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Negative Pressure Regulates BMP-9 Expression Through the MAPK/ERK5 Signaling Pathway and Thereby Promotes Fracture Healing

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Abstract

Negative pressure wound therapy (NPWT) has been widely used in wound repair and tissue regeneration, and its mechanistic role in fracture healing is gaining increasing attention. Bone morphogenetic protein-9 (BMP-9), a recognized and highly effective osteoinductive factor, plays a crucial role in bone repair. However, the regulatory mechanism of its expression under negative pressure remains unclear. This study aimed to investigate whether negative pressure wound therapy (NPWT) accelerates fracture healing by upregulating BMP-9 and osteocalcin (OCN) expression through activation of the MAPK/ERK5 signaling pathway. A tibial fracture model was established in Sprague-Dawley (SD) rats. The rats were randomly divided into the control group, the Model+Gauze group, the Model+Gauze+BIX group, the Model+NPWT group, and the Model+NPWT+BIX group. Fracture tissue was obtained 14 days after surgery for molecular and histological analysis. Real-time fluorescence quantitative PCR results showed that mRNA expression of BMP-9, OCN, MEK5, and ERK5 in the Model+NPWT group was significantly higher than that in the other groups (P<0.05). Western blot analysis was consistent with this finding, demonstrating a significant increase in protein expression. Further immunohistochemistry revealed that OCN expression in the fracture area of the Model+NPWT group was significantly increased, suggesting a stimulatory effect on osteoblastic activity. This study demonstrates that NPWT may upregulate BMP-9 and OCN expression by activating the MAPK/ERK5 signaling pathway, thereby enhancing the osteogenic response at the fracture site and ultimately promoting fracture repair. This study provides a new perspective on the molecular mechanisms by which NPWT promotes fracture healing and provides a theoretical basis for its clinical application in fracture treatment.(**International Journal of Biomedicine. 2026;16(1):33-40.**)

Keywords: negative pressure • wound therapy • bone morphogenetic protein-9 • osteocalcin

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Abbreviations

ALP, alkaline phosphatase; **BMP-9**, bone morphogenetic protein-9; **ERK5**, extracellular-signal-regulated kinase 5; **MSCs**, mesenchymal stem cells; **MAPK**, mitogen-activated protein kinase; **NPWT**, negative pressure wound therapy; **OCN**, osteocalcin; **SD**, Sprague-Dawley.

Introduction

Fracture is one of the most common orthopedic diseases in clinical practice, and its repair process is regulated by multiple factors, including inflammatory response, cell migration, angiogenesis, and osteoblast differentiation.[1] Although most fractures heal with traditional treatment methods, in complex situations such as open fractures, concomitant infections, large bone defects, and osteoporosis in the elderly, there is a risk of delayed healing or even complete failure to heal.[2] Therefore, exploring methods that can effectively promote fracture repair and enhance the capacity for bone regeneration has always been a hot topic in bone tissue engineering and translational medicine research. In recent years, negative pressure wound therapy (NPWT) has been widely used in managing chronic wounds, diabetic foot, and postoperative wounds due to its

advantages of accelerating wound healing, promoting tissue perfusion, reducing edema, and inducing cell remodeling.[3,4] The therapeutic mechanism of NPWT is not limited to physical drainage but also includes the regulation of the local cell mechanical environment, thereby activating related signaling pathways and inducing tissue regeneration. In the field of orthopedics, researchers have begun to pay attention to whether NPWT can further promote bone tissue repair by regulating related signaling pathways. Zhu et al.[5] found that NPWT can enhance blood supply and osteoblast activity in the fracture area, shorten fracture-healing time, and promote bone-bridge formation. Li et al.[6] also observed that NPWT can promote angiogenesis in a diabetic trauma model, indirectly improving the physiological environment for fracture repair.

