Biological effects of extracorporeal shockwave therapy in tendons: A systematic review

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Abstract. Extracorporeal shockwave therapy was initially used for kidney stone disintegration and its application was then extended to calcific tendinitis. The therapeutic field expanded and included numerous types of tendinopathies, from shoulder to plantar fascia. The clinical benefits were documented in trials and the effects and mechanisms were studied on models including animal and human tendons. The present systematic review outlines a large spectrum of biological effects. First, an optimal dose is adapted for each species and each tendon; exceeding the optimal dose may lead to structural injury. Furthermore, the biological effects may be grouped into neovascularization induction, cellularity and extracellular matrix changes, metalloprotease and cytokine modulation, as well as lubricin production. As a result, the remodeled tendon displays improved biomechanical properties to resist stress.

Introduction

Extracorporeal shock wave therapy (ESWT) was first used for kidney stones, as a method to disintegrate them (1). At the beginning of the 1990s, it was used as a non-invasive procedure to successfully treat calcific tendinitis. From then on, further tendon disorders were effectively addressed, with subsequent research work on their biological and clinical effects.

Basically, there are two types of ESWT: Focused and radial shockwave therapy. Focused ESWT is extensively used in clinical practice; it comprises high-energy pressure pulses that converge to a focal point, where maximal pressure is reached. They have an initial high positive pressure wave (up to 80 MPa) with a rapid raise time (30-120 nsec), followed by a negative wave (5-10 MPa). The pulse duration is short, 5 μsec (2). Wave energy is released at tissue interfaces that have different acoustic impedances, causing compressive and shear loads. Microscopic gas bubbles develop and collapse in the interstitial fluid of tissues, a phenomenon called cavitation. It produces high localized stresses, a mechanical stimulation (3). Radial ESWT is characterized by a diverging pressure field, with a maximal pressure at the source (4). Certain researchers agree that radial ESWT cannot be described as real extracorporeal shockwaves as they lack their physical features and proposed to name them radial pressure wave therapy, as a distinct form of therapy (5).

Energy flux density (EFD) determines the energy flow through an area perpendicular to the direction of wave propagation and its units are mJ/mm^2. The classification of ESWT includes low (<0.08 mJ/mm^2), medium (<0.28 mJ/mm^2) and high (<0.60 mJ/mm^2) EFD (6).

Microscopically, tendons are composed of cells, tenocytes and extracellular matrix (ECM) that contains collagen, elastin and ground substance. Tenocytes, spindle-shaped cells, are responsible for matrix maintenance and repair and occupy 5% of the tissue volume. ECM contains mainly type I collagen, while type III collagen is the next-most abundant and critical in pathologic tendons and tendon-healing processes (7).

Tenocytes may convert mechanical stimulation into a biochemical response, leading to the release of growth factors and cellular adaptation (8). The process is known as mechanotransduction and may lead to maintenance, remodeling or degeneration of the tendon through regulation of anabolic and catabolic genes. The mechanisms of action remain to be fully elucidated.

The therapeutic field of ESWT is continuously expanding, as research adds new opportunities. Post-stroke spasticity was addressed with comparable efficacy with botulinum toxin and both types of ESWT, with the radial form providing the best short- and medium-term results (9).

Materials and methods

A comprehensive literature search was performed in the online databases PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Cochrane Library (https://www.cochrane.org/Both) using combinations of the following key words: 'extracorporeal shockwave therapy', 'biological effect' and 'tendon'. through. The search included papers available as an abstract since 1988

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up to December 2021. The inclusion criteria were as follows: i) Studies on human or animal tendons, either *in vivo* or *in vitro*, with ESWT application; and ii) full-text available at the authors' institution. Studies with quantitative assessment of pain and function were excluded.

A total of two authors (DP, DC) independently identified 7,120 titles that were manually checked to exclude reviews and clinical trials and to retain only *in vivo* or *in vitro* studies on human and animal tendon tissue. After exclusion of duplicates, the full-text of 23 records was examined (Fig. 1) and the following data were extracted and entered in an Excel document: i) First author name and year; ii) type of tendon, human or type of animal, *in vivo* or *in vitro*; iii) ESWT doses; and iv) moments of the analysis. Further data on outcomes were extracted: v) Definition of the optimal dose; vi) neoangiogenesis; vii) histopathologic changes; viii) biochemical changes; and ix) mechanical properties.

Data regarding the optimal dose were analyzed in an attempt to obtain information regarding the following points: Optimal dose for achieving the maximum biologic effect, possible consequences of failing to apply the optimal dose and factors that influence the optimal dose. Furthermore, the investigation was focused on biological effects of ESWT on angiogenesis, cellularity, ECM and biomechanical properties of the tendons.

