# How Shock Waves Works on Musculoskeletal Tissues? The Contribution of Italian School

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## University of Naples "Federico II" and University of Verona – School of Medicine: The Role of ESW on Production of Nitric Oxide (NO) Increase of NO production

NO plays a critical role in inflammation, and this school has proved that ESW increases NO production in cells [1]. Using human umbilical vein endothelial cells (ECs) as a model system, the authors observed that ESW, at low-energy density, rapidly induced an enhancement of eNOS activity. In these cells, eNOS activity is modulated by tyrosine- and serine-phosphorylation. ESW shifted eNOS to a less-tyrosine-phosphorylated form, without affecting its serine-phosphorylation, thus accounting for its rapid enzyme activation. LPS/IFN-gamma treatment of human umbilical vein ECs induced a rapid inhibition of eNOS activity and concomitant NF-kappaB activation which were efficiently counteracted by ESW treatment. The results of this study indicate that the molecular mechanism of clinically observed anti-inflammatory action of ESW should include tyrosine-dephosphorylation of eNOS, a successive increase in NO production, and suppression of NF-kappaB activation.

## University of Turin - School of Medicine: The Role of ESW on Stem Cells Proliferation and Differentiation Proliferation and differentiation of fibroblasts

Experimental data [2] confirm that ESW treatment promotes and improves the repair process through accelerated timing of RNA expression for transforming growth factor (TGF)-β1, collagen I, and collagen III (relative to untreated fibroblasts).

## Differentiation of adipose-derived stem cells into osteoblast-like cells by ESW activity on osteogenic medium

Human adipose-derived stem cells (hASCs) are a promising cell type for bone tissue engineering, given their potential to differentiate into osteoblast-like cells. Interactions among biochemical and mechanical signals result in bone formation and repair. In this process, stem cells have a crucial role. The ESW treatment increased Runt-related transcription factor 2, ALP, and bone morphogenetic protein-2 expression, as well as ALP activity and calcium deposits with respect to untreated cells. Moreover, ESWs induced ROS formation and both ERK and Smad (small "mothers against" decapentaplegic) phosphorylation. Recent studies [3, 4] show the effects of ESWs on osteogenic differentiation in an in vitro model using hASCs and define the mechanisms involved in this process.

## Modulation of stem cell differentiation toward myofibroblasts

Mesenchymal stem cells are precursors of myofibroblasts, cells deeply involved in promoting tissue repair and regeneration. However, since myofibroblast persistence is associated with the development of tissue fibrosis, the use of tools that can modulate stem cell differentiation toward myofibroblasts is central. Shock waves inhibit the development of a myofibroblast phenotype; they downregulate the expression of the myofibroblast marker alpha-smooth muscle actin and the extracellular matrix protein type I collagen. Functionally, stem cells acquire a more

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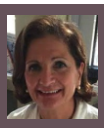
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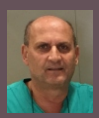
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fibroblast-like profile characterized by a low contractility and a high migratory ability. Shock wave treatment reduces the expression of integrin alpha 11, a major collagen receptor in fibroblastic cells, involved in myofibroblast differentiation [5].

### Differentiation and proliferation of osteoblasts on scaffold

ESW was used to improve the ability of human osteoblasts to colonize scaffolds and to induce their osteogenic property. The calcium deposits and differentiation markers studied have demonstrated that shock waves increase osteoblast migration and penetration into scaffolds: this study may provide an important starting point for the introduction of shock waves to boost bone formation through osteoblast stimulation in diseases characterized by bone defects [6].

### University of Milan - School of Medicine: The Role of ESW on Regeneration of Tendon Cells and Ecs Regenerative effect of SW on tendon cells

The regenerative effect of SW on tendon cells has been shown by De Girolamo et al. [7] in an adherent culture model of primary human tendon cells. This setting replicates the natural cell-to-cell interactions between cells and the extracellular matrix. This is crucial for the mechanotransduction given by changes in mechano-sensitive membrane proteins. The first effect observed in his study was the expression of scleraxis, a transcription factor specific for tenocytes, and their progenitors. It contributes to regulating the expression of COL1 A1 in tendon fibroblasts and the differentiation of mesenchymal cells into fibroblasts. Furthermore, the consequential peak of interleukin IL 1β (day 1), not related to the degradation of ECM, IL-6 (day 2), and IL-1 (day 2), together with TGFβ and vascular endothelial growth factor (VEGF) (day 2). Tumor necrosis factor α (TNFα) was not affected by ESW treatment. This pathway agrees perfectly with the healing inflammatory mechanism, where the initial acute response is followed by the rise of the anti-inflammatory cytokine IL-10 responsible for the self-resolving phase of inflammation together with the start of the healing mechanism highlighted by TGFβ and VEGF.