During fracture repair, bone morphogenetic protein-9 (BMP-9), a member of the TGF-β family, has been increasingly recognized in recent years as one of the cytokines with the greatest osteogenic induction potential. Compared with the traditionally used BMP-2 and BMP-7, BMP-9 has a stronger ability to induce mesenchymal stem cells (MSC) differentiation into osteoblasts and to sustainably upregulate osteogenic markers, such as ALP, OCN, and Runx2.[7-9] Mostafa et al.[10] showed that the regulatory transduction mechanism of BMP-9 involves not only the Smad-dependent pathway but also further enhances the expression of transcription factors and the synthesis of osteogenic proteins by activating non-classical signaling axes, such as the MAPK family. In particular, the MAPK/ERK5 (mitogen-activated protein kinase/ extracellular-signal-regulated kinase 5) signaling pathway plays a crucial role in osteogenic protein synthesis.[11-13] Although BMP-9 is widely recognized for its role in promoting fracture healing, little research has examined whether it is preferentially activated by negative pressure. Furthermore, little has been reported on whether its differentiation and proliferation are regulated by the MAPK/ERK5 signaling pathway under negative pressure, and whether negative pressure preferentially activates this pathway to regulate BMP-9. To this end, we established an SD rat tibial fracture model and used an NPWT intervention strategy. Combining immunohistochemistry, qPCR, and Western blot, we investigated the expression of key factors, including BMP-9, OCN, MEK5, ALP, and ERK5, and analyzed the effects of negative pressure on fracture repair. To examine whether negative pressure regulates BMP-9 and OCN via the MAPK/ERK5 signaling pathway and thereby promotes fracture healing, we included the ERK5 pathway inhibitor BIX02189 as a functional intervention.

This study is expected to reveal the function and role of BMP-9 and the MAPK/ERK5-BMP-9 axis under negative pressure, providing a theoretical basis and potential targets for interventions to repair clinical bone tissue.

Materials and Methods

Experimental Animals and Groups

Sixty healthy 8-week-old SD rats (weighing 220–250 g), half male and half female, were purchased from Hubei Provincial Laboratory Animal Center (License number: SCXK-2022-0011). All animals were housed in a SPF environment at 22–25°C, 50%–60% humidity, and a 12-h day/night cycle, with free access to food and water. To ensure animal welfare, humane endpoints were established, including weight loss exceeding 20%; severe infection or injury that could not be healed; noticeable pain or discomfort that could not be alleviated by medication; or the animal exhibiting excessive mental or behavioral stress during the experiment. All animals were sacrificed under deep anesthesia with sodium pentobarbital (50 mg/kg, intraperitoneal) before cervical dislocation.

If any of these conditions were observed, the experiment would be terminated immediately, and the animals would be euthanized in accordance with procedures approved by the ethics committee. After 7 days of acclimation, the animals were randomly assigned to 5 groups (n=12): Control group: no treatment; Model+Gauze group: fracture model established and covered with saline gauze; Model+Gauze+BIX group: ERK5 pathway inhibitor BIX02189 was added to the model group; Model+NPWT group: negative pressure treatment; Model+NPWT+BIX group: negative pressure treatment plus ERK5 inhibitor intervention. The experiment was carried out from March to July 2024.

Tibial Fracture Model Construction and Negative Pressure Treatment

After the animals were anesthetized (sodium pentobarbital, 40 mg/kg, i.p.), a longitudinal incision was made in the proximal tibia of the right hind limb to expose the bone surface. A surgical drill (Elbo EL-21) was used to create a bone defect approximately 3mm in the mid-tibial region (Figure 1A). Subsequently, a 0.8mm diameter Kirschner wire (Jiangsu Kangda) was inserted into the medullary canal for internal fixation. The fracture ends were reduced, and X-rays confirmed the successful model (Figure 1B). NPWT treatment was performed using a disposable, medical, negative-pressure sealing and drainage dressing (Jiangsu Yaguang Medical Technology Co., Ltd., model YX9800-01) connected to a continuous negative-pressure aspirator (set to –125 mmHg) (Figure 1C). The dressing was changed every 3 days for 14 days. The control group was covered with moist sterile gauze. BIX02189 (MCE, HY-12056) was dissolved in DMSO and diluted with 25% DMSO. The Model+Gauze+BIX group and the Model+NPWT+BIX group were intraperitoneally injected daily at a dose of 5 mg/kg for 7 days.

