A review of the cellular and molecular effects of extracorporeal shockwave therapy

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Summary
Extracorporeal shockwave therapy (ESWT) is a novel therapeutic modality and its use in promoting connective tissue repair and analgesic effect has been advocated in the literature. It is convenient, cost-effective, and has negligible complications; it therefore bypasses many of the problems associated with surgical interventions. This paper reviews the proposed mechanisms of action in promoting tissue repair and regeneration as well as analysing its efficacy providing an analgesic effect in clinical applications. Further research will be required to not only identify the underlying mechanisms more precisely, but will also be critical for ensuring consistency across the literature so that the most beneficial treatment protocol can be developed. Extracorporeal shockwave therapy stands as a promising alternative modality in promoting tissue repair.

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Introduction

Extracorporeal shockwave therapy (ESWT) is an acoustic pressure disturbance that can be converted into mechanical energy (1, 2). It was originally used in lithotripsy in the 1980s whereby high-energy shockwaves were used to mechanically break up kidney stones in the urinary system (3). An observed side effect of the lithotripsy treatment was increased bone mineral density in the treatment area which subsequently led to its application in orthopaedics where it was demonstrated that ESWT increased osteogenic activity to enhance fracture healing (4). Extracorporeal shockwave therapy seems a promising therapy for musculoskeletal disorders in both human and veterinary medicine as it is non-invasive and it bypasses many of the risks and costs associated with surgical intervention (2). As a result, research into the mechanisms and effects of ESWT has advanced rapidly and it has been increasingly used to treat bone injuries and other soft tissue disorders of the musculoskeletal system in humans and animals.

Extracorporeal shockwave therapy is emerging as a popular choice of treatment in human medicine and its increasing acceptance in Europe, South America, Asia and North America demonstrates its potential within the orthopaedic field (2). The U.S. Food and Drug Administration approved the first ESWT device for use in human patients as a safe treatment modality for plantar fasciitis in 2000 and for lateral epicondylitis in 2002. Extracorporeal shockwave therapy has shown beneficial therapeutic effects in treating plantar fibromatosis, calcific tendinitis of the shoulder, and lateral epicondylitis of the elbow (5–16). Functional restoration was achieved in 88% of subjects suffering from calcific tendinitis of the shoulder and in 90% of those with lateral epicondylitis of the elbow, demonstrating that ESWT is a useful alternative to surgical intervention (15, 17). In contrast, Wilner and Strash showed that ESWT had little or no benefit in improving both pain and function for lateral elbow pain, highlighting the controversy surrounding the use of ESWT and that further research is required (18).

It has more recently been used to treat chronic Achilles tendinopathies and patellar tendinopathies (11, 19-26). Patellar tendinopathies improved in both pain and function and produced superior results when compared to surgical intervention (22, 24–26). Extracorporeal shockwave therapy has been used as a non-invasive option to treat avascular necrosis of the femoral head and non-union or delayed bony union (27–35). Angiogenesis and bone remodelling were demonstrated after ESWT for avascular necrosis of the femoral head along with significant improvements in hip function although the long-term effects are yet to be established (28–32).

Reports in the clinical veterinary literature suggest that ESWT is a beneficial treatment for a variety of diseases, although results are equivocal and may depend on the condition. In early studies, it was used successfully to treat osteoarthritis of the hip in dogs but outcomes were less clear in dogs with longstanding stifle joint arthritis (36, 37). It has been shown to be beneficial

in the treatment of suspensory desmitis in horses (38, 39). Additionally, a recent pilot study demonstrated that ESWT improved cartilage and subchondral bone quality in a rabbit osteochondrosis dissecans model (40). After a collagenase-induced injury to the suspensory ligament in horses, ESWT resulted in a significantly faster healing rate and smaller lesions after three treatments over nine weeks (41). More recently, dogs having tibial plateau levelling osteotomy surgery were administered focused ESWT or sham treatment and ESWT was found to be associated with better radiographic healing scores at four weeks, although the differences between treatment groups were non-significant at eight weeks (42). Likewise, after tibial tuberosity advancement surgery, ESWT as an augmentative treatment with autogenous bone grafts improved osteotomy gap density at four weeks, but the improvement was insignificant at eight weeks in comparison to grafts alone, ESWT alone, or no augmentative therapy (43). In contrast, kinetic gait analysis identified a 10% increase in vertical force measurements, alongside improvements in pain and subjective gait score after radial ESWT to one limb with osteoarthritis of the hip, in comparison to the control limb which did not change (44).

