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Review Article

Cracking the code: Understanding ESWT's role in bone fracture healing

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ABSTRACT

Bone non-union has always been a research hotspot in the field of orthopedics. Non-unions are often accompanied by symptoms such as pain, deformity, and dysfunction, which can significantly affect patients' quality of life and cause related socioeconomic problems. Clinically, there are various treatments available for non-unions, and the main treatment methods are divided into surgical and non-surgical treatments. At present, surgery is the most widely used treatment for bone non-unions and has a high healing rate. However, even after surgery, some patients still face the problem of bone non-union. Furthermore, a small number of patients have surgical contraindications and could not tolerate surgery. Therefore, alternative treatments are needed to improve outcomes for patients with bone fractures. Extracorporeal shock wave therapy (ESWT) is a non-invasive treatment method with similar efficacy and better safety compared with surgery. Nevertheless, the exact mechanism for ESWT to treat patients with bone non-union are still not well understood. This article reviews the mechanisms of ESWT in promoting bone fracture healing by regulating osteoblasts and osteoclasts, providing a theoretical foundation for the clinical application of ESWT.

The Translational Potential of this Article: This review provides a comprehensive overview of the mechanisms underlying ESWT on promoting bone fracture healing by regulating osteoblasts and osteoclasts. The information provided in this article can offer a novel non-invasive method for clinicians to treat bone non-union.

1. Introduction

Bone fracture is the most prevalent large organ traumatic injury to human body, and the repairment of bone fracture is a regenerative process [1,2]. Among the bone fracture patients, approximate 5–10 % of patients suffer from bone non-union [3,4]. Bone non-unions refers to fractures that fail to heal within nine months, and there is no progress in bone healing for three consecutive months [5]. Patients with non-unions undergo pain and dysfunction limitations, decreasing the patient's activities and impairing quality of life [6,7]. Most of non-unions are managed successfully through surgical treatment which is "golden standard" with healing rates from 74 % to 95 % [8–11]. Due to individual factors, some patients cannot achieve good therapeutic effects after surgery. Moreover, a portion of patients who have contraindications of surgery cannot undergo surgical intervention. Therefore, there is an urgent need for effective non-surgical treatments that can achieve satisfactory results without serious complications.

Until now, there are various conservative treatment methods for non-union fractures, such as extracorporeal shock wave therapy (ESWT), ion resonance, and ultrasound, which have been widely used in clinical treatment. For example, ion resonance can effectively promote the healing of the ankle fractures and significantly reduce complications [12]. At present, increasing evidence indicates that there is significantly positive effect of ESWT in treating bone non-unions [13]. For example, in a clinical study involving 126 patients with a long bone non-union, the effectiveness of ESWT and surgical treatment is compared, and the

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results indicate that the two methods have success rates of 71 % and 74 % respectively [8]. In addition, an animal experiment also shows that ESWT can stimulate the formation of new bone [14].

ESWT has become a successful, non-surgical, and economical strategy for treating bone non-unions [15,16]. The interaction between osteoblasts and osteoclasts plays a pivotal role in facilitating effective repair of bone non-unions [17]. It has been proved that ESWT can promote fracture healing through influencing osteoblasts and osteoclasts, but there is no consensus on the exact mechanism [18,19]. Therefore, this review aims to comprehensively summarize the application and mechanism of ESWT in promoting fracture healing by regulating osteoblasts and osteoclasts.

2. The role of osteoblasts and osteoclasts in bone fracture healing

Bone fracture healing is a complex process coordinated by interactions among cells, growth factors, and biological signaling pathways. The process of bone healing is categorized into three stages: the initial reaction phase, the subsequent repair phase, and the final remodeling phase [20]. The first stage of bone healing is characterized by inflammation and the formation of fracture hematoma. In the second stage of bone healing, there is an increase in the population of fibroblasts, chondrocytes, and osteoblasts. Additionally, callus tissue forms in and around the site of fracture. The broken ends are connected by collagen fibers, and osteoblasts start to form spongy bone. Subsequently, fibrocartilaginous callus is converted into woven bone by endochondral ossification [21,22]. In the third stage of bone healing, bone remodeling unit consists of a closely-coupled group of osteoclasts and osteoblasts, which plays a key role in maintaining the integrity of the bone by replacing old, damaged bone with new, stronger bone, and regulating the balance of calcium and phosphate in the body (Fig. 1) [23,24].

Osteoblasts and osteoclasts play key roles during the bone fracture healing process, and have garnered significant clinical interest over the recent years [25]. Osteoblasts are originally from osteoprogenitor cells differentiated from mesenchymal stem cells (MSCs), which is in charge of promotion of bone formation, and a part of osteoblasts differentiates into osteocytes to support bone structure and bone metabolism [26,27]. Moreover, osteoblasts synthesize and secrete collagen and non-collagen proteins that regulate bone mineral deposition, turnover and bone cell activity [24]. In addition, osteoclasts are derived from the monocyte/macrophage lineage and are the sole cells with the ability to absorb bone tissue [28]. Osteoclasts are mainly responsible for resorbing damaged bone, which facilitates osteoblasts in rebuilding the bone shape and structure [29]. Therefore, osteoblasts and osteoclasts are promising therapeutic targets for promoting bone healing.