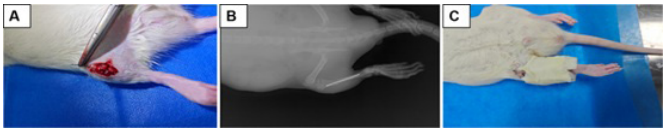

Figure. 1. *Rat tibial fracture model establishment and negative pressure treatment. (A) A 3 mm bone defect was created by drilling in the mid-tibial region of the rat. (B) X-ray imaging demonstrates the successful establishment of a tibial fracture model. (C) The wound in the negative pressure wound treatment group was covered with a vacuum-sealed drainage dressing. Negative pressure was applied continuously at -125 mmHg.*

Tissue Sample Collection

On Day 14 after the intervention, the animals were killed by cervical dislocation. All animals were deeply anesthetized with an intraperitoneal injection of 50 mg/kg sodium

pentobarbital before cervical dislocation. The callus tissue at the fracture site was collected and divided into 2 parts: one part was fixed with 4% paraformaldehyde for 48 hours and embedded in paraffin for immunohistochemistry; the other part was quickly frozen in liquid nitrogen and stored at -80°C for qPCR and Western blotting.

Real-Time Fluorescence Quantitative PCR Detection

Total RNA was extracted using a RNeasy Mini kit (Qiagen AB, Sollentuna, Sweden) and a thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). RNA was reverse transcribed into cDNA using the First Strand cDNA Synthesis kit (Fermentas; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocols. Primer sequences were:

BMP-9 forward primer, 5′- AGACCGTGCTTGTGAAGACAT -3`′ and reverse primer, 5′- CACGATGGCGTGTTTGGTG -3′

ALP forward primer, 5′- TGGACGGTGAACGGGAGAACG -3′ and reverse primer, 5′- TGAAGCAGGTGAGCCATAGGG -3′

OCN forward primer, 5′-GCCCTGACTGCATTCTGCCTC-3′and reverse primer, 5- TCACCACCTTACTGCCCTCCT -3′

GAPDH forward primer, 5′-ACAGCAACAGGGTGGTGGAC-3′ and reverse primer, 5′- TTTGAGGGTGCAGCGAACTT -3′

RT-PCR was performed using a SYBR qPCR mix (2×; Toyobo Co., Ltd., Osaka, Japan) and an RT-PCR detection system (Bio-Rad Laboratories Inc.). Thermocycling parameters were an initial denaturation for 1min at 95°C, followed by 40 denaturation cycles at 95°C for 15 s, annealing at 60°C for 15 s, and elongation at 72°C for 60 s. Samples were run in triplicate. Relative gene expression was analyzed with reference to GAPDH expression and the $2^{-\triangle\triangle Ct}$ method.

Western Blot Assay

Protein was extracted using RIPA lysis buffer (Meilunbio, MA0151) supplemented with PMSF (1 mM) and phosphatase inhibitors (Beyotime, P1260). Tissue homogenization was followed by centrifugation at 12,000 rpm for 15 min at 4°C, and the supernatant was collected. Protein concentration was determined using the BCA assay (Beyotime, P0012). Forty micrograms of protein were separated by 10% SDS-PAGE and electrophoresed at 100 V for 90 min. The membrane was then transferred to a PVDF membrane (Millipore, IPVH00010) at a constant voltage of 300 mA for 90 min. After blocking with 5% skim milk powder (diluted in TBST) for 1 hour, the membranes were incubated overnight at 4°C with the following primary antibodies: BMP-9 (Affinity, DF7758, 1:1000); ALP (Affinity, DF6225, 1:1000); OCN (Affinity, DF12303, 1:1000); MEK5/p-MEK5, ERK5/p-ERK5, and Nur77/p-Nur77 (all Affinity products); and internal control: GAPDH (Hangzhou Xianzhi, AB-P-R001, 1:10,000).. Secondary antibodies were used: HRP-conjugated goat anti-rabbit IgG (Boster, BA1051, 1:10,000). Incubation was performed at room temperature for 1 hour. Color was developed with an ECL solution (Affinity, KF8003) and exposed on an SH-523 imaging system. Grayscale values were analyzed in ImageJ, and GAPDH was used as an internal control for normalization.