Extracorporeal shockwave therapy is known to have a number of mechanisms of action on connective and other tissues. Nitric oxide (NO) is a free radical that acts as a second messenger in many pathways and plays an important role in healing (45). Shockwave stimulates an anti-inflammatory response, possibly by enhancing either non-enzymatic or enzymatic production of NO, thereby keeping local NO concentration at physiological levels in the early stages of inflammation (46). Bone morphogenetic proteins (BMP) induce cartilage and bone formation, and ESWT increases BMP expression (47–50). Extracorporeal shockwave therapy significantly increases transforming growth factor β1 (TGF-β1) production in newly formed bony union as well as promoting bone marrow osteoprogenitor growth (45, 48, 51). This response promotes bone healing as differentiation into osteoblasts occurs with subsequent osteoid production. Osteoblasts produce alkaline phosphatase, an enzyme that assists in bone mineralisation, and increased alkaline phosphatase activity has been found post ESWT treatment suggesting enhanced osteoblastic activity and thus an increase in new bone formation (49–52). Extracorporeal shockwave therapy significantly increases the release of vascular endothelial growth factor (VEGF) promoting the formation of neo-vessels and angiogenic factors (32, 49, 53). This is associated with a significant increase in bone strength and bony union formation and enhanced angiogenesis and bone remodelling have been observed, improving function (48, 53, 54).

Extracorporeal shockwave therapy as an augmentative treatment

Mechanotransduction

The transient pressure disturbances of shockwaves release pulsed energy and pressure which act mechanically upon the bone via mechanotransduction. Although the mechanisms of action of ESWT remain unknown, it is possible that ESWT and mechanical loading induce an osteogenic effect via similar mechanisms. A recent review proposed that there is no unique pathway in which bone responds to mechanical loading, which may also be true for ESWT (55). Mechanical loads generate tensile and compressive forces, causing fluid flow within the canaliculi of cortical bone (56). Osteocytes housed within the lacunar-canalicular system are exposed to this fluid movement, which is thought to stimulate stretch-activated cation nonselective channels (57). Activation of such channels can stimulate other osteocytes and osteoblasts via gap junctions to induce multiple cellular responses via growth factor release, in that there was a direct increase in the production of TGF-β1 from human osteoblast-like cells (56, 57). Furthermore, inhibition of these channels significantly inhibited TGF-β1 mRNA levels, which is an important mediator of ESWT-induced bone formation (57). Although this experiment was performed *in vitro*, it provides a possible explanation into how mechanotransduction can stimulate growth factor release, which can subsequently promote osteogenesis, as discussed later. In addition, mechanically-stimulated membrane hyperpolarization can occur via small calcium-activated potassium channels and it has been shown that ESWT can also induce membrane hyperpolarization, lending support to the idea that ESWT and mechanical stimulation act via similar mechanisms on bone (58, 59). This also suggests that multiple channels may be involved in not only transducing the effects of ESWT but also in growth factor release.

Animal models help to elucidate the mechanisms of ESWT. Pertussis toxin inhibits protein interaction with G-protein coupled receptors on the cell membrane (60). Pertussis toxin injection into the segmental femoral defects of rats combined with ESWT significantly reduced ESWT-promoted TGF-β1 and BMP-2 production, callus formation and fracture gap healing, which subsequently limited ESWT-induced bone repair, suggesting that osteogenic growth factors are critical in mediating ESWT-induced osteogenesis. It is also suggested that membrane perturbation can initiate G-protein responses regardless of growth factor binding, which can then stimulate signalling pathways that promote bone formation.