3. The therapeutic mechanism of ESWT

The shock wave passing through the human body can generate various mechanical stress effects at the interface between tissues, thereby facilitating tissue regeneration and healing [30]. There is evidence that ESWT can produce various effects at three aspects: physical, chemical and biological levels [31]. First, at the physical level, shock wave generates both positive and negative pressure. The positive pressure facilitates the process of absorbing, reflecting, refracting, and transmitting energy to tissues, and the negative pressure produces cavitation on the tissue interfaces, leading to the generation and

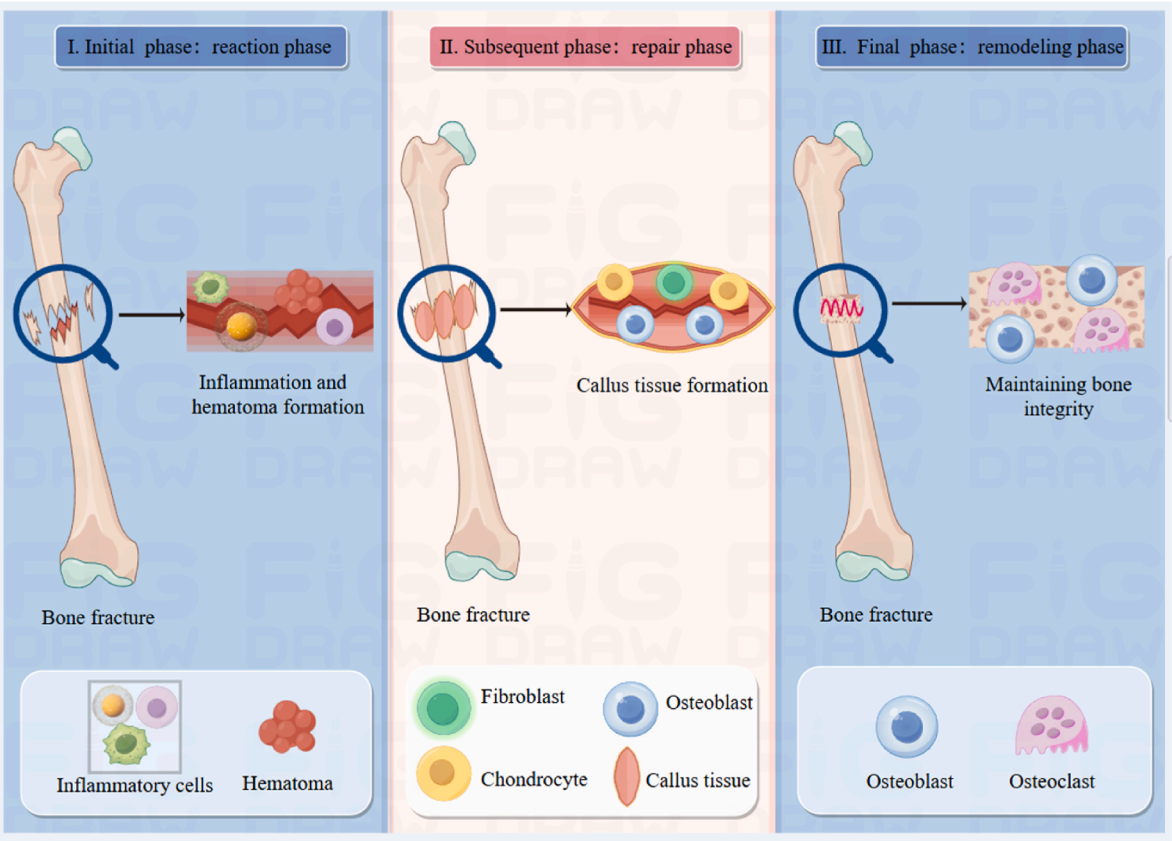

Fig. 1. The process of bone healing is divided into three distinct phases: the initial reaction phase, the subsequent repair phase, and the final remodeling phase. The first phase of bone healing is characterized by inflammation and the formation of fracture hematoma. During the second phase of bone healing, there is an increase in the population of fibroblasts, chondrocytes, and osteoblasts, leading to the formation of callus tissue in and around the fracture site. In the third phase of bone healing, bone remodeling unit consists of a closely-coupled group of osteoclasts and osteoblasts, which plays a key role in maintaining the integrity of the bone (By Figdraw).

implosion of air bubbles, thus generating a second shock wave or fluid microjets [31,32]. The cavitation enhances the cellular membrane permeability and biological molecules ionization [33]. Second, at the chemical level, shock wave can regulate transmembrane ion channels in cells, thus mediating intracellular calcium flux [34]. The transmembrane current alterations triggered by enhanced influx of $Ca2^{+}$ can deliver the information of shock wave impulses from the extracellular environment to the cytoplasm [35]. Third, at the biological level, shock wave has been found to play various biological roles, including angiogenesis improvement, wound healing acceleration, bone non-union healing promotion, nerve regeneration, as well as inflammation suppression (Fig. 2) [36].

4. The mechanism of ESWT in promoting bone fractures healing

Following traditional fracture treatment, some patients with bone fractures did not achieve satisfactory therapeutic effects. In order to help these patients, ESWT has been proposed for bone healing. Focused shock wave and radial shock wave are two main types of ESW, and they differ in many aspects, such as energy source, energy intensity, depth of maximal energy and applicable fracture site (Table 1) [37–39]. Up to now, both types of shock wave have been used in the clinical treatment of bone fracture.

Table 1
Focused shockwave and radial shockwave are two main forms of ESW, and they differ in many aspects, such energy source、energy intensity、depth of maximal energy and applicable fracture site.