Immunohistochemistry (IHC) Staining

For immunohistochemistry staining, paraffin sections were routinely deparaffinized and rehydrated with graded alcohols. Antigens were retrieved with 0.25% trypsin at 37°C for 20 minutes. Endogenous enzymes were blocked with 3% H_2O_2 for 10 minutes, followed by blocking with 10% goat serum for 30 minutes. The primary antibody against osteocalcin (LSBio, LS-C83497, 1:100) was incubated overnight at 4°C. The next day, a secondary antibody conjugated with HRP (Tongling Biotechnology) was added, and DAB (Servicebio, G1212) was used for color development. The sections were counterstained with hematoxylin, dehydrated, and mounted. The stained areas appeared brownish-yellow. Positive intensity was measured using a Nikon Fi3 microscope, and the percentage of positive area was analyzed using Image-Pro Plus 6.0.

Statistical analysis

All data are presented as the means ± SD, and statistical significance was assessed by one-way analysis of variance (ANOVA). SPSS 18.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis. Differences between groups were considered statistically significant at $P<0.05$.

Results

Changes in BMP-9, ALP, and OCN mRNA Expression

In this study, the qPCR was used to measure gene mRNA levels. The results showed that BMP-9 mRNA expression was significantly higher in the Model+NPWT group than in the Model+Gauze group ($P<0.01$), suggesting that NPWT activated BMP-9 transcription (Figure 2E). In contrast, BMP-9 expression was significantly decreased in the Model+Gauze+BIX group, which was treated with the ERK5 pathway inhibitor BIX02189, indicating that the MAPK/ERK5 pathway positively regulates its expression. ALP and OCN mRNA expression levels were also significantly higher in the Model+NPWT group than in the Model+Gauze group ($P<0.05$ or $P<0.001$), indicating that NPWT not only affects the expression of inducible factors but also broadly promotes mid- and late-stage osteogenic differentiation (Figures 2F and 2G). BIX02189 intervention significantly inhibited the MAPK/ERK5 signaling pathway, leading to decreased ALP and OCN expression.

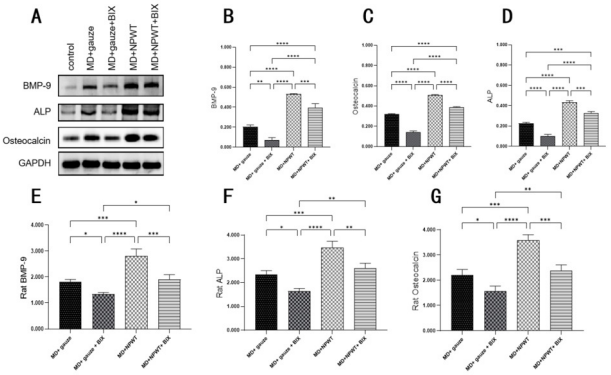

Figure. 2. *Expression changes of BMP-9, ALP, and OCN in fracture healing. (A) Representative protein blots showing the BMP-9, OCN, and ALP levels in each group. (B) Statistical analysis of BMP-9 protein levels in each group. (C) Statistical analysis of OCN protein levels in each group. (D) Statistical analysis of ALP protein levels in each group. (E) BMP-9 gene expression levels in each group were detected by qRT-PCR. (F) ALP gene expression levels in each group were detected by qRT-PCR. (G) OCN gene expression levels in the two groups were detected by qRT-PCR. The control group was normal bone tissue of rats without fractures, which was only used for normalization analysis and not presented in the statistical analysis graph. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.*