Extracellular matrix components such as collagen I can bind to integrins such as α2β1 found on the surface of osteoblasts, which can regulate specific pathways essential for osteoblast differentiation (61). Sun and colleagues used cultured bone marrow-derived human mesenchymal stem cells to demonstrate that ESWT promotes bone healing by releasing significant amounts of adenosine triphosphate (62). This ATP release activated P2X7 receptors which initiated downstream signalling via p38 kinase, driving hMSCs into the osteoblastic lineage. Evidence strongly suggests that the mechanotransduction of ESWT occurs in a similar manner to that of mechanical loading, however further exploration is critically needed to understand better the complexities of the mechanisms of ESWT.

Mitogen-activated protein kinase pathways

A large body of literature indicates that mechanotransduction by ESWT stimulates

signalling molecules that activate cytoplasmic protein kinases, collectively recognized as the MAPK cascade (63). This highly complex network transmits and amplifies ESWT-induced osteogenic growth factor signals from the cell membrane to intracellular targets within the nucleus, and this regulates numerous cellular responses, namely osteogenic proliferation and differentiation of mesenchymal stem cells (64, 65).

Ras is a monomeric G-protein that transduces extracellular stimuli into signalling pathways initiating a variety of biological responses (66). Receptor tyrosine kinases (RTK) are major cell surface receptors activated through ligand binding, such as by growth factors including fibroblast growth factor (FGF). Binding initiates autophosphorylation and the subsequent interaction of two proteins, GRB2 and Sos which activate Ras (67). The ESWT-induced membrane hyperpolarization is associated with Ras activation suggesting that hyperpolarization mediates growth factor release, which in turn activates RTK, downstream Ras and the MAPK cascade. Receptor tyrosine kinases inhibition suppressed extracellular signal-related kinase (ERK) activation (51, 59). Furthermore, cells containing Ras in the active state can initiate further downstream signalling without ligand binding to RTK (67). This also suggests that ESWT-induced hyperpolarization may alter the state of Ras independent of RTK binding.

These results suggest that ERK and p38 kinase mediate downstream signalling, which promotes bone formation, in that increased alkaline phosphatase activity indicates osteoblastic activity, collagen I increases demonstrate increased bone matrix (osteoid) secretion, and increased osteocalcin demonstrates osteoid mineralization and therefore bone formation. In addition, ESWT-induced mesenchymal cell aggregation, hypertrophic cartilage, and osteoblasts in particular expressed phosphorylated ERK (63). Wang and colleagues demonstrated that ERK stimulates the growth, maturation, and differentiation of mesenchymal progenitor cells into osteoblasts (51). In contrast, inhibiting ERK activation inhibits osteogenic differentiation highlighting the importance of ERK in driving mesenchymal stem cells into osteogenic lineages and its role in ESWT-induced fracture repair (65). These findings suggest that inhibiting a component of the MAPK pathway affects subsequent downstream signalling and disrupts osteogenesis. This highlights that specific and coordinated signalling is essential for successful bone repair, which ESWT appears to promote.

Runt-related transcription factor 2 (RUNX2), formerly known as Cbfa1, is an essential osteogenic transcription factor that plays a vital role in determining the osteoblastic lineage while regulating osteogenic cell growth and maturation (51, 68). A RUNX2 deficiency prevents MSC differentiation into osteoblastic phenotypes, which subsequently inhibits bone mineralization, illustrating its fundamental role in osteogenesis (69). Although RUNX2 is predominantly considered as a bone marker, studies have demonstrated RUNX2 expression in chondrocyte progenitors and that it plays an important role in promoting both chondrogenesis and chondrocyte maturation, essential for endochondral bone repair (68, 70). Runt-related transcription factor 2 also regulates osteocalcin mRNA expression in that reduced RUNX2 activation also reduced osteocalcin expression (51, 71).

It has been shown that ESWT increases RUNX2 expression, and stimulates the MAPK cascade as discussed above (49, 50). The MAPK pathways also appear to play an important role in mediating RUNX2 activity (61). These findings suggest a link between RUNX2 and the MAPK cascade in that ESWT could indirectly promote RUNX2 activity via downstream signalling of the MAPK cascade, however further exploration of this hypothesis is needed.