The different characteristics of focused shockwave and radial shockwave	Focused shockwave	Radial shockwave
Energy source	Electro-hydraulic、piezo-electric、electro-magnetic devices	Ballistic devices
Energy intensity	Higher	Lower
Depth of maximal energy	Deep tissue	Superficial tissue
Applicable fracture site	Fracture of deep and superficial bones	Fracture of superficial bones

ESWT-mediated promotion of fracture healing may work through multiple mechanisms. First, the cavitation effect of ESWT can produce microfractures leading to fracture hematoma formation, thereby promoting osteogenic response and reactivating bone growth [32,40,41]. Second, studies show that ESWT can promote osteoblast formation, preserve the osteogenic potential of MSCs, and enhance the differentiation of osteoblasts [42,43]. Third, ESWT could prevent the production

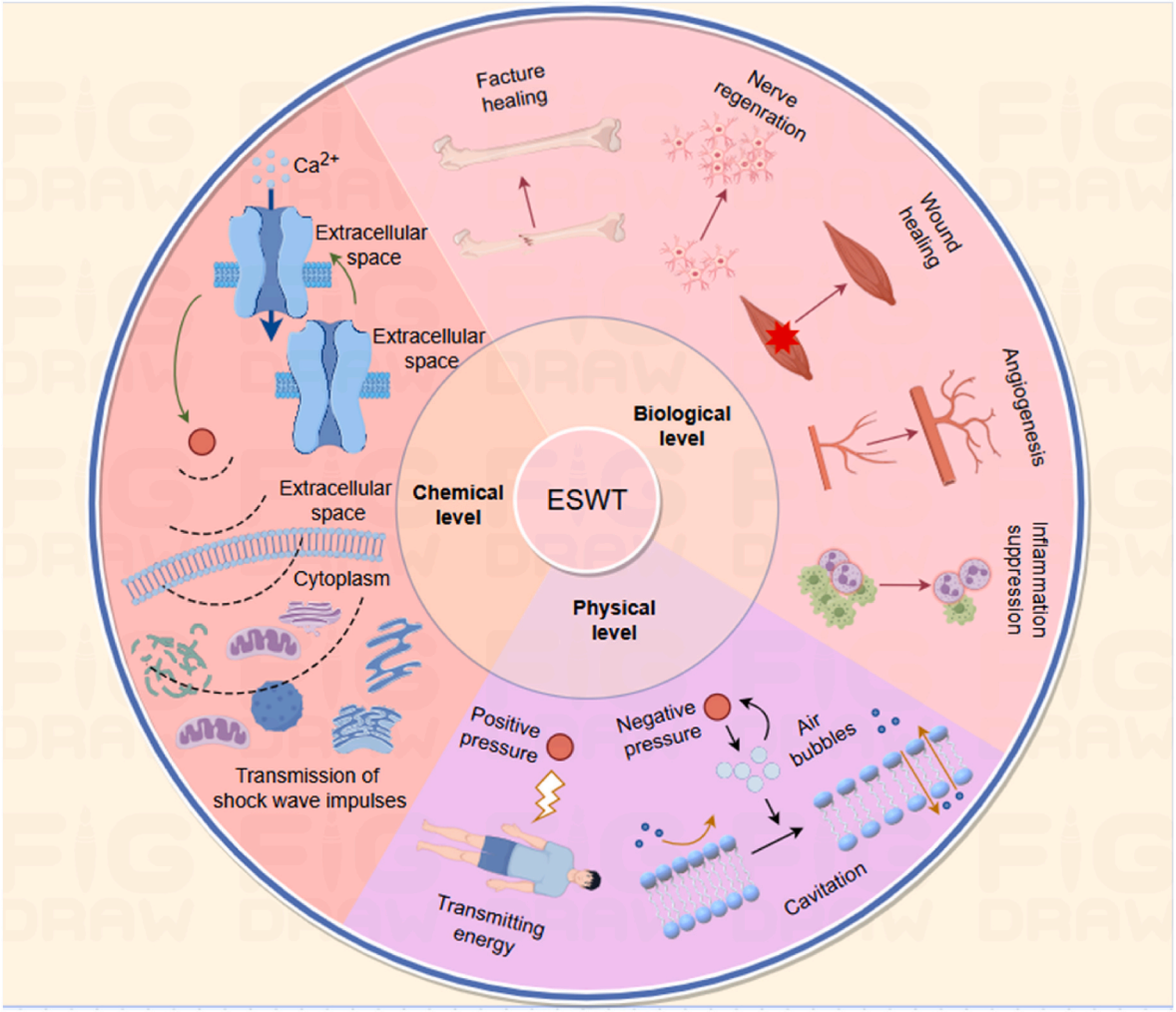

Fig. 2. ESWT plays a significant role in physical, chemical, and biological levels. At the physical level, shock wave can transmit energy to tissues and produce cavitation to enhance the cellular membrane permeability and biological molecules ionization. At the chemical level, ESWT can deliver the information of shock wave impulses from the extracellular environment to the cytoplasm by enhanced influx of $Ca2^{+}$. At the biological level, shock wave plays various biological roles, including angiogenesis improvement, wound healing acceleration, bone non-union healing promotion, nerve regeneration, as well as inflammation suppression (By Figdraw).

and differentiation of osteoclasts by targeting NF-κB signaling pathway in vitro [19]. Therefore, ESWT is deemed to promote fracture healing through regulating osteoclasts and osteoblasts.

The subsequent sections of this article will delve into the impact of ESWT on the function of osteoblasts and osteoclasts during the process of fracture healing (Fig. 3).

5. ESWT facilitate fracture healing by promoting the formation and differentiation of osteoblasts

ESWT led to an increase in osteoblast formation and differentiation, which was associated with elevated levels of various factors (including, Runx2, Osterix, BMP, TGF-β1, NO, and FAK), as well as activated cellular signaling pathways such as MAPK and Wnt. Furthermore, we have summarized the applications of ESWT in promoting the formation and differentiation of osteoblasts in Table 2.