Changes in Protein Expression of BMP-9, ALP, MEK5, ERK5, NUR77, and OCN

To further validate these transcriptional changes at the protein level, this study used a Western blot to examine the protein expression levels of BMP-9, ALP, MEK5, ERK5, and OCN in each group. The phosphorylation levels of MEK5 and ERK5 were also measured. The results showed that the protein expression trends of BMP-9, ALP, and OCN were consistent with those of their mRNA counterparts. BMP-9 protein expression was significantly higher in the NPWT group than in the Model+Gauze group (P<0.01), while that in the Model+Gauze+BIX group was significantly lower than in the NPWT group (P<0.05) (Figure 2A, 2B). Furthermore, ALP and OCN protein expressions were significantly increased in the NPWT group, with statistically significant differences, (P<0.01) suggesting that NPWT enhances the expression of proteins associated with fracture repair (Figure 2A, 2C, 2D). BIX02189 treatment significantly decreased the expression levels of these proteins. Further analysis of key proteins in the MAPK/ERK5 signaling pathway, MEK5 and ERK5, as well as their phosphorylated forms (p-MEK5 and p-ERK5), and the downstream transcription factors Nur77 and p-Nur77, revealed significant upregulation of p-MEK5, p-ERK5, and p-Nur77 expression in the Model+NPWT group (P<0.01 or P<0.001, respectively) (Figures 3A, 3C, 3E, and 3G). However, phosphorylation levels of p-MEK5 and p-ERK5 were significantly decreased in the Model+NPWT+BIX group. Significant changes in the phosphorylation levels of p-MEK5 and p-ERK5 were also observed between the Model+Gauze+BIX and Model+Gauze groups (Figures 3A, 3C, and 3E). This demonstrates that the ERK5 pathway inhibitor BIX02189 can significantly inhibit the MAPK/ERK5 signaling pathway, whereas NPWT can activate it and its downstream factors.

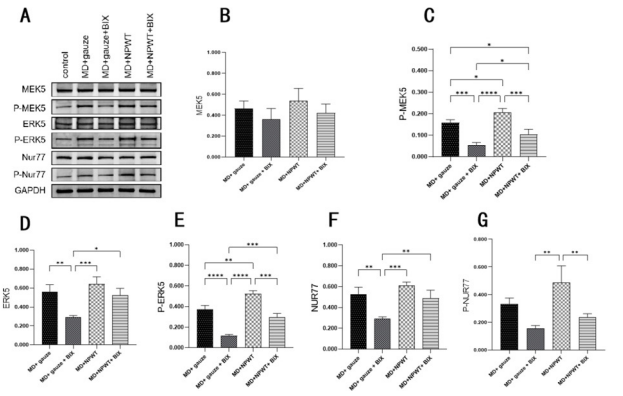

Figure. 3. *Altered expression of MEK5, P-MEK5, ERK5, P-ERK5, NUR77, and P-NUR77 during fracture healing. (A) Representative Western blots showing the protein levels of MEK5, P-MEK5, ERK5, P-ERK5, NUR77, and P-NUR77 in each group. (B) Statistical analysis of MEK5 protein levels in each group. (C) Statistical analysis of P-MEK5 protein levels in each group. (D) Statistical analysis of ERK5 protein levels in each group. (E) Statistical analysis of P-ERK5 protein levels in each group. (F) Statistical analysis of NUR77 protein levels in each group. (G) Statistical analysis of P-NUR77 protein levels in each group. The control group represents normal bone tissue from rats without fractures and is used only for normalization analysis and is not shown in the statistical analysis graphs.*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.*

Immunohistochemistry

To validate the spatial regulation of NPWT on bone formation in the fracture area, this study used immunohistochemistry to detect OCN expression at the tissue level. Immunohistochemistry results showed that under 100× and 400× magnifications, a distinct brown OCN-positive signal was visible in the fracture end tissue of the Model+NPWT group. In the Model+NPWT+BIX group, the intensity and distribution of OCN-positive signals were significantly lower than those in the NPWT group, further suggesting that activation of the ERK5 signaling pathway plays a key role in NPWT-induced osteogenesis (Figure 4). Immunohistochemical observations were consistent with the trends of qRT-PCR and Western blot assays, further confirming that NPWT enhances local bone formation by upregulating OCN expression, which is positively correlated with BMP-9 expression.

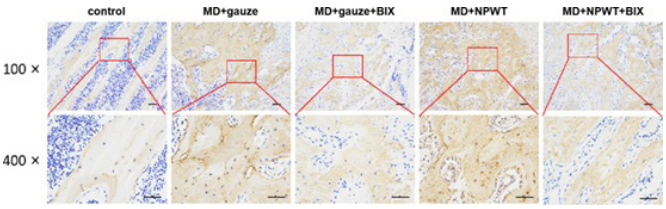

Figure. 4. *Histological changes in each group. At 100× and 400× magnification, distinct brown OCN-positive signals were observed in the fracture tissue of the Model+NPWT group, widely distributed in the newly formed trabeculae and callus areas (red frame). Scale bar, 50 μm.*

Discussion

Fracture repair is a complex biological process involving the activation and regulation of multiple signaling pathways. In recent years, with the application of vacuum sealing drainage technology in clinical fracture treatment, its role in promoting bone repair has gradually attracted attention. Although studies have demonstrated a link between vacuum sealing and fracture healing,[14,15] the related signaling pathways are rarely reported. This study aimed to establish an SD rat tibial fracture model and systematically explore whether NPWT promotes fracture healing by preferentially activating the MAPK/ERK5 signaling pathway, thereby regulating the expression of osteogenic factors BMP-9 and OCN.