Extracorporeal shockwave therapy stimulates Ras-induced production of the free radical superoxide, which critically mediates the downstream effects of Ras in regulating cytosolic ERK and the subsequent co-ordination of the MAPK pathway (51, 72). Inhibiting Ras abrogates superoxide synthesis and inhibiting superoxide reduces ERK activation and subsequent osteoprogenitor growth highlighting the importance of superoxide in mediating the early stages of ESWT-induced osteogenesis (51, 72). These studies show that the MAPK cascade plays a critical role in providing specificity and amplification of osteogenic signalling and that the dysregulation of components in the cascade can alter essential cellular processes.

Vascular endothelial growth factor

Evidence suggests that angiogenesis considerably enhances the repair process. It has been widely advocated in the literature that ESWT can significantly increase the production of angiogenic factor VEGF (49, 54, 73).

Runt-related transcription factor 2 is required for vascular invasion into the soft cartilagenous callus during fracture repair in that RUNX2 deficiency in osteogenic cells prevents VEGF expression and vascular invasion (68, 70, 74, 75). The ESWT-induced MAPK cascade regulates hypoxia inducible factor 1-alpha (HIF-1α), which subsequently binds to VEGF promoter and increases VEGF-A mRNA expression (72, 76). It has been shown that RUNX2 and HIF-1α interact together to mediate angiogenesis however VEGF can still be induced via the HIF-1α response in RUNX2-null cells (75).

This illustrates both the importance and the complex interactions of RUNX2, VEGF, HIF-1α and VEGF in mediating angiogenesis. However, it also highlights the need for further work to elucidate how ESWT modulates these molecules to promote angiogenesis and subsequently influence the healing process.

Vascular endothelial growth factor can promote endothelial cell sprouting in a paracrine manner resulting in the formation of new blood vessels (76). It is suggested that improved blood supply plays a role in inflammation and is associated with the influx of growth factors which mediate osteogenesis. Extracorporeal shockwave therapy-induced bone healing is abrogated following VEGF-inhibition, suggesting that VEGF possesses a dual role in mediating both angiogenesis and osteogenesis (73). It appears that VEGF mediates osteoblastic activity and therefore contributes to osteogenesis, although the functional role of VEGF is highly complex.

Vascular endothelial growth factor-A is a particular focus in the literature regarding fracture repair. Vascular endothelial growth factor-A demonstrated chemotactic and proliferative effects on primary human osteoblasts (PHO) in a dose-dependent manner, and it can also directly promote the differentiation of primary osteoblasts *in vitro* (77, 78). Vascular endothelial growth factor-A also enhances fracture healing by promoting osteoid production and bone formation (76). This is supported by the increased bone mineralization associated with the increased expression of VEGF-A mRNA (76, 79). In addition, VEGF inhibition decreased nodule formation and alkaline phosphatase production in primary human osteoblasts (PHOs) (78). From the studies discussed, it is possible that VEGF may be one of the potential pathways through which ESWT modulates both angiogenesis and the essential processes of osteogenesis to promote healing, but further work is required to confirm this.

Extracorporeal shockwave therapy-induced BMP production (potent osteoinductive proteins) uniquely interacts with VEGF. Vascular endothelial growth factor inhibition significantly limited BMP-2 induced bone formation and although VEGF alone did not augment bone regeneration, when coupled with BMP-4, bone formation was significantly enhanced (80, 81). In contrast, despite VEGF-A inhibition, BMP-induced osteoblast differentiation and mineralization still occurred, however the BMP-induced angiogenic response was attenuated (82). This suggests that BMP-induced osteoblastic production of VEGF-A is important in coupling angiogenesis to bone formation, but is not required for BMP-induced osteoblast differentiation. Furthermore, BMP-2 neutralization had no effect on VEGF-A expression, however such findings may be limited by the study period suggesting that interactions may not have occurred within the 24 hour time-frame (72).