Results and Discussion

Studies retrieved. A summary of the studies (n=23) and their key features/findings is provided in Table I. According to the subjects, studies in the literature were able to be classified as *in vivo* or *in vitro* studies. *In vivo* studies had been performed on rats [6 papers (10-15)], rabbits [5 papers (6,16-19)], horses [one paper (20)], ponies [2 papers (21,22)] and dogs [one paper (23)], an on normal tendons [9 papers (6,12,14,16,17,19,21-23)] and diseased tendons [6 papers (10,11,13,18,20,24)]. The experimental model for diseased tendons was either a ruptured tendon, in the form of a complete and sutured rupture or a partial rupture [2 papers (10,13)] or a collagenase-induced tendinopathy [4 papers (11,15,18,20)]. Two studies were performed on human tissue, namely one on human Achilles tendon, using microdialysis of the peritendinous environment, and one on rotator cuff tendinopathy with surgical cure. In general, one limb was subjected to ESWT application and the contralateral limb was used as a control.

In vitro studies had been performed on cultured tendon cells from either normal or diseased tendons. A total of three papers reported results on cultured normal tendon cells and three papers compared cultured normal cells with tendinopathic cells. It is noteworthy that *in vitro* studies were not able to be directly translated to *in vivo* conditions, although they shed light on important mechanisms of action.

Finding the optimal dose. Information was structured according to whether the experimental model was *in vivo* or *in vitro*, and the focus was on cell viability and ECM alterations.

In vivo, for normal rabbit Achilles tendon, Rompe *et al* (6) determined three main EFD levels with different effects. Doses of up to 0.28 mJ/mm^2 did not damage the tendon and adjacent structures, or the alterations were transitory and reversible within four weeks. However, a dose of 0.60 mJ/mm^2 produced marked damage to the tendon and paratenon, with fibrinoid necrosis within the tendon and inflammatory and reparative peritendinous reactions that were still present at 4 weeks (6). In the normal rabbit quadriceps tendon, a lower EFD (0.35 mJ/mm^2) left no structural alteration at 10 days, a medium EFD (0.5 and 0.9 mJ/mm^2) produced edema in the paratenon at 10 days that resolved at 28 days, and a higher EFD (1.2 mJ/mm^2) induced marked modifications in cells and ECM at 10 days. Researchers opined that the quadriceps tendon is more 'resistant' to ESWT than the Achilles tendon (16). In rats, normal Achilles tendon exposed to 0.15 mJ/mm^2 and 1,000 or 1,500 pulses exhibited no structural alteration at 3 weeks. A higher dose of 0.20 mJ/mm^2 and 2,000 pulses produced collagen disorganization, inflammatory mononuclear reaction and an increased number of capillaries (12).

In vitro, in normal Achilles rat tenocyte cultures, doses of 0.36 mJ/mm^2 and 50, 100 and 250 pulses did not alter normal cell viability, whereas the same EFD with a higher number of pulses and an EFD of 0.68 mJ/mm^2, irrespective of the number of pulses, reduced the proportion of viable cells. The cell viability suppression was found to be dose-dependent (25). Normal human tendon cell cultures exhibited no modification when exposed to 0.08, 0.14 and 0.17 mJ/mm^2, either at 500 or 1,000 pulses. Furthermore, certain energy levels were found to augment cell viability: 0.14 mJ/mm^2 and 1,000 pulses was found to increase the number of viable cells at 24 and 48 h (26), and a dose of 0.17 mJ/mm^2 and 1,000 pulses promoted cell viability at 7 and 10 days after treatment, as measured by the increased content of DNA (27). For both tendinopathic and normal human cell cultures, 0.17 mJ/mm^2 and 250 or 500 pulses did not alter cell viability, whereas a higher number of pulses (1,000 and 2,000) led to cell death. Furthermore, a regimen with 500 pulses was indicated to promote cell proliferation (28). The variation among the reported doses may be explained by the variability of the model on which they were applied, as cultures were harvested from different human tendons.

As for the ECM, in an *in vivo* study on normal ponies' tendons, 0.14 mJ/mm^2 and 600 pulses produced a rapid stimulation of matrix turnover (3 h) and an increase of enzymatic cleavage of collagen. In the long term (6 weeks), damaged collagen was decreased, presumably replaced by mature collagen (22).

Corroborating the results of the published papers, it may be concluded that there is a variability of optimal doses between *in vivo* and *in vitro* studies, among different tendons in the same species and among the same tendons in various species. There is a clear dose-related effect of ESWT, both in terms of cell viability and ECM.