BMP-9, a member of the TGF-β superfamily, is considered one of the factors with the greatest known osteogenic potential.[16,17] It can not only effectively induce MSCs to differentiate into osteoblasts but also upregulate a variety of osteoblast-related genes, including *Runx2*, *OCN*, and *ALP*.[7,18,19] Park et al.[20] reported that BMP-9 has a stronger osteogenic effect than BMP-2 and BMP-7, providing a theoretical basis for its application in bone tissue engineering and fracture repair. In this study, the results of PCR and Western blot showed that after negative pressure treatment of the fracture ends of rats, the expression of BMP-9 mRNA and protein levels in the callus tissue of the fracture ends was significantly higher than that in the gauze group and the blank group, and the difference was

statistically significant. This demonstrates that the osteogenic potential of the fracture ends was significantly enhanced after NPWT treatment, thereby improving the fracture-healing microenvironment and promoting fracture healing.

It is worth noting that BMP-9 promotes callus formation, osteoblast maturation, and trabecular reconstruction, as well as ectopic bone formation and angiogenesis.[12,23-25] In this study, a complete fracture model was used. Under the conditions of bone-end contact and periosteum retention, the function of BMP-9 is more reflected in regulating the activity and differentiation level of osteoblasts in the fracture area. This expression background under this physiological environment enables us to more accurately evaluate the role of BMP-9 in the natural process of fracture repair. NPWT, as a non-drug, exogenous factor-free, physiological stimulation method, induces BMP-9 expression, suggesting that this technology may intervene in the molecular chain of bone repair by activating endogenous factors. Our results confirmed that NPWT preferentially promotes BMP-9 overexpression and thereby enhances fracture healing. The plasma concentration of ALP is a biochemical indicator of bone formation, and its changes are the core biomarkers of bone formation and mineralization during fracture healing.[21] The study by Moss et al.[22] showed that ALP activity began to increase significantly 2 weeks after surgery, indicating enhanced osteoblast activity and the initiation of mineralization. In this study, ALP activity was consistent with that of BMP-9. At 2 weeks, RNA and protein levels in the negative-pressure group were significantly higher than those in other groups, demonstrating that negative pressure enhances osteoblast activity and accelerates bone formation. In this study, immunohistochemical results also confirmed that the callus tissue content in the negative pressure group was significantly higher than in the gauze and blank groups, indicating that negative pressure enhances osteoblast activity.

Osteogenesis is an essential stage in fracture healing. OCN, as a marker of osteogenic transformation, is widely used to reflect the degree of bone formation and mineralization activity.[26-29] Zhang et al.[30] observed in a mouse ectopic osteogenesis model that OCN expression was strong in BMP-9-induced bone-like tissue and was positively correlated with new bone density. Studies have shown that BMP-9 can not only regulate early osteogenic genes such as *Runx2* and *COL1A1*, but also significantly upregulate OCN expression, thereby promoting osteoblast maturation and bone matrix calcification.[20,31,32] In this experiment, immunohistochemical OCN-positive staining in the NPWT group was significantly enhanced, indicating accelerated local bone formation. At the same time, the mRNA and protein expression of OCN in the callus of the negative pressure group was significantly higher than that in the gauze group and the blank group. This shows that NPWT can significantly increase OCN expression, promoting osteoblast maturation and accelerating bone formation and fracture healing. On the other hand, OCN expression in the negative pressure group showed a positive correlation with BMP-9, indicating that the significantly elevated OCN expression and the promotion of osteoblast maturation and fracture healing were inseparable from BMP-9 regulation. It also showed that negative pressure preferentially activated BMP-9 to participate in fracture repair, while regulating OCN to promote the conversion of bone tissue into osteoblasts, thereby accelerating fracture healing.