Transforming growth factor beta superfamily

The TGF-β superfamily consists of various proteins and growth factors. Both TGF-β and BMP bind and activate transmembrane serine/threonine kinase receptors that initiate intracellular signalling via phosphorylation of Smad signalling proteins and induce direct transcriptional responses within the cell nucleus (83, 84). It is not entirely clear how TGF-β and BMP mediate such diverse effects by signalling through the same Smad-protein pathway. However, increasing evidence suggests that considerable cross-talk and interaction occurs between Smad-dependent and Smad-independent pathways, namely with p38 kinase and the MAPK cascade (47, 84, 85). This is further supported in that p38 kinase and ERK appeared to mediate TGF-β and BMP-2 signals in osteoblasts demonstrating that highly complex interconnecting pathways are involved in mediating the effects of these osteogenic factors (86). A recent study showed that ESWT applied to human adipose-derived stem cells induced ERK and Smad phosphorylation, whilst increasing BMP-2, RUNX2 and ALP expression, resulting in differentiation into osteoblast-like cells (50). Although this study is limited in that it was conducted on human adipose-derived stem cells, it does lend support to the involvement of Smad proteins and the MAPK cascade and moreover their importance in mediating other signals downstream, such as BMP-2, to effectively drive the osteoblastic lineage and induce bone formation.

It is suggested that these pathways (Smad, p38 kinase and MAPK) converge at the RUNX2 gene, and thus promote mesenchymal precursor cell differentiation into osteoblasts (50, 84, 86, 87). However, Nishimura et al. demonstrated that after inhibiting Smad1 and Smad5 interactions with RUNX2, osteogenic processes and subsequent osteocalcin activity were still induced, suggesting that Smad interactions are not critical for RUNX2 action, but can enhance RUNX2 activity (88). It was also demonstrated that dominant-negative RUNX2 appeared to inhibit BMP-2 induced osteoblastic differentiation, suggesting that RUNX2 is essential for mediating BMP signalling. Given the association between ESWT and BMP expression, also discussed in the next section, it is reasonable to postulate that ESWT acts at least in part through effects on RUNX2 signalling to promote osteogenesis and subsequently enhance fracture repair.

Transforming growth factor-β is actively involved in recruiting osteoblast precursors and in driving differentiation towards an osteoblastic lineage (84, 89). Furthermore, TGF-β stimulates the proliferation of MSC, osteoprogenitor cells, chondrocytes and is also involved in extracellular matrix production. Wang et al. demonstrated that EWST applied to rat femoral defects promoted colony-forming-unit-osteoprogenitor (CFU-O) which was associated with increased production of TGF-β1 (52). Colony-forming-unit-osteoprogenitor produced measurable increases in ALP activity suggesting that osteogenic differentiation and osteoblastic activity was occurring, further indicated by the formation of bone nodules. Extracorporeal shockwave therapy-promoted TGF-β1 activity is further supported by Wang et al. and associated with subsequent increases in ALP activity and bone nodule formation. These findings all suggest that ESWT-induced TGF-β1 production mediates osteoprogenitor growth and differentiation (51).

►Figure 1 proposes the potential mechanisms of action of ESWT on bone, which includes the possible downstream signalling pathways that have been discussed. Further research is required to confirm these mechanisms.

Bone morphogenetic proteins

It is commonly accepted that BMP are potent stimulators of osteogenic activity and mediate essential processes of bone regeneration such as MSC differentiation into osteogenic lineage, proliferation and osteoblastic maturation, therefore greatly promoting osteogenesis (47, 90, 91). Bone morphogenetic protein-2 to BMP-7 are members of the TGF-β superfamily and play unique functional roles in orchestrating morphogenetic signals to induce bone formation. Bone morphogenetic proteins increase ALP activity and osteocalcin expression, demonstrating osteoblastic activity and bone matrix mineralization and illustrate the roles of BMP in actively promoting bone formation and enhancing fracture repair (91, 92). This is supported by a recent study on bone marrow stromal

cells in that ESWT significantly increased BMP-2 levels, ALP activity and osteocalcin mRNA expression, associated with an increase in mature mineralized bone nodules compared to the control group (49). In mice lacking BMP-2, the initial stages of fracture repair are inhibited despite the presence of other osteogenic stimuli and an *in vitro* study demonstrated that cells deficient in the BMP-2 receptor could not differentiate into osteoblasts (91, 93). This suggests that not only is BMP-2 critical in driving the differentiation towards the osteoblastic lineage, but that it appears to be the most potent osteogenic stimulus governing fracture repair.

