hernán I, et al. Extracorporeal shock waves, a new nonsurgical method to treat severe burns. *Burns* 2010; 36:844-849.
103. Larking AM, Duport S, Clinton M, et al. Randomized control of extracorporeal shock wave therapy versus placebo for chronic decubitus ulceration. *Clin Rehabil* 2010; 24:222-229.
104. Ottomann C, Stojadinovic A, Lavin PT, et al. Prospective Randomized Phase II Trial of Accelerated Reepithelialization of Superficial Second-Degree Burn Wounds Using Extracorporeal Shock Wave Therapy. *Ann Surg* 2012; 255:23-29.