5.1. The osteoblast-specific transcription factors

The differentiation of osteoblasts is regulated by osteoblast-specific transcription factors, namely Runx2 and Osterix (Osx) [44,45]. Runx2 is a Runt-associated gene that guides the differentiation of MSCs into osteoblast lineages and suppresses their differentiation into adipocyte and chondrocyte lineages, which plays a key role in vertebrate osteoblast differentiation [46–49]. In addition, Osx controls osteoblast lineage commitment and the following osteoblast proliferation and differentiation, which is necessary for bone formation [50]. Hence, the heightened levels of Runx2 and Osx may enhance the differentiation of MSCs into osteoblasts, ultimately aiding in the recovery of fractures.

Accumulating evidence indicate that ESWT can enhance the formation and differentiation of osteoblasts by controlling the levels of Runx2 and Osx. Akt is one of the important players in the signaling of crucial bone anabolic factors, which strengthens osteogenic transcription factors (including Osx and Runx2) function and transcriptional activity [51,52]. Following ESWT, the level of Akt activity at the fracture site of tibial defect rats significantly increases [53]. Moreover, in a rat model, ESWT can promote the expression of Runx2 and Osx during osteogenic induction, thus boosting the proliferation and differentiation of BMSCs into osteoprogenitor cells, and ultimately stimulating bone formation [43]. After ESWT, Runx2 expression on human umbilical cord blood mesenchymal progenitor cells (HUCB-MPCs) was significantly increased. The majority of the MPCs derived from the HUCB exhibited differentiation towards the osteogenic lineage, resulting in an increase in bone alkaline phosphatase activity and formation of bone nodules. This further confirms that ESWT can activate osteoblastic lineage by increasing the expression of Runx2 [54]. Ultimately, ESWT can enhance the healing of fractures through the stimulation of osteoblast-specific transcription factors such as Runx2 and Osx.

5.2. Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) were deemed as a valuable factor for treatment of bone fracture. Specifically, BMPs were identified as organic components of bone matrix, playing a crucial role in all stages of bone fracture healing, such as promoting the differentiation of MSCs into osteoblasts, stimulating osteoblast proliferation and enhancing osteoblast function, increasing the expression of bone formation markers, namely, osteocalcin (OC) and bone-specific alkaline

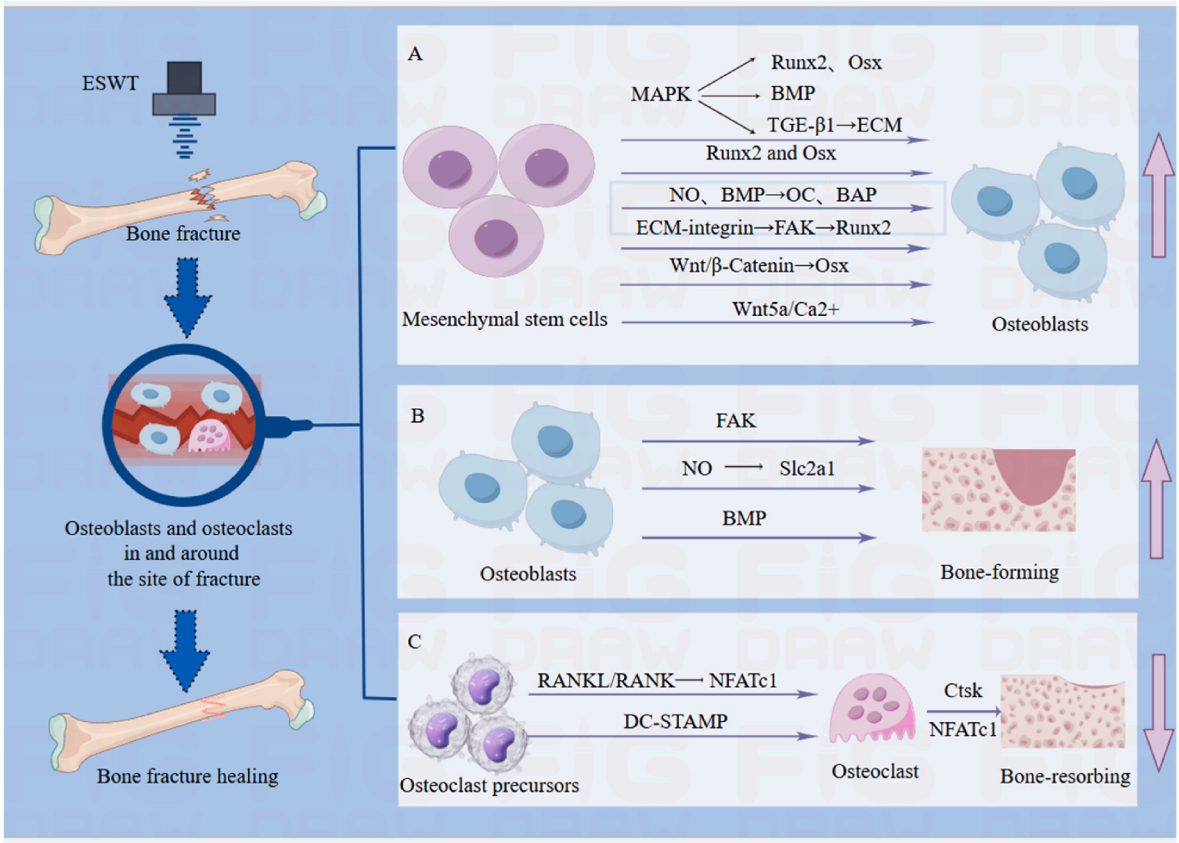

Fig. 3. ESWT promotes fracture healing by regulating the formation and differentiation of osteoblasts and osteoclasts in and around the fracture site. **A, B.** ESWT promotes bone formation by enhancing osteoblast proliferation and differentiation, which was associated with elevated levels of various factors (including, Runx2, Osx, BMP, TGF-β1, NO, and FAK), as well as activated cellular signaling pathways such as MAPK and Wnt. **C.** In addition, ESWT inhibits bone resorption by suppressing the formation and differentiation of osteoclasts through modulation of the RANKL/OPG ratio and key markers for osteoclast differentiation (DC-STAMP, NFATc1 and Ctsk) (By Figdraw).