Extracorporeal shockwave therapy significantly increased BMP-2, BMP-3, BMP-4 and BMP-7 mRNA expression in segmental femoral defects in rats (94). Within one week, post-ESWT, BMP-2, BMP-3 and BMP-4 were expressed within aggregating mesenchymal stem cells and immature chondrocytes. This illustrates not only the osteoinductive capacity of BMP, but their importance in mediating chondrogenesis. Within four weeks post-ESWT, there was greatest expression of BMP-2, further supporting that it is the most potent BMP. Expression of BMP-3 and BMP-4 appeared to be greatest in the later stages of the healing process with BMP-7 expression most prominent in the final stages of remodelling. This demonstrates that the temporal expression of BMP depends on the stage of cell morphology, highlighting the different functional roles of BMP, and their importance in ESWT-induced bone regeneration (95). Further studies support that ESWT measurably increases BMP expression, particularly BMP-2 (50, 53, 73).

Bone morphogenetic protein-3 appears to inhibit chondrocyte differentiation, suggesting that BMP-3 would be down-regulated during bone regeneration to enable soft callus formation (96). Bone morphogenetic protein-3 also appears to antagonize other BMP in that BMP-2-mediated differentiation into osteoblasts was inhibited (97). Bone morphogenetic protein-3 knockout mice demonstrated an increase in trabecular bone compared to controls suggesting that BMP-3 negatively regulates osteogenesis. These studies conflict with other findings in that ESWT increased BMP-3 expression, highlighting the need for further research in order to achieve a better understanding of the functional roles of BMP (94).