### Vascular induction

Regarding the vascular induction promoted by ESW, Sansone et al. [8] demonstrated in a three-dimensional (3D) Matrigel culture model of a human microvascular EC line (HMEC-1), 12 h after the SW treatment, a statistically significant increase in capillary connections with respect to the control group was observed. HMEC-1 is a HMEC-1 that retains the morphological and functional characteristics of human microvascular cells, producing the Von Willebrand factor and forming tubular structures in three-dimensional solid support, mimicking the formation of capillary vessels. Hence, they are different from ECs and can form larger vessels. Unfocussed low energy and a low number of shots demonstrated interesting results in terms of neo-vessel and capillary bifurcation. More, preliminarily in the 3 h following the treatment, the downregulation in genes involved in the apoptotic processes (BAX, BCL2LI, GADD45A, and PRKCA), in the cell cycle (CDKN2C, CEBPB, HK2, IRF1, and PRKCA), oncogenes (JUN and WNT1), cell adhesion (ICAM-1), and proteolytic systems (CTSD, KLK2, and MMP10) has been observed. This can be explained as a preparatory phase for sprouting new vessels, or the "early reactive response" to the SW stimulus able to improve successively, 12 h after, the differentiation of endothelial progenitors cells in EC and comparable to the behaviors of the laminar shear stress flow, characterized by an anti-apoptotic effect. Furthermore, the county of capillary connection was higher for low EFD levels of 0,01 mJ/mm2 and at 200 shocks (200), confirming the hypothesis of better regenerative vascular-mediated effects in tissue regeneration applying low energy and a low number of shots.

### University of Rome "La Sapienza"- School of Medicine: The Role of ESW on Tenocytes and Chondrocytes Activities

### Tenocytes proliferation and collagen synthesis

In their study, Vetrano et al. [9] investigated the effects of ESWT on primary cultured human tenocytes. The authors found that ESWT promoted cell proliferation and collagen synthesis, which are essential processes for tendon healing and regeneration. These findings suggest that ESWT could potentially be used as a therapeutic approach for tendon-related disorders.

### Tendon repair

Leone et al. [10] studied the effects of ESWT on the functional activities of ruptured human tendon-derived tenocytes in vitro. The researchers found that ESWT improved cell migration, proliferation, and collagen production, which are critical for tendon repair. This study further supports the potential use of ESWT for the treatment of tendon injuries and disorders.

### Tendon-derived stem cells

In a subsequent study, Leone et al. [11] examined the impact of ESWT on the in vitro-induced differentiation of human tendon-derived stem/progenitor cells (hTSPCs). The authors found that ESWT enhanced the differentiation of hTSPCs into tenocytes, suggesting that this treatment modality could enhance the regenerative potential of tendon-derived stem/progenitor cells.

### Chondrocyte regeneration and CD44 expression

In their most recent study, Vetrano et al. [12] investigated the combined effects of hyaluronic acid (HA), platelet-rich plasma (PRP), and ESWT on human chondrocyte regeneration in vitro. The authors observed that ESWT, HA, and PRP treatments promoted chondrocyte regeneration, with ESWT-mediated increase of CD44 expression enhancing the susceptibility of chondrocytes to HA treatment. These findings indicate that combining ESWT with other regenerative treatments may enhance the therapeutic potential for cartilage repair.

### University of Bari - School of Medicine: The Role of ESW on Cytokines, Osteoblasts Activities Chondrocytes activity

By stimulating the chondrocytes [13], there is an increase in anti-inflammatory cytokines (such beta1 integrin and Il-10) and a reduction in inflammation mediators (such as N-Cadherin, B-Caternin, and TNFα), both in healthy subjects and in arthritic. These results would provide the rationale for the treatment of cartilage tissue pathologies, from chondropathies to osteoarthritis.

### Osteoblasts and osteoclasts activity

Treatment of the osteoblasts resulted in an increase in the expression of Bax and activation of the cyclin E2/CDK2, indicating a proliferative and differentiating effect of the osteoblasts [14]. At the same time, we found a reduction of the receptor activator NF kappa B ligand (RANKL)/osteoprotegerin (OPG) ratio, suggesting an inhibition of osteoclasteogenesis. These effects are consistent with clinical applications in skeletal tissue pathologies, such as union delays and bone edema.

When the stimulation was conducted on osteoblasts taken from the subchondral bone of arthritic subjects, we verified a significant increase in IL10 and in the expression of CD29 and CD105 [15]. Therefore, in osteoarthritis, shock waves could allow to remodulate the adaptations that occur at the level of the subchondral bone, such as cortical sclerosis, the formation of osteophytes, and geodic cysts, inhibiting the differentiation of osteoclasts and promoting bone repair.