Table 2
The summary of the applications of ESWT in promoting the formation and differentiation of osteoblasts.

Author	Time	Subject	Shock wave type	Parameter	Factors	Conclusion
Buarque de Gusmao, C.V. et al.	2019	Animal experiment	Focus	0.12 mJ/mm^2; 500 impulses	FAK Akt	ESW can increase the expression of Akt, FAK and TGF - b1
Chen, Y. et al.	2017	vitro experiment	Focus	10 kV; 250, 500, 750, and 1000 impulses	Runx2 Osx	ESW can induce the expression of Runx-2 and osteogenic markers ALP, Col1, and Osterix in BMSCs, thereby promoting the osteogenic differentiation of BMSCs
Wang, F.S. et al.	2004	vitro experiment	/	0.16, 0.24, and 0.42 mJ/mm^2; 200 impulses	Runx2	ESWT enhanced osteogenic activity of HUCB-MPCs
Wang, F.S. et al.	2003	Animal experiment	Focus	0.16 mJ/mm^2; 500 impulses	BMP	ESW promotes segmental defect healing by enhancing the expression of BMP
Muzio, G. et al.	2014	vitro experiment	Focus	0.22 mJ/mm^2;100 impulses	BMP	The osteogenesis of ESW is directly related to the increased expression of BMP
Chen, Y.J. et al.	2004	Animal experiment	/	0.16 mJ/mm^2; 500 impulses	TGF-β1	TGF-β1 is likely to play a mitogenic role in mesenchymal stem cell growth.
Wang, F.S. et al.	2002	vitro experiment	/	0.16 mJ/mm^2; 0, 250, 500, 750, 1000, 1500 and 2000 impulses	TGF-β1	ESW promotes the growth of bone marrow stromal cells and the formation of bone nodules by increasing TGF-β1 expression.
Wang, C.J. et al.	2009	clinical experiment	/	28 kV; 6000 impulses	NO TGF-β1 VEGF BMP-2	ESW promoted bone healing was associated with significant increases in serum NO level and osteogenic growth factors.
Wang, C.J., F.S. Wang, and K. D. Yang	2008	Animal experiment	Focus	0.47 mJ/mm^2;2000 impulses	VEGF eNOS PCNA BMP	ESW romoted bone healing was associated with increased numbers of neo-vessels and angiogenic and osteogenic growth factors including VEGF, eNOS, PCNA, and BMP
Yin, T.C. et al.	2011	vitro experiment	/	0.18 mJ/mm^2;250 impulses	MTT VEGF alkaline phosphatase BMP2 RUNX2 osteocalcin	ESW can promote bone formation of MSCs by enhancing NO expression
Chen, Y.J. et al.	2004	Animal experiment	/	0.16 mJ/mm^2;500 impulses	MAPK	ESW increases ERK and p38 phosphorylation to promote mitosis in callus
Wang, F.S. et al.	2002	Animal experiment	/	0.16 mJ/mm^2;0, 250, 500, and 1000 impulses	CBFA1	ESW leads to CBFA1 phosphorylation by activating ERK, which promotes the growth and maturation of bone progenitor cells into bone nodules.
Xu, J.K. et al.	2012	vitro experiment	Focus	5, 10, 15, and 20 kV; 250, 500, 750, and 1000 impulses	FAK Integrin	ESW phosphorylates FAK by activating integrins, thereby promoting osteoblast adhesion and migration
Hu, J. et al.	2016	vitro experiment	/	0.16 mJ/mm^2;500 impulses	FAK	ESW activates the ERK1/2 pathway by phosphorylation of FAK
Yu, L. et al.	2017	Animal experiment	Radial	0.2, 0.4, and 0.6 bar; 1000 impulses	Wnt5a/Ca2$^+$	ESW with optimal energy can activate the Wnt5a/Ca2$^+$ signaling pathway
Wang, C.J. et al.	2014	Animal experiment	Focus	0.22 mJ/mm^2;800 impulses	DKK-1 PCNA VEGF BMP-2	ESWT is effective in the amelioration of osteoporotic OA of the knee in rats.

phosphatase (BAP) etc [55,56].

ESWT can stimulate cytokines associated with bone formation like BMP, thereby promoting bone regeneration [57]. Following eight weeks of ESWT in a rat model with a midshaft femoral fracture, the mRNA expression of BMP-2, 4, and 7 in the fractured femur was significantly elevated, leading to woven bone remodeling and newly generated medullary cavity. This demonstrates that BMPs play key roles in promoting bone fracture healing following ESWT [58]. Following ESWT, the MG63 human osteoblast-like cells show an 8.5-fold increase of BMP-4 expression and a two-fold increase of BMP-7 expression, resulting in an upregulation of alkaline phosphatase and osteocalcin mRNA expression in MG63 human osteoblast-like cells. This demonstrates that ESWT can enhance osteoblast activity by increasing BMP expression [59]. Collectively, ESWT may enhance osteoblast activity by up-regulating BMPs, thereby promoting bone regeneration.