Neovascularization. It is widely accepted that neovascularization is important for healing, as it removes by-products and increases the oxygen content and the number of inflammatory and reparatory cells.

In vivo, in normal rabbit tendons, significantly increased neo-vessels were observed at 4 weeks after one session of 0.12 mJ/mm^2, 500 pulses, which persisted up to 12 weeks. Angiogenesis-related markers [endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor (VEGF) and proliferating cell nuclear antigen (PCNA)]

were significantly increased at one week. eNOS and VEGF displayed higher levels up to 8 weeks and decreased up to 12 weeks, whereas PCNA continued to have increased levels at 12 weeks (17). In normal dog Achilles tendon-bone junction, application of one session of 0.18 mJ/mm^2, 1,000 pulses, was followed by an increase of new capillaries at 4 weeks (17-fold) and 8 weeks (16-fold), most of them located adjacent to the peritendineum. New capillaries were also noted in the adjacent tendon tissues. Muscularized vessels, in a proportionate number to capillaries, were found at 4 weeks and persisted at 8 weeks, mostly in the peritendineum and the adjacent tendon. Both capillaries and muscularized vessels are indicative of neovascularization. No alteration of normal cancellous bone architecture was reported (23). By contrast, a study on normal insertional patellar tendon (rabbit) indicated no alteration in the number of capillaries at 6 weeks after one session of different ESWT regimens (1,000 or 2,000 pulses, 0.18, 0.27 or 0.36 mJ/mm^2) (19).

Achilles tendinopathy (rabbit) displayed new capillaries in the peritendinous tissue together with larger blood vessels, including differentiated arterioles and venules, at 4 weeks after 2-weekly sessions of 0.29 mJ/mm^2, 1,500 pulses (18). A similar result was reported for the tendinosis of superficial digital flexor tendon (horse) after 3 ESWT sessions, each one 3 weeks apart, with 0.14 mJ/mm^2, 1,500 pulses. At five weeks after treatment completion, there were significantly more capillaries in the treated tendons (20). In the study model of a cut Achillean tendon (rat), one session of 500 pulses at 0.19 mJ/mm^2 produced a significant increase in the number of capillaries after 20 days (13). In humans with grade III rotator cuff tendinopathy (Riley classification), one session of 4,000 pulses at 0.3 mJ/mm^2 increased the vascular volume area with evidence of nodular neo-angiogenesis in deeper tissue layers. For grade IV tendinopathy, evidence of new vessel formation was not significant, mostly due to the small number of cases (24).

In normal human cell cultures, ESWT induced a strong and significant release of TGF-β and VEGF, with a maximum at day 2 and persistence of higher levels at day 7. VEGF is stimulated by the release of certain cytokines (IL-6 and IL-10) and is an important promotor of neo-angiogenesis, contributing to the healing process (27).

Cellularity. In the regeneration process, the cornerstone becomes the activated tenocyte that behaves like a modified form of fibroblast or fibrocyte.

In the *in vivo* tendinopathic model, ESWT induced acceleration of the healing process, increasing the number of blast-like forms at 4 weeks. At 16 weeks, the healing process was complete, with mature tenocytes in a parallel array (18). Increased expression of PCNA, a protein associated with the S-phase of a dividing cell and a marker of tenocyte proliferation, was documented up to 12 weeks. The presence of immature forms, blast-like, recruited from the peritendon into the lesion, was evident at one week; at 4 weeks, the mature forms (spindle-shaped tenocytes) were arranged into tendon bundles (11).

Tenocyte metabolism is accelerated with intense production of growth factors (TGF-β1 and IGF-1) involved in ECM biosynthesis. Tenocytes displayed an increased number of nuclei and were actively producing ECM (20). Within 6 weeks after ESWT application, TGF-β1 and IGF-1 were significantly increased compared to the control, contributing to tendon healing (11).

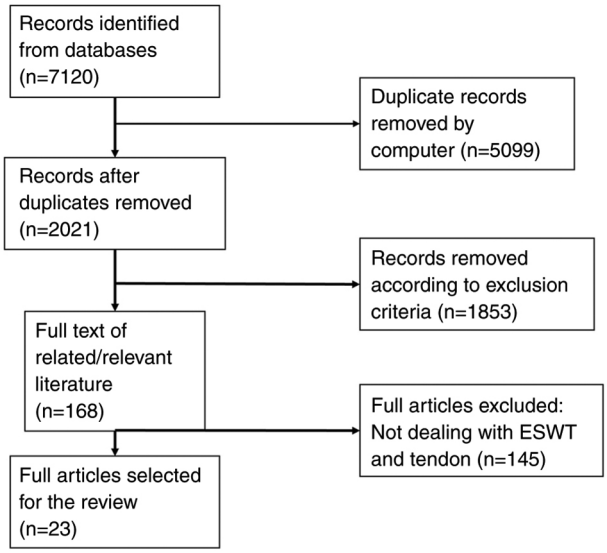

Figure 1. Flowchart of study selection. ESWT, extracorporeal shock wave therapy.