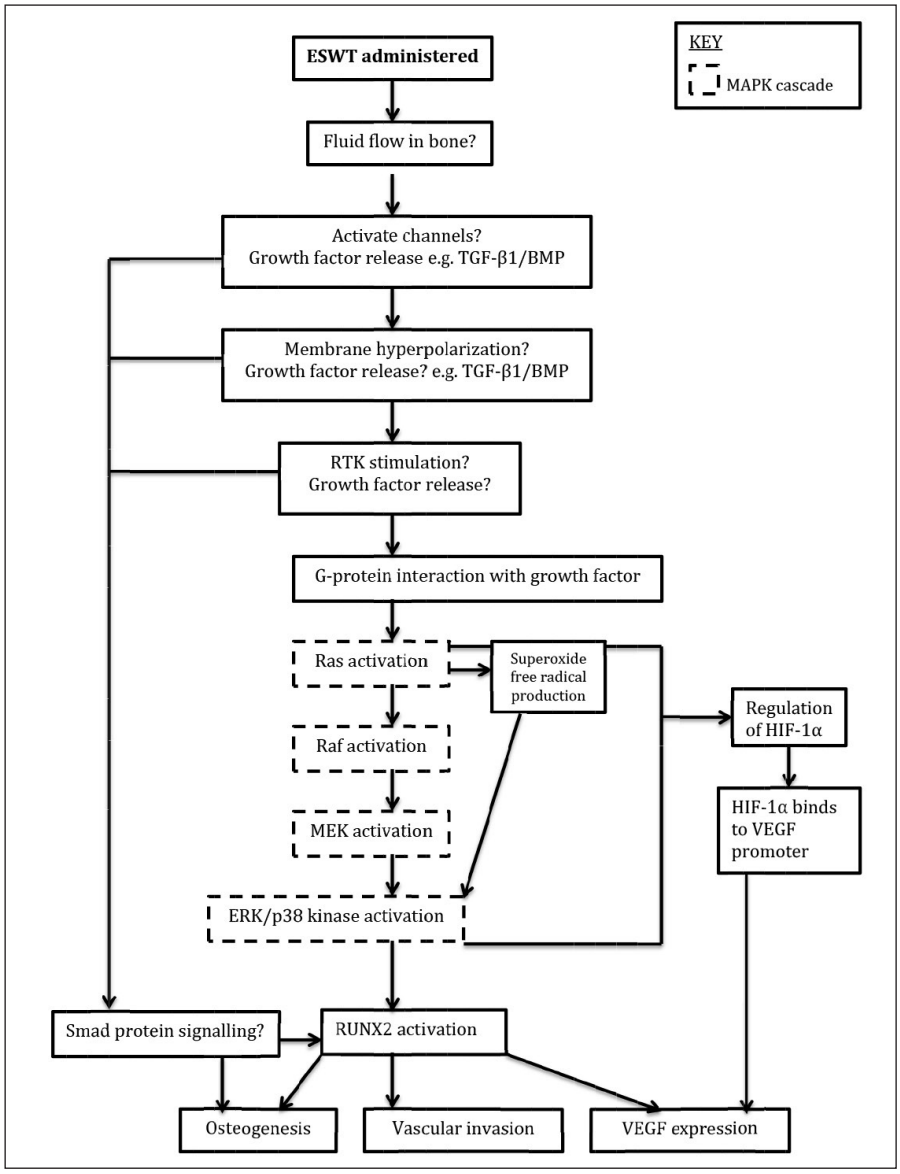

Figure 1 Proposed mechanisms of action of extracorporeal shockwave therapy.

Fibroblast growth factor and prostaglandin E2

Fibroblast growth factor (FGF) is expressed in mesenchymal stem cells, maturing osteoblasts and chondrocytes and is thought to play a role in promoting TGF-β expression in both osteoblasts and chondrocytes (90). Extracorporeal shockwave therapy stimulates significant increases in FGF-2 levels, associated with a time-dependent increase in TGF-β1 in human fibroblasts and osteoblasts (98). Although TGF-β1 increases were not significantly different from the control group, the effect of ESWT was markedly higher in osteoblasts than in chondrocytes, with almost a 10-fold higher increase in FGF-2 concentration, suggesting that osteoblasts act as the target cells for ESWT. Fibroblast growth factor–2 has a potent mitogenic role in promoting MSC proliferation and differentiation along with chondrocyte proliferation (68, 99). In addition, local

rhbFGF application to defective bone demonstrates increases in callus volume and bone mineral content (100). These findings illustrate the importance of FGF in bone regeneration subsequently in fracture healing. Fibroblast growth factor can bind to RTK and activate Ras and subsequent MAPK signalling. This is further supported in that FGF-2 increased ERK1/2 phosphorylation, which resulted in RUNX2 phosphorylation and subsequent osteocalcin promoter activity (101). The ERK1/2 inhibitors blocked FGF-2 action and its resultant downstream signalling pathways demonstrating that FGF-2 plays an important role in regulating both RUNX2 and bone formation.

When ESWT was applied to primary human osteoblasts, from normal human cancellous bone, it induced proliferation and differentiation of osteoblasts and upregulation of genes involved in osteoblast differentiation, namely prostaglandin E2-receptor EP3 (PGE2-EP3) and BMP-2 inducible kinase (102). Prostaglandin E2 has briefly been mentioned previously. It is primarily produced by osteoblasts, yet it activates osteoblasts, playing a crucial role in regulating bone formation and metabolism (103, 104). It has also been shown to have anabolic effects on bone *in vivo* (105). This highlights not only the importance of PGE2 in promoting bone formation, but that ESWT can aid to induce such an effect. This is illustrated by the significantly higher mineralization found in the ESWT group compared to the control group, helping to show further the proposed osteogenic effects of ESWT (102). Furthermore, this study lends support to the importance of BMP-2 and its upregulation post-ESWT, as shown by numerous other studies as discussed.

Other mechanisms of action

A secondary, analgesic effect of ESWT has been demonstrated. Recent studies, primarily in equine subjects, have reported an acute transient period of analgesia post-ESWT with immediate effect and lasting for two to four days (106–109). Such effects have been reflected by transient functional improvements in lameness with an increased walking ability and have been evaluated using different methods such as measuring ground reaction forces with force platforms (109). One equine study used a constant-voltage, variable-current pulse stimulator to determine the nociceptive threshold of the horse when the subject first responded and demonstrated a small cutaneous analgesic effect lasting for three days post-ESWT (108).