5.3. Transforming growth factor-beta 1

Transforming growth factor-beta 1 (TGF-β1) is a widely distributed growth factor that is able to modulate the proliferation, migration, differentiation, and survival of various cell types [60]. As one of the most important factors in the bone environment, TGF-β1 can affect cells of osteoblast lineage. In the bone formation process, TGF-β1 recruits MSCs, and enhances their proliferation and early osteogenic differentiation by promoting extracellular matrix (ECM) protein production, thus facilitating bone formation [61]. Therefore, TGF-β1 is considered to be one of the key cytokines involved in fracture treatment.

Accumulating evidence demonstrated that application of ESWT enhanced bone growth by activating the TGF-β1 pathway. For example, after ESWT, high levels of TGF-β1 expression were observed in the bone defect area, along with a substantial aggregation of MSCs. Subsequent histological examination revealed that stem cells gradually differentiated into osteoblasts and chondrocytes, thus promoting the intensive intramembranous, endochondral ossification, and closure of the bone gap [62]. TGF-β1 also can promote BMSCs towards osteoprogenitors during the repair of fractures in vivo [63,64]. Furthermore, the level of TGF-β1 and the number of osteoprogenitors are significantly increased in ESWT group than untreated group, and osteoprogenitors formation was significantly correlated with production of TGF-β1. ESWT can upregulate the expression of TGF-β1, thereby effectively promoting the differentiation of rat BMSCs towards osteoprogenitors [65]. In general, ESWT can enhance bone healing through upregulating the production of TGF-β1.

5.4. Nitric oxide

Nitric oxide (NO) is a signaling molecule generated from L-arginine by three different nitric oxide synthase (NOS) enzymes, including neuronal NOS (nNOS), endothelial NOS (eNOS), and an inducible form of NOS (iNOS) [66,67]. NO plays various positive roles in the process of bone fracture healing. For example, NO facilitates the osteogenic differentiation of BMSCs and pre-osteoblasts by promoting the mRNA expression of osteoblastic genes such as BAP, OC, and collagen-1 [68–71]. In addition, NO promotes glycolysis to enhance osteoblasts

differentiation and activity by upregulating multiple glycolytic genes such as Slc2a1 [72]. Therefore, NO bears an application potential to promote bone fracture healing.

Increasing evidence suggests that ESWT can promote the generation of NO, thus promoting bone healing. For instance, ESWT significantly increased the serum NO level in long bone non-union patients, thus promoting callus formation and bone gap closure. This demonstrates a significant increase in serum NO levels associated with ESWT promotion of bone healing [73]. In a rabbit bone fracture model following ESWT, the number of osteogenic growth markers, such as eNOS, is significantly increased, and both bone strength and cortical bone formation are obviously improved in comparison with non-ESWT group [74]. Moreover, pretreatment with NOS inhibitors can reverse the osteogenic effect of ESWT, indicating the key role of NO in ESWT–mediated osteogenic effect on BMSCs from femoral head osteonecrosis [75]. Therefore, we can reasonably summarize that ESWT can promote osteoblast differentiation by increasing the levels of NO, thus promoting fracture healing.

5.5. Mitogen-activated protein kinases

The Mitogen-activated protein kinases (MAPKs) are essential mediators for several important cellular processes including growth, differentiation, and apoptosis, which are responsible for converting extracellular stimuli into cellular responses [76–78]. In mammalian cells, ERK and p38 are family members of MAPKs [79]. ERK and p38 can not only activate osteogenic factors such as BMP and TGF-β, but also upregulate the expression of Runx2 and Osx, thereby inducing proliferation and differentiation of osteoblasts [80–84]. In summary, the efficacy of MAPKs in the treatment of bone fractures has been gradually recognized, thus becoming valuable therapeutic focus.

Increasing evidence has shown that MAPKs are crucial in the fracture healing process following ESWT. After ESWT treatment, ERK and p38 are activated in the callus tissue of rat bone defects, leading to MSCs aggregation and intramembranous/endochondral ossification at the defect site. This indicates that MAPKs can respond to ESWT stimulation, triggering mitogenic and osteogenic responses at the bone defect site [85]. In addition, ESWT induces O^{2-} generation by promoting tyrosine kinase-regulated ERK activation, leading to phosphorylation of Runx2, which promotes the growth and maturation of osteoprogenitor cells [86]. In summary, the abovementioned findings reveal the importance of MAPKs in the bone tissue healing process triggered by ESWT.

5.6. Focal adhesion kinase

Focal adhesion kinase (FAK) is an important intracellular non-receptor tyrosine kinase that plays a key role in signal transduction, which is mainly activated by integrins [87]. Integrins are multifunctional cell-surface adhesion molecules, which can activate FAK phosphorylation by interacting with the ECM, leading to the initiation of a series of signaling pathways [88,89]. Firstly, The ECM-integrin signals in the process of bone formation can activate FAK phosphorylation, which subsequently triggers Runx2 phosphorylation and thereby enhances osteogenic differentiation of MSCs [90]. Secondly, phosphorylated FAK is also involved in osteoblast adhesion, migration, and bone formation [91–93]. The lack of FAK in osteoblasts and osteocytes can postpone the process of bone tissue healing and remodeling [94]. Taken together, FAK signaling pathway plays a key role in the bone tissue regeneration.