In vitro, in normal cell cultures (rats), ESWT at an optimal dose promoted tenocyte proliferation as indicated by upregulation of PCNA as early as 6 h. At 48 h, there was no more stimulatory effect. At 24 h, there was increased expression of TGF-β1 as a mark of increased ECM production and increased production of NO by fibroblasts. NO production was increased at 24 h by certain values of EFD (50 and 100 pulses and 0.36 mJ/mm^2), while a higher number of pulses (250 and 500) did not affect NO production (12). Normal human cell cultures did not display any alteration of NO levels after ESWT. Thus, the role of NO in promoting tendon healing remains to be fully elucidated (27).

One session of ESWT increased the proliferation of either healthy or ruptured tendon-derived human cultured cells, with a more prominent effect on the ruptured tendon-derived cells (a ratio of 1.75). ESWT induced a migratory phenotype in both types of culture, particularly evident for cells derived from ruptured tendons, triggering cell mobility (29).

Tissue regeneration requires maintenance of the cell phenotype. Phenotyping drift, a natural tendency in cultured rabbit and avian tenocytes, has also been demonstrated in human Achilles tendon cultures, as a tendency to shift from an elongated shape toward an ovoid shape. The phenomenon may be responsible for altered protein synthesis *in vivo*, increasing the ratio of type III:I collagen and inducing tendon pathologies (30). In normal human tendon cultures, one session of ESWT was proved to prevent the phenotyping drift, maintaining the normal tenocyte secretory profile of type I collagen (26).

The cellular compartment of the human tendon includes, besides tenocytes, a population of stem cells, human tendon-derived stem/progenitor cells (hTSPCs), characterized by several phenotypes and a potential for multilineage differentiation (osteoblasts, adipocytes, chondrocytes, tenocytes) (31,32). In cultures of both human normal and diseased

Table I. Studies on biological effects of ESWT on human and animal tendons.

Author, year	Studied tissue/ organism	ESWT doses, sessions	Study time-points	Optimal dose	Neoangiogenesis	Cellularity	ECM	Mechanical properties	(Refs.)
Rompe, 1998	42 rabbits, normal Achilles tendon, *in vivo*	One session, 1,000 pulses, 0.08, 0.28 and 0.60 mJ/mm^2	1, 7, 14 and 28 days after	0.08 and 0.28 mJ/mm^2 caused no damage; with 0.60 mJ/mm^2, fibrinoid necrosis, inflammation and reparative peritendinous reactions occurred	N/A	N/A	N/A	N/A	(6)
Orhan, 2001	48 rats, cut and sutured Achilles tendon, *in vivo*	One session 500 shocks, 0.12 mJ/mm^2	2 and 3 weeks	N/A	N/A	Week 2: Intense inflammatory reaction;week 3: Improved, organized healing	Increased hydroxyproline formation (days 3 and 9)	N/A	(10)
Wang, 2002	8 dogs, normal Achilles tendon-bone junction, *in vivo*	One session, 0.18 mJ/mm^2, 1,000 pulses	Before treatment and at 4 and 8 weeks after	N/A	New capillaries at 4 weeks (17-fold increase) and at 8 weeks (16-fold); muscularized vessels at 4 and 8 weeks	N/A	N/A	N/A	(23)
Maier, 2002	36 rabbits, normal quadriceps tendon, *in vivo*	1,500 pulses at 0.35, 0.5, 0.9 or 1.2 mJ/mm^2	10 and 28 days	0.35 mJ/mm^2: No histologic alteration at 10 days 0.5 and 0.9 mJ/mm^2: Minimal edema in the paratenon that disappeared at 28 days; 1.2 mJ/mm^2: Modified tendon structure at 10 days	N/A	N/A	N/A	N/A	(16)

Table I. Continued.

