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Basic Study

Low intensity pulsed ultrasound reduces alveolar bone resorption during orthodontic treatment *via* Lamin A/C-yes-associated protein axis

Wu T *et al*. LIPUS reduces bone resorption

Abstract

BACKGROUND

The bone remodeling of orthodontic treatment for malocclusion often requires a long duration around two to three years, which also may lead to some complications such as alveolar bone resorption or tooth root resorption. Low-intensity pulsed ultrasound (LIPUS), a noninvasive physical therapy, has been shown to promote bone fracture healing. It's also reported that LIPUS could reduce the duration of orthodontic treatment, however, how LIPUS regulates the bone metabolism during orthodontic treatment process is still unclear.

AIM

To investigate the effects of LIPUS on bone remodeling in orthodontic tooth movement (OTM) model and explore the underlying mechanisms.

METHODS

A rat model of OTM was established, and alveolar bone remodeling and tooth movement rate were evaluated *via* micro-computed tomography and staining of tissue sections. *In vitro*, human bone marrow mesenchymal stem cells (hBMSCs) were isolated to detect their osteogenic differentiation potential under compression and LIPUS stimulation by quantitative reverse transcription polymerase chain reaction, western blot, alkaline phosphatase (ALP) staining and Alizarin red staining. The expression of yes-associated protein (YAP1), the actin cytoskeleton, and the Lamin A/C nucleoskeleton were detected with or without YAP1 small interfering RNA (siRNA) application *via* immunofluorescence.

RESULTS

The force treatment inhibited the osteogenic differentiation potential of hBMSCs; moreover, the expression of osteogenesis markers decreased, such as type 1 collagen (COL1), runt-related transcription factor 2, ALP and osteocalcin (OCN). LIPUS could

rescue the osteogenic differentiation of hBMSCs with increased expression of osteogenic marker inhibited by force. Mechanically, the expression of LaminA/C, F-actin and YAP1 was downregulated after force treatment, which could be rescued by LIPUS. Moreover, the osteogenic differentiation of hBMSCs increased by LIPUS could be attenuated by YAP siRNA treatment. Consistently, LIPUS increased alveolar bone density and decreased vertical bone absorption *in vivo*. The decreased expression of COL1, OCN and YAP1 on the compression side of the alveolar bone was partially rescued by LIPUS.

CONCLUSION

LIPUS can accelerate tooth movement and reduce the alveolar bone resorption by modulating the cytoskeleton-Lamin A/C-YAP axis, which may be a promising strategy to reduce orthodontic treatment process.

Key Words: Low intensity pulsed ultrasound; Bone resorption; Osteogenesis; Cytoskeleton-Lamin A/C-YAP axis; Bone marrow mesenchymal stem cells; Orthodontic tooth movement

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Core Tip: Low-intensity pulsed ultrasound can promote local alveolar bone remodeling and reduce vertical alveolar bone resorption and consequent gingival recession by regulating the osteogenic ability of bone marrow mesenchymal stem cell by upregulating the expression and nuclear translocation of yes-associated protein decreased by mechanical stress *via* affecting the cytoskeleton and nuclear skeleton.

INTRODUCTION

Orthodontic treatment for malocclusion usually lasts 2-3 years, which brings great challenges to patient compliance and increases the risk of many complications, such as alveolar bone resorption, gingivitis, and other destructive diseases of the periodontal supporting tissue[1]. The alveolar bone loss caused by long-term orthodontic treatment may be due to imbalanced osteogenic and osteoclast activity.

Low-intensity pulsed ultrasound (LIPUS) usually refers to a pulse-emitted ultrasonic wave with an intensity between 30 and 100 mW/cm^2, which is a noninvasive physical mechanical energy source[2]. It is delivered in the form of an acoustic wave and applied to tissue and cells to regulate biochemical functions[3]. As revealed by several clinical trials and animal experiments *in vivo*, LIPUS can reduce the fracture healing time[4], effectively treat delayed fracture union[5] and bone nonunion[6], and is safer and noninvasive than other treatments.