Research has demonstrated that FAK is crucial in enhancing osteoblast differentiation following ESWT. For example, after ESWT, integrin expression increases in rat osteoblasts, and subsequent integrin-induced FAK phosphorylation further activates ERK1/2, leading to increased osteoblast adhesion, distribution, and migration, ultimately promoting bone healing [92]. Moreover, MSCs transfected with FAK short interfering RNA (siRNA) did not exhibit increased mineralized matrix deposition and Runx2 phosphorylation expression following ESWT [94]. The above experiments demonstrate that FAK can activate multiple signaling pathways in ESWT to promote bone fracture healing process. In conclusion, ESWT-induced enhancement of osteogenesis is mediated through activation of the FAK signaling pathway.

5.7. Wnts

Wnts are a family composed of various secreted glycoproteins that play significant roles in basic bone metabolism, including proliferation, differentiation, and apoptosis of osteoblasts [95–97]. There are two types of Wnt signaling pathways: the classical pathway and the nonclassical pathway. Wnt/β-catenin signaling pathway (the classical pathway) enhance the expression of Osx, thus leading to BMSCs differentiate into osteoblasts rather than adipocytes [98]. In addition, as one of the main non-canonical signaling pathways, the Wnt-calcium (Wnt-Ca^{2+}) pathway can be activated by Wnt5a, and Wnt5a is a representative ligand in the nonclassical Wnt pathway [99]. Wnt5a-activated Wnt/Ca^{2+} (Wnt5a/Ca^{2+}) signaling pathway can inhibit adipogenesis, thereby facilitating the differentiation of BMSCs towards osteoblast lineage cells [100–102]. Therefore, Wnt signaling pathway may be one of the key targets involved in the treatment of fracture healing.

ESWT can promote bone generation via the classical or nonclassical Wnt signaling pathway. For example, ESWT can indirectly promote the osteogenic differentiation of BMSCs by activating the Wnt5a/Ca^{2+} signaling pathway in the subchondral bone plate of osteoarthritis rats, thus noticeably promoting the thickness of tibial condyle subchondral bone plate [103]. In addition, DKK-1 is a suppressant of the typical Wnt/β-catenin pathway, which exerts its function through blocking the combination between Wnt ligands and their receptors [104]. In rats with osteoarthritis and osteoporosis, ESWT improve bone mineral density, bone strength, and subchondral plate thickness by decreasing the levels of DKK-1 [105]. Therefore, ESWT can enhance the differentiation of MSCs into osteoblasts and bone formation by regulating the expression of Dkk-1 and the Wnt/β-catenin signaling pathway [105]. All in all, ESWT can regulate the differentiation of MSCs to facilitate fracture healing through the classical or nonclassical Wnt signaling pathway.

6. ESWT facilitate fracture healing by inhibiting the formation and differentiation of osteoclasts

6.1. Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) and Osteoprotegerin (OPG)

Osteoclasts are differentiated from the monocyte–macrophage lineage and are multinucleated cells responsible for bone resorption [55]. Excessive and hyperactive osteoclast activity causes premature cartilage resorption and subsequently diminishes bone formation during the process of fracture healing [106]. Therefore, the regulation of osteoclast activity is crucial for effective and adequate bone repair. The degree of osteoclast activity is influenced by the equilibrium between the level of Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) and Osteoprotegerin (OPG) [107]. RANKL secreted by osteoblasts binds with RANK (receptor of RANKL) on osteoclast precursors to promote mononuclear osteoclast precursor fusion, followed by multinucleated osteoclast maturation [108,109]. OPG is primarily synthesized by osteoblasts and functions as a soluble receptor for RANKL, acting as a decoy receptor, thereby inhibiting RANKL/RANK signaling and negatively regulating osteoclastogenesis [110]. All in all, the ratio of RANKL/OPG has a significant impact on regulating the formation and activation of RANKL-induced osteoclasts [111,112]. Therefore, we can summarize that RANKL plays a negative role in bone formation, while OPG plays a positive role in bone formation. Reducing the ratio of RANKL/OPG can inhibit osteoclast generation and prevent bone resorption, which is considered as a promising therapeutic target for the treatment of bone nonunion.

In recent years, accumulating evidence indicate that ESWT inhibited bone resorption through regulating the level of RANKL and OPG. For

instance, following ESWT, the RANKL/OPG ratio is decreased in murine osteoblasts, indicating that ESWT can inhibit osteoclast generation [18]. Furthermore, ESWT improves the structure of trabecular bone and increases bone strength in rats with osteoporosis by reducing RANKL expression and inhibiting osteoclast activity. This suggests that ESWT can reduce bone resorption and promote bone formation [113]. In comparison with osteoporotic fracture rats without any treatment, the level of OPG is obviously increased, and ossification was higher in osteoporotic fracture rats treated by ESWT [114]. Taken together, ESWT can influence osteoclast activity through regulating RANKL and OPG, thereby inhibiting bone resorption and promote fracture healing (Fig. 4).