Author, year	Studied tissue/ organism	ESWT doses, sessions	Study time-points	Optimal dose	Neoangiogenesis	Cellularity	ECM	Mechanical properties	(Refs.)
Wang, 2003	50 rabbits, normal Achilles tendon, *in vivo*	One session 500 shocks, 0.12 mJ/mm^2	24 h; 1, 4, 8 and 12 weeks	N/A	Neovascularization increased at 4 weeks, persisting at 12 weeks (eNOS, VEGF, PCNA)	N/A	N/A	N/A	(17)
Chen, 2004	48 rats, collagenase-induced Achilles tendinosis, *in vivo*	One session, 200 shocks, 0.16 mJ/mm^2	1, 2, 4, 6 and 12 weeks after session	N/A	N/A	Tenocyte proliferation (PCNA); tenocyte stimulation (TGF-β1, IGF-1)	N/A	Restauration of mechanical load-to-failure and stiffness of healing tendons	(11)
Hsu, 2004	18 rabbits, Collagenase-induced patellar tendinopathy, *in vivo*	Two weekly sessions, 1,500 pulses, 0.29 mJ/mm^2	4 and 16 weeks after treatment	N/A	4 weeks: New capillaries in the peritendinous tissue, larger blood vessels, differentiated arterioles and venules	4 weeks: Tenocyte stimulation; 16 weeks: Increased healing process	Higher HP levels at weeks 4 and 16; higher pyridinoline levels (up to 10 times at 4 weeks)	4 and 16 weeks: Higher ultimate tensile load than control that increased from 4 to 16 weeks	(18)
Orhan, 2004	32 rats, normal Achilles tendon, *in vivo*	One session of 1,000 pulses at 0.15 mJ/mm^2, 1,500 pulses at 0.15 mJ/mm^2, 2,000 pulses at 0.20 mJ/mm^2	3 weeks	0.15 mJ/mm^2, at 1,000 and 1,500 pulses, no alteration of structure 0.20 mJ/mm^2 and 2,000 pulses: Alteration of tendon structure	N/A	N/A	N/A	N/A	(12)
Orhan, 2004	48 rats, ruptured Achilles tendon, *in vivo*	One session 500 pulses, 0.19 mJ/mm^2	20 days	N/A	Increased number of capillaries	N/A	Absent or minimal adhesions that did not distort the configuration of the tendon	The mean force required to rupture the tendon was higher	(13)

Table I. Continued.

Author, year	Studied tissue/ organism	ESWT doses, sessions	Study time-points	Optimal dose	Neoangiogenesis	Cellularity	ECM	Mechanical properties	(Refs.)
Kersh, 2006	6 horses, collagenase-induced tendinosis of superficial digital flexor tendon, *in vivo*	3 sessions, 1,500 shocks, 0.14 mJ/mm^2 (3 weeks interval between sessions)	5 weeks after completion	N/A	Neovascularization (significantly more capillaries); increased metachromasia within the intima and subintima of larger arteries	Increased number of fibroblasts (not significant); increase in the number of tenocyte nuclei; tenocytes were actively producing ECM	N/A	N/A	(20)
Bosch, 2007	6 ponies, normal superficial flexor tendon; normal common digital extensor tendon, *in vivo*	Focused session ESWT, 600 shocks, 0.14 mJ/mm^2	After 3 h and 6 weeks	N/A	N/A	At 3 h: Higher synthesis rate of GAG and collagen; at 6 weeks: Decreased GAG and total collagen	N/A	N/A	(21)
Chao, 2008	Rats, cultured Achilles tendon normal cells, *in vitro*	One session of focused ESWT 0.36 or 0.68 mJ/mm^2 50, 100, 250 or 500 pulses	24, 48 and 96 h	Optimal dose: 100 pulses at 0.36 mJ/mm^2 that maintained cell viability	N/A	Tenocyte proliferation (PCNA); increased tenocyte synthesis (TGF-β1, IGF-1)	N/A	N/A	(25)
Han, 2009	Human, cultured Achilles tendinopathy and normal FHL tendon, *in vitro*	One session 0.17 mJ/mm^2, 250, 500, 1,000 or 2,000 pulses	72 h	Optimal dose for cell viability: 500 pulses; optimal dose for cell proliferation: 500 pulses	N/A	N/A	Normal tenocytes: Increase of IL-1; diseased tenocytes: Decrease of MMP-1, MMP-13 and IL-6	N/A	(28)

Table I. Continued.