LIPUS has also gained attention in the field of orthodontics. *In vivo*, LIPUS can increase the distance of the teeth movement[7]; a retrospective clinical study also showed that LIPUS reduced the duration of invisible treatment by an average of 49%[8]. In a clinical study, buccal alveolar bone thickness and height did not respond to LIPUS during maxillary arch expansion[9]. In a tooth movement model in rats, LIPUS application increased the number of osteoclasts on the compression side[10]. The increased osteoclasts may lead to alveolar bone resorption and periodontal supporting tissue destruction. It's unclear whether LIPUS regulate the alveolar bone remodeling.

Bone marrow mesenchymal stem cells (BMSCs), as bone marrow-derived stem cells, exhibit self-renewal capacity and multiple differentiation potential and can differentiate into multiple types of cells, tissues and organs under certain conditions[11]. LIPUS could regulate MSC growth[12], and can promote chondrogenesis of MSCs seeded on 3D printed scaffolds[13]. Besides, MSCs encapsulated in hydrogels of certain stiffness shows enhanced osteogenesis ability under LIPUS[14]. Few researches reported the effect of LIPUS on cells loaded with compression. Whether LIPUS could regulate the property of MSCs under compression force to control the alveolar bone remodeling during orthodontic treatment and the underlying mechanism need to be investigated.

Here, we show that LIPUS could accelerate the orthodontic tooth movement (OTM) and increase alveolar bone density and decreased vertical bone absorption *via* Lamin A/C-yes-associated protein (YAP) axis. This study shed light LIPUS is promising strategy to accelerate the orthodontic treatment with little side effects.

MATERIALS AND METHODS

Cell culture

The human jawbone tissue sampling protocol gained approval from the Ethical Guidelines of Hospital of Stomatology, Peking University (No. PKUSSIRB-202385020) and was carried out after providing informed consent. Briefly, we obtained nucleated cells from the jaw bone through 60-min digestion using collagenase type I (2 mg/mL; Worthington Biochem, Lakewood, NJ, United States) and dispase II (4 mg/mL; Roche Diagnostic, Indianapolis, IN, United States) for an hour at 37 °C. Single-cell suspensions of nucleated cells from the jaw bone were subsequently acquired using 70-μm cell strainers (BD Bioscience, United States). Then, single cells were seeded onto 100-mm dishes at 1 to 1.5 × 10^6 cells/mL. BMSCs were isolated and cultured following the previously reported protocol[15,16]. BMSCs were cultivated in Minimum Essential Medium α (VivaCell, Shanghai, China) supplemented with 15% fetal bovine serum (Gibco, Grand Island, NY, United States) and 1% penicillin-streptomycin solution (Cytiva, Shanghai, China) at 37 °C with 5% CO_2, and the medium was changed at 3-d intervals.

Transfections of small interfering RNA

Before transfection, the cell culture medium was changed to standard conditions without penicillin-streptomycin solution. The cells were then transfected with control small interfering RNA (siRNA) or YAP1 siRNA (Ribobio, Guangzhou, China) *via* a ribo*FECT*™ CP Transfection Kit (Ribobio, Guangzhou, China) in accordance with the manufacturer's instructions. Quantitative reverse transcription polymerase chain

reaction (qRT-PCR), western blotting and immunofluorescence staining were subsequently performed to measure the knockdown rate.

Osteogenic differentiation, alkaline phosphatase activity and alizarin red staining

BMSCs (1 × 10^5/mL) were seeded onto 6-well plates and cultured until the cell confluence reached 70%-80% before the medium was changed to osteogenic differentiation medium supplemented with l-ascorbic acid (50 μg/mL; Sigma, Missouri, United States), β-glycerophosphate (10 mmol/L; Sigma, St. Louis, Missouri, United States) and dexamethasone (0.1 μM; Sigma, Missouri, United States). During osteogenic induction of stem cells, the induction differentiation medium was changed every 3 d.