6.2. Dendritic cell-specific transmembrane protein (DC-STAMP), nuclear factor of activated T-cells 1 (NFATc1) and Cathepsin K (Ctsk)

Dendritic cell-specific transmembrane protein (DC-STAMP), nuclear factor of activated T-cells 1 (NFATc1) and Cathepsin K (Ctsk) are key markers for osteoclast differentiation. The DC-STAMP protein serves as a regulator of osteoclast cell fusion, facilitating intercellular fusion among osteoclasts [115]. After cell fusion, immature osteoclasts become large, multinucleated, mature osteoclasts capable of bone resorption [28,116]. Knocking down DC-STAMP totally inhibits the differentiation of osteoclasts, indicating that DC-STAMP plays a crucial role as the key regulator in osteoclastogenesis [117–121]. The transcription factor NFATc1 is a key downstream component of the RANKL/RANK signaling pathway. NFATc1 regulates osteoclast formation and osteoclast resorptive activity by inducing a series of osteoclast-specific genes, such as Ctsk and osteoclast-associated receptor [122,123]. Ctsk, as a cysteine protease member of the cathepsin lysosomal protease family, exhibits high expression in osteoclasts and effectively degrades type 1 collagen (type 1 collagen accounts for 90 % of the bone organic matrix) [124]. Therefore, DC-STAMP, NFATc1 and Ctsk play key roles in bone resorption, making them promising therapeutic targets for the treatment of bone nonunion. ESWT inhibits osteoclast differentiation in vitro and decrease the expression of Ctsk, NFATc1 and DC-STAMP, thus reducing migration and survival rate of osteoclasts [19]. Taken together, ESWT can influence osteoclast differentiation through various pathways, thereby inhibiting bone resorption.

7. Summary

This review provides an overview of how ESWT promotes fracture healing by regulating osteoblasts and osteoclasts. Research conducted both in living organisms and in laboratory settings has demonstrated that ESWT can have a beneficial impact on either enhancing the healing of fractures or restarting the unsuccessful bone fracture healing. In addition, until now, there are no well-established standard parameters for using ESWT to treat bone nonunion in different types of patients or different fracture sites. Therefore, further comprehensive and large-scale studies are needed to form guidelines of ESWT therapy. The information provided in this article can not only provide a reference for clinicians to make treatment decisions in clinical practice, but also motivate researchers to further study the therapeutic effects and potential mechanisms of ESWT in promoting fracture healing. In summary, ESWT is a reliable and promising interventional method for promoting fracture healing, which still requires the further investigation in the future.

Ethics approval and consent to participate

Not applicable.

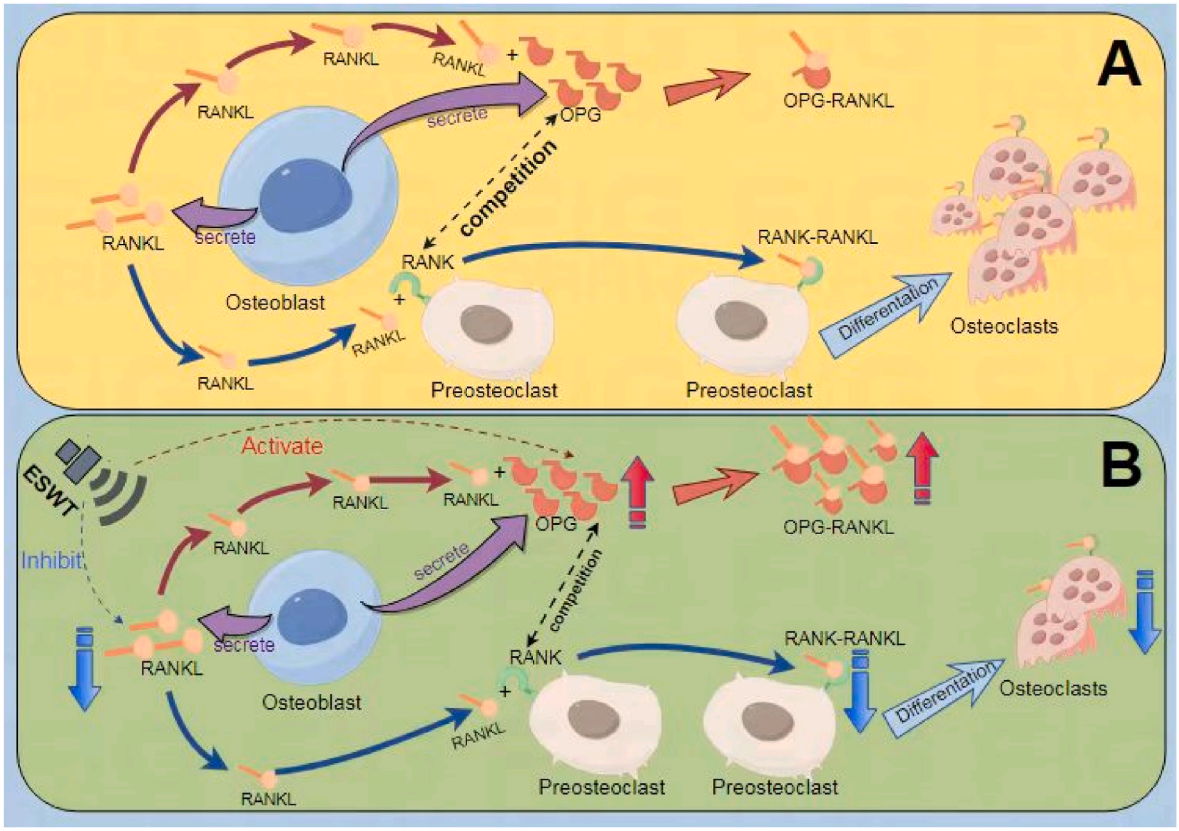

Fig. 4. A. Osteoblast can secrete RANKL and OPG, and RANKL can compete with OPG to combine with RANK on preosteoclasts to regulate the differentiation from preosteoclast to osteoclast. **B**. Following ESWT, there is an increase in OPG expression and a decrease in RANKL expression, reducing the combination RANKL and RANK, thus inhibiting differentiation from preosteoclast to osteoclast, which is beneficial for bone healing (By Figdraw).

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

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Declaration of interest statement

The authors declare that they have no competing interests to declare.

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The authors declare that artificial intellegence is not used in this study.

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