Author, year	Studied tissue/ organism	ESWT doses, sessions	Study time-points	Optimal dose	Neoangiogenesis	Cellularity	ECM	Mechanical properties	(Refs.)
Bosch, 2009	6 ponies, normal superficial digital flexor tendon; normal common digital extensor tendon, *in vivo*	Focused session ESWT, 600 shocks, 0.14 mJ/mm^2	After 3 h and 6 weeks	N/A	N/A	3 h: Tenocyte activation, structural disorganization	3 h: Increased collagen cleavage; 6 weeks: No collagen damage	N/A	(22)
Zhang, 2011	12 Rats, normal knee tendon, *in vivo*	2 different doses: 3,000 pulses of 0.15 or 0.4 mJ/mm^2	4 days	N/A	N/A	N/A	Increased lubricin in tendons, dose-dependent	N/A	(14)
Vetrano, 2011	Human, cultured normal semitendinosus cells, *in vitro*	One session 0.08, 0.14 and 0.17 mJ/mm^2, 500 and 1,000 pulses	1, 4, 8 and 12 days	None of the regimens affected viability of cells	N/A	None of the regimens affected viability of cells. 1,000 pulses, 0.14 mJ/mm^2 promoted tenocyte proliferation, prevented phenotype drift	Increased collagen synthesis, particularly type I	N/A	(26)
Penteado, 2011	30 rabbits, normal patellar tendon, tibial insertion, *in vivo*	One session, 6 different regimens (0.18, 0.27 or 0.36 mJ/mm^2, 1,000 and 2,000 pulses)	6 weeks	N/A	No difference in blood vessels	N/A	N/A	N/A	(19)
Leone, 2012	Human, cultured tendinopathic cells from Achilles tendon. Normal semitendinosus tendon, *in vitro*	One session, 0.14 mJ/mm^2, 1,000 pulses	1 and 4 days	N/A	N/A	ESWT induced no morphological variations (normal and diseased); ESWT induced proliferation of tenocytes (normal	ESWT reduced the increased concentrations of Col I and Scx in diseased cultures	N/A	(29)

Table I. Continued.

Author, year	Studied tissue/ organism	ESWT doses, sessions	Study time-points	Optimal dose	Neoangiogenesis	Cellularity	ECM	Mechanical properties	(Refs.)
						and diseased), more in diseased; ESWT induced cell migration in both cultures after trauma, more in diseased (wound repair)			
Branes, 2012	Human, 31 rotator cuff tendinopathy (10 treated and 21 controls), *in vivo*	One session, 0.3 mJ/mm^2, 4,000 pulses	After session	N/A	ESWT increased VVA, nodular neo-angiogenesis for grade III in deeper layers, no response for grade IV	N/A	N/A	N/A	(24)
Yoo, 2012	45 rats, collagenase-induced Achilles tendinopathy, *in vivo*	4 sessions, 1,000 pulses, 0.085 mJ/mm^2, days 5, 8, 12 and 15	Days 7, 12, 19, 26 and 33 after baseline	N/A	Day 33: No inflammatory cells or fibrotic tissue	Increase in fibrillary diameter and fibrillary adhesion force	N/A	N/A	(15)
de Girolamo, 2014	Human, cultured normal tendons, *in vitro*	One session, 1,000 pulses, 0.17 mJ/mm^2	Days 1, 2, 4 and 7 after treatment	Increased cell viability	N/A	N/A	Increased expression of Scx and type I collagen, increased IL-β1, IL-6 and IL-10	N/A	(27)
Waugh, 2015	Human, normal (n=19) and tendinopathic (n=10) tendons, *in vivo*	One session, 2,500 pulses, 0.064 mJ/mm^2	1, 2, 3 and 4 h	N/A	N/A	Elevated levels of IL-6 and IL-8 post ESWT remained higher at 4 h; elevated levels of pro-MMP-9, without active form increase	N/A	N/A	(34)

Table I. Continued.

Author, year	Studied tissue/ organism	ESWT doses, sessions	Study time-points	Optimal dose	Neoangiogenesis	Cellularity	ECM	Mechanical properties	(Refs.)
Leone, 2016	Human, cultured normal semitendinosus and ruptured Achilles, *in vitro*	One session, 1,000 pulses, 0.14 mJ/mm^2	21 days	N/A	Increased tenocyte proliferation, migration and collagen synthesis, more intense in ruptured tendons	N/A	N/A	N/A	(33)

eNOS, endothelial nitric oxide synthase; VEGF, vascular endothelial growth factor; PCNA, proliferating cell nuclear antigen; GAG, glycosaminoglycan; HP, hydroxyproline; VVA, vascular volume area; Scx, scleraxis; N/A, information not available; ESWT, extracorporeal shock wave therapy; ECM, extracellular matrix.

tendon cells, ESWT was able to enhance hTSPC differentiation toward a tenocytic-like lineage, visible at day 21 (33).

ECM. On normal tendons (ponies), ESWT application was followed by a rapid increase of degraded collagen at 3 h and a decrease at 6 weeks. The levels of glycosaminoglycans (GAG) at 3 h were unchanged and decreased at 6 weeks. However, no changes in hyaluronic acid, DNA and total collagen levels were noted either at 3 h or at 6 weeks. The results pointed to an anabolic response. The degraded collagen increase at 3 h may reflect an accelerated collagen turnover (slightly improbable for such a short duration) or direct physical damage. Overall, the anabolic response decreased at 6 weeks (21). On normal cultured human cells, ESWT increased the collagen content, predominantly type I (26).