Although negative pressure can preferentially mobilize BMP-9 to the fracture site, the underlying molecular mechanism remains unclear. Studies have shown that the transduction and expression of BMP-9 are regulated by multiple signaling pathways, including the canonical Smad-dependent pathway and the non-canonical MAPK pathway. [10,33-36] Among these, the MAPK/ERK5 pathway has recently been shown to play an essential role in the early stages of bone formation. Studies have shown that MEK5 activation of ERK5 can promote the expression of osteogenic transcription factors such as MEF2C and c-Fos, thereby regulating the activity of downstream osteogenic factor genes.[37-39] MEK5, as its upstream kinase, can specifically phosphorylate and activate ERK5, thereby controlling the transcriptional activity of its target transcription factors. In this study, immunohistochemistry showed that after negative-pressure treatment of the fracture ends, the protein expression levels of ERK5 and MEK5 in the NPWT group were significantly higher than those in the gauze and blank groups. At the same time, the expression of p-MEK5 and p-ERK5 was significantly upregulated in the Model+NPWT group, indicating that negative pressure preferentially activates the MAPK/ERK5 signaling pathway and promotes ERK5 phosphorylation.

In addition, ERK5 signaling plays a unique role in osteogenesis regulation. Under BMP-9 stimulation, activation of the ERK5 pathway can significantly enhance the expression of transcription factors such as Runx2 and Osterix, thereby driving MSC differentiation into mature osteoblasts.[40-43] In this study, we found that BMP-9 increased significantly after negative pressure treatment, proving that it has activated ERK5 signaling and thereby increased the overexpression of downstream factors. At the same time, MEK5, ERK5, BMP-9, and OCN in the negative pressure group + inhibitor group were significantly higher than those in the gauze+inhibitor group, demonstrating that BMP-9 stimulates ERK5 signaling and increases the overexpression of osteogenesis-related factors, thereby promoting fracture healing. It also showed that NPWT promotes the overexpression of ERK5 and MEK5, suggesting that this intervention may integrate the BMP-9 regulatory chain with ERK5 as the hub.

The therapeutic effect of NPWT in fracture healing stems not only from its fluid- drainage and decompression functions, but also from the activation of cellular mechanical signaling through micro-negative-pressure mechanical stimulation and the tension environment it creates. Multiple studies [5,44,45] have shown that NPWT can regulate local tissue stress distribution and cytosol circulation, thereby activating mechanosensitive elements (such as integrins, FAK, and YAP/TAZ) in osteoblasts and endothelial cells, and upregulating the expression of a series of osteogenesis-related signaling molecules. The MAPK/ERK family itself is a core component of the mechanical stimulation response pathway and is highly activated under stressful environments, which is consistent with the mechanical stimulation by negative pressure. This

may also be related to the preferential activation of the MAPK/ERK signaling pathway by negative pressure. Wen et al.[46] showed that cyclic tensile stress can significantly enhance ERK5 phosphorylation in MSCs and induce the expression of bone-specific markers. In this study, we observed that the expression levels of ERK5 and MEK5 in the NPWT group were significantly higher than those in the other 3 groups, suggesting that the ERK5/MEK5 signaling pathway may respond to the mechanical stimulation of negative pressure and the stress signals induced by stretching, thereby activating the signaling pathway and regulating related factors.

In summary, this study, based on an SD rat fracture model, systematically demonstrated for the first time that NPWT may enhance the osteogenic response at the fracture site by activating the MAPK/ERK5 signaling pathway and upregulating BMP-9 and OCN expression. This mechanism was validated through multiple experimental approaches, including changes in mRNA expression and protein levels, as well as enhanced OCN expression at the tissue level, establishing a complete chain of evidence from signaling to functional manifestation. If this strategy is combined with NPWT, it may open new therapeutic avenues for patients at high risk of severe fractures, nonunion, and bone infections.

Ethical Statement

All animal experiments in this study were approved by the Laboratory Animal Ethics Committee of Xinjiang Medical University (IACUC-20240227-18, approval date: February 27, 2024) and strictly adhered to the Standards for the Administration of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals.

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Conflicts of Interest

The authors declare that they have no competing interests.

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