The use of radial wave in the stimulation of osteoblasts [16] resulted in a statistically significant reduction of type 1 collagen, osterix, bone sialoprotein and RANKL expression, osteocalcin, and osteopontin. These modulations correspond to an inhibition of osteoblastogenesis, suggesting that the radial wave has no indication in the treatment of fractures. On the other hand, a reduction in the RANKL/OPG ratio was found, which leads to an inhibition of osteoclastogenesis. We can hypothesize the application of this type of device in pathologies of a more proliferative nature.

**Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has/her given his consent for his/her images and other clinical information to be reported in the Journal. The patient understands that his/her name and initials will not be published, and due efforts will be made to conceal his identity, but anonymity cannot be guaranteed.

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## References


1. Mariotto S, Cavalieri E, Amelio E, Ciampa AR, de Prati AC, Marlinghaus E, et al. Extracorporeal shock waves: From lithotripsy to anti-inflammatory action by NO production. Nitric Oxide 2005;12:89-96.
2. 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 TGF-beta1 and for collagen Types I and III. Acta Orthop 2009;80:612-7.
3. Catalano MG, Marano F, Rinella L, de Girolamo L, Bosco O, Fortunati N, et al. Extracorporeal shockwaves (ESWs) enhance the osteogenic medium-induced differentiation of adipose-derived stem cells into osteoblast-like cells. J Tissue Eng Regen Med 2017;11:390-9.
4. Simplicio CL, Purita J, Murrell W, Santos GS, Dos Santos RG, Lana JF. Extracorporeal shock wave therapy mechanisms in musculoskeletal regenerative medicine. J Clin Orthop Trauma 2020; 11 Suppl 3:S309-18.
5. Rinella L, Marano F, Berta L, Bosco O, Fraccalvieri M, Fortunati N, et al. Extracorporeal shock waves modulate myofibroblast differentiation of adipose-derived stem cells. Wound Repair Regen 2016;24:275-86.
6. Muzio G, Vernè E, Canuto RA, Martinasso G, Saracino S, Baino F, et al. Shock waves induce activity of human osteoblast-like cells in bioactive scaffolds. J Trauma 2010;68:1439-44.
7. De Girolamo L, Stanco D, Galliera E, Viganò M, Lovati AB, Marazzi MG, et al. Soft-focused extracorporeal shock waves increase the expression of tendon-specific markers and the release of anti-inflammatory cytokines in an adherent culture model of primary human tendon cells. Ultrasound Med Biol 2014;40:1204-15.
8. Sansone V, D' Agostino MC, Bonora C, Sizzano F, De Girolamo L, Romeo P. Early angiogenic response to shock waves in a three-dimensional model of human microvascular endothelial cell culture (HMEC-1). J Biol Regul Homeost Agents 2012;26:29-37.
9. Vetrano M, D'Alessandro F, Torrisi MR, Ferretti A, Vulpiani MC, Visco V. Extracorporeal shock wave therapy promotes cell proliferation and collagen synthesis of primary cultured human tenocytes. Knee Surg Sports Traumatol Arthrosc 2011;19:2159-68.
10. Leone L, Vetrano M, Ranieri D, Raffa S, Vulpiani MC, Ferretti A, et al. Extracorporeal Shock Wave Treatment (ESWT) improves in vitro functional activities of ruptured human tendon-derived tenocytes. PLoS One 2012;7:e49759.
11. Leone L, Raffa S, Vetrano M, Ranieri D, Malisan F, Scrofani C, et al. Extracorporeal Shock Wave Treatment (ESWT) enhances the in vitro-induced differentiation of human tendon-derived stem/progenitor cells (hTSPCs) Oncotarget 2016;7:6410-23.
12. Vetrano M, Ranieri D, Nanni M, Pavan A, Malisan F, Vulpiani MC, et al. Hyaluronic Acid (HA), Platelet-Rich Plasm and Extracorporeal Shock Wave Therapy (ESWT) promote human chondrocyte regeneration in vitro and ESWT-mediated increase of CD44 expression enhances their susceptibility to HA treatment. PLoS One 2019;14:e0218740.
13. Moretti B, Iannone F, Notarnicola A, Lapadula G, Moretti L, Patella V, et al. Extracorporeal shock waves down-regulate the expression of interleukin-10 and tumor necrosis factor-alpha in osteoarthritic chondrocytes. BMC Musculoskelet Disord 2008;9:16.
14. Tamma R, dell'Endice S, Notarnicola A, Moretti L, Patella S, Patella V, et al. Extracorporeal shock waves stimulate osteoblast activities. Ultrasound Med Biol 2009;35:2093-100.
15. Iannone F, Moretti B, Notarnicola A, Moretti L, Patella S, Patella V, et al. Extracorporeal shock waves increase interleukin-10 expression by human osteoarthritic and healthy osteoblasts in vitro. Clin Exp Rheumatol 2009;27:794-9.
16. Notarnicola A, Tamma R, Moretti L, Fiore A, Vicenti G, Zallone A, et al. Effects of radial shock waves therapy on osteoblasts activities Musculoskelet Surg 2012;96:183-9.


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