Tendon regeneration is based on collagen synthesis, initially in the form of type III, followed by a maturation process and transformation in type I. Type I collagen appears like well organized, banded fibrils and provides high tensile strength. Type III collagen takes a woven pattern. Hydroxyproline is an aminoacid component of collagens accounting for ~13% and represents a reliable index for newly formed collagen. Pyridinoline is a crosslink residue of collagen and reflects its rate of degradation.

In the cut and sutured tendon model (rats), ESWT increased the hydroxyproline content, i.e., collagen synthesis, from 2- to 3-fold at days 3 and 9 compared to the control (10). The healing process under ESWT was followed by minimal or absent adhesions that did not disturb the configuration of the tendon.

In the model of collagenase-induced tendinopathy (rats), the optimal dose of ESWT reversed the increased concentrations of DNA, GAG and hydroxyproline to normal. Fiber bundles displayed a regular arrangement at 12 weeks. Higher doses elicited inhibitory effects on biochemical characteristics and an irregular array of fiber bundles (11). In the same model of tendinopathy (rabbits), the ESWT-treated tendons displayed higher levels of hydroxyproline and pyridinoline at 4 and 16 weeks, as an indication of intensive collagen synthesis and maturation (18).

At the nanostructural level, ESWT increased the collagen fibril diameter and the fibrillary adhesion force, accelerating healing (15).

In vitro, normal human cultured tissue displayed increased expression of scleraxis at 24 h, which decreased later, and an increased expression of type I collagen at day 4 that returned to normal levels at day 7 (27). Human tendinopathic cultures were characterized by higher gene expression of type I collagen and scleraxis, a possible indication of the repair process; ESWT induced a reduction of their levels toward normal. The process may be interpreted as an impairment effect of ESWT on cell differentiation in favor of cell proliferation and migration to ensure regeneration (29).

Metalloproteases and cytokines. Cultures from normal tendon subjected to one session of ESWT exhibited increased levels of specific interleukins (IL-β1, IL-6 and IL-10), with a maximum at day one for IL-β1 and IL-6 and at day 2 for IL-10, and persistence of higher values at day 7. In this context, IL-β1 induced IL-6 release, which, in turn, promoted IL-10 increase, an anti-inflammatory cytokine to limit the inflammation process (27).