There is little focus of this temporary ESWT-induced analgesic effect within the literature and the mechanisms of this are currently unknown. However, some studies that were primarily conducted on rats, and therefore demonstrate that this analgesic effect occurs in other species, provide insight into possible neurophysiological mechanisms underlying this transient period of analgesia following ESWT. Extracorporeal shockwave therapy reduces immunoreactive calcitonin-gene related peptide (CGRP) in dorsal root ganglion (DRG) neurons from 61% to 18% in a rat study (110-112). Maximal inhibition of CGRP expression was seen at four days which was associated with improved walking ability and as CGRP plays a role in pain transmission this suggests that ESWT may stimulate an analgesic effect through neuropeptide depletion (110–113). Studies have demonstrated rapid degeneration of nerve fibres post-ESWT along with significant reductions in sensory nerve conduction velocities compared to controls (110, 114-117). This suggests that ESWT-induced peripheral nerve modulation could contribute to analgesia. Multiple applications of ESWT have been shown to cause slower re-innervation of nerve fibres and increased neuronal injury with increasing dosage of ESWT (114, 118). These findings suggest a dose-dependent analgesic effect associated with ESWT. Biological markers for axonal regeneration indicate that complete re-innervation of disrupted nerve fibres can occur after ESWT (111, 114, 115). This suggests that desensitization of the treated area by ESWT would occur, clinically recognized by reduced lameness, whereas subsequent re-innervation which suggests sensitivity of the area would increase, providing a possible explanation for the reported transient analgesic effect of ESWT (107–110, 115).

In contrast, conflicting studies have demonstrated no modulatory effect of ESWT on neuronal activity highlighting the equivocal evidence regarding the mechanisms of this analgesic effect (119–121). In addition, the acute period of analgesia post-ESWT, reflected by improvements in gait peaked at two days, suggesting that the analgesic effect demonstrated would not be due to nerve fibre destruction as this effect would be expected to be immediate (109). Furthermore, although other data that supports this proposed temporary analgesia where functional improvements decreased after 72 hours, the authors did not attribute such findings to the effect of ESWT according to skin sensitivity tests and thermographic imaging (122). The direct comparison of the analgesic effects of ESWT across studies is limited in that the responses of 'clinically sound' animals may vary from animals suffering from conditions such as insertional desmopathies and defective osseous structures causing lameness (76, 92, 106–109, 123). Furthermore, large inconsistencies across studies such as the dosage of ESWT applied and the methods used to evaluate analgesia further hinder elucidating this effect. Due to the paucity of knowledge and discrepancies of this ESW-induced analgesia across the literature, further exploration of this phenomenon is indicated.

Conclusion

There is a large body of literature proposing to define the mechanisms by which ESWT stimulates connective tissue repair and this review has attempted to summarize these mechanisms. This review focused on what appears to be the key players in promoting repair, namely RUNX2, BMP, VEGF and the MAPK cascade. Understanding the exact roles of these factors is critical in not only clarifying how ESWT works, but also gaining a firmer grasp of how repair may become impaired initially. However, this review also highlights the complexities in trying to elucidate the effect of ESWT on a cellular and molecular level, demonstrating that considerable research is still required to gain a further understanding of these pro-

cesses. Inconsistencies of method and dosage are largely responsible for variations in the results of treatment with ESWT that are reported in the literature. The clinical studies are largely uncontrolled. Considerably more research is required to determine a treatment protocol based on knowledge of the cellular mechanisms purported to underlie its effect, and this is fundamental to achieving consistency of approach both within and between studies. Not only will this facilitate data comparison between studies, it will establish an appropriate, evidence-based treatment strategy. Furthermore, the conflicting and inconsistent findings surrounding the phenomenon of ESWT-induced analgesia highlights that further research is required in this area. Could this analgesic effect play an influential role in accelerating return to function, enabling increased loading which in turn could enhance tissue repair? This postulation needs exploring and could help to elucidate the mechanisms underlying the clinical effects of ESWT.

Despite these unknowns and inconsistencies, the use of ESWT in promoting bone healing has been advocated in the literature with increasing clinical success, supporting the use of ESWT as an effective alternative modality in soft and hard tissue repair, and as an analgesic.

Conflict of interest

The authors do not have any conflicts of interest to declare.

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