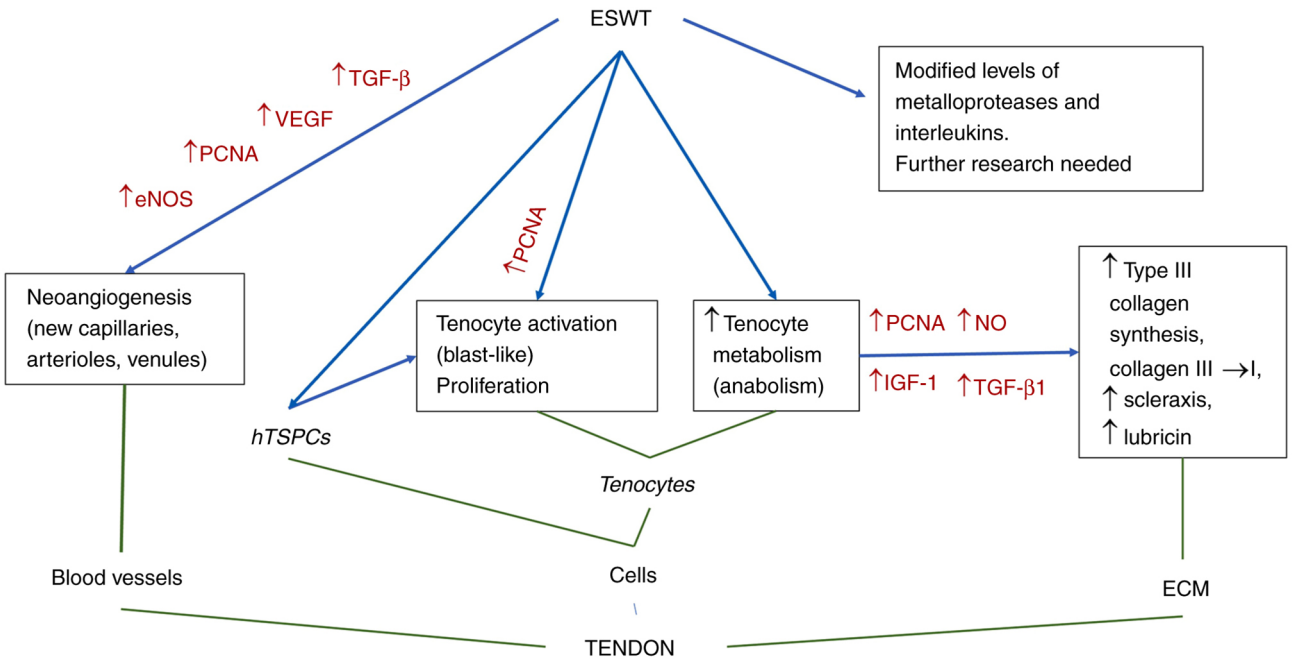

Figure 2. Main biological effects of ESWT application on tendon. ESWT, extracorporeal shock wave therapy; eNOS, endothelial nitric oxide synthase; PCNA, proliferating cell nuclear antigen; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor-β; NO, nitric oxide; IGF-1, insulin-like growth factor 1; ECM, extracellular matrix.

It was postulated that excreted MMPs and cytokines, particularly interleukins, from diseased tenocytes are breaking the ECM and induce tendon damage. Decreasing levels of these substances may reflect healing. Increased excretion of IL-6 and MMP-1, MMP-2 and MMP-13 was found in diseased cultured tenocytes; ESWT application reduced the higher levels of MMP-1, MMP-13 and IL-6 at 72 h, possibly contributing to a metabolic normalization. In normal cultured human tenocytes, only IL-1 levels were increased after ESWT, an event that necessitates further research (28).

Analysis of the microdialysate from the peritendinous space of human normal and tendinopathic patellar and Achilles tendons revealed that tendinopathy was associated with high levels of IL-6 and IL-8. ESWT application was followed by an increase of these two interleukins, in both normal and diseased tendons within the interval from 1 to 4 h. IL-β1 and IL-2 variations were not significant pre- and post-treatment. Pro-MMP-9, a latent form, increased after ESWT, without any change in the active form levels. The lack of significance of MMP activity changes raised the possibility of grouping individuals as responders and non-responders, with a proportion of responders of 60% in the general population (defined as exhibiting a minimum 5-fold increase in any MMP level at any point post-ESWT) (34).

The large spectrum of results of the two papers on IL and MMP levels deserves further investigation.

Lubricin production. Lubricin, a lubricating molecule, found in diarthrodial joints, is produced also by tenocytes enhancing the gliding of the tendons. ESWT increased lubricin expression and production in a dose-dependent manner in normal rat knee tendons and the effect was visible in the short term (4 days) (14).

Biomechanical properties of tissue after ESWT application. In the model of collagenase-induced tendinopathy, ESWT was followed by restoration of mechanical load-to-failure and stiffness of healing tendons, with a higher ultimate tensile load that increased from 4 to 16 weeks (11,18). The mean force required to rupture the tendon was higher 20 days after one session of ESWT (13).

ESWT, a method derived from urinary lithotripsy, represents a new therapeutic approach for tendon pathology, with encouraging results on pain and function. The biological mechanisms that sustain its clinical results have been extensively studied both *in vitro* and *in vivo*.

Research from the last 20 years indicated that there is a certain optimal dose for the maximum therapeutic effect for each studied species and for each studied tendon. Tendon- and species-specificity is a trait of the therapy. Energies that exceed the optimal value reduce cell viability and disorganize the ECM in a dose-dependent manner, with deleterious consequences on tendon structure.

Scientific papers agreed on several structural alterations that promote tendon healing. ESWT induces neovascularization in the tendinopathic tissue, with extensive capillary formation from the peritendinous structures. The inflammatory reaction that followed the collagenase-induced tendinopathy was accelerated by ESWT.

The therapy acts on both cellular and extracellular compartments of the tendon structure. Tenocyte proliferation and activation as blast-like forms with enhanced protein synthesis together with accelerated mobility reconstruct the normal structure. Accelerated collagen turnover, with type III collagen synthesis and subsequent type I collagen maturation, was documented in the ECM. As a result, biomechanical properties of the treated tendons improved significantly in comparison with non-treated tendinopathic structures. The mechanisms are outlined in Fig. 2.

An important clinical effect on plantar fasciitis, a structure that may be assimilated to a tendon, is worth mentioning.

Biological studies on plantar fascia are currently lacking; the analgesic effect of ESWT is comparable to that of autologous blood-derived products in the medium-term (6 months). Among the forms of ESWT, the success rate is higher with medium- and high-intensity ESWT (35,36).

Future research will focus on the role of cytokines and metalloproteases that mediate, at a cellular level, the degenerative process and its evolution under ESWT application.

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Competing interests

The authors declare that they have no competing interests.

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