After induction for 14 d, alkaline phosphatase (ALP) staining (Beyotime, Shanghai, China) were conducted in accordance with specific protocols, and so as Alizarin Red staining (Sigma, St. Louis, Missouri, United States) after induction for 21 d. For the quantification of mineralization, we dissolved red matrix sediment in 10% cetyl-pyridinium chloride (Macklin, Shanghai, China), and the absorbance of the solution was measured to determine the degree of mineralization quantitatively[17].

Application of a compression force

Compression force was applied to the BMSCs to mimic stress during orthodontic movement. Briefly, a rounded glass sheet (30 mm in diameter) was placed over cell layers close to confluence in a 6-well plate. Stainless steel beads were placed above the glass sheet to adjust the static pressure to 1 g/cm^2. The cells were under static compression for 24 h.

LIPUS treatment

In this study, the LIPUS device was obtained from the Institute of Acoustics (Chinese Academy of Sciences, Beijing, China). The device has circular transducers with an area of 9.07 cm^2 to match the area of a well in a 6-well plate. The cells were stimulated with LIPUS following the following specifications: 1.5 MHz frequency, 0.2 pulse duration

ratio, 30 mW/cm^2 incident intensity, and 1.0 kHz repetition rate. Stimulation was applied for 20 min every day *in vivo* and *in vitro* until the rats and cells were harvested, and the control group and force group were treated with pseudo-LIPUS. *In vivo*, the rats under anesthesia were placed at a constant location, after which the transducer was pressed against the side of the cheek closest to the maxillary first molar. *In vitro*, we attached the transducer to the bottom of the plate corresponding to the well.

qRT-PCR

After 7 d of osteogenic induction, we utilized TRIzol reagent (Invitrogen, California, United States) to extract total cellular RNA following previous protocols[18]. Table 1 displays all the primers used. cDNA synthesis kits (Takara Bio, Tokyo, Japan) were used to prepare first-strand cDNA from RNA through reverse transcription in accordance with specific protocols. A Viia™ 7 Real-time PCR System (Applied Biosystems, Washington, Rhode Island, United States) was used for qRT-PCR, which was carried out in triplicate.

Western blotting

After 7 d of osteogenic induction, RIPA buffer containing 1 mM phenylmethanesulfonyl fluoride (Solarbio, Beijing, China) was added for cell lysis, followed by centrifugation at 12000 × *g* for 20 min at 4 °C. Thereafter, the supernatant was quantified with a BCA assay (Beyotime, Shanghai, China). Total protein was added to loading buffer, and the mixture was subsequently boiled for 10 min at 100 °C. Thereafter, the samples were stored at -20 °C. Equal amounts of total protein (30 μg) were loaded on sodium dodecyl sulfate-polyacrylamide gels and separated prior to electroblotting onto polyvinylidene difluoride membranes (Millipore, Billerica, Massachusetts, United States). After blocking with 5% BSA, the membranes were incubated overnight on a shaker at 4 °C. The primary antibodies used were rabbit anti-type 1 collagen (COL1) and anti-osteocalcin (OCN) (1:500; Abcam, Cambridge, Massachusetts, United States), rabbit anti-ALP (1:500; Invitrogen, California, United

States), and mouse anti-GAPDH (1:1000; ProteinTech, Cook, Illinois, United States). Finally, HRP-labeled secondary antibodies (Zsgb-Bio, Beijing, China) were added for another 1-h incubation, followed by visualization *via* enhanced chemiluminescence (NCM Biotech, Suzhou, China). ImageJ software was used for band intensity analysis.

Immunofluorescence staining

After 7 d of osteogenic induction, 4% paraformaldehyde (PFA) was added for 15 min to fix the cells, after which the cells were rinsed with phosphate buffered saline (PBS) three times prior to 10 min of permeabilization with 0.5% Triton X-100 and washing with PBS. Thereafter, 5% BSA was added to the block cells for an hour before they were incubated with primary antibodies (1:200; Abcam, Cambridge, Massachusetts, United States) against YAP1, Lamin A/C and F-actin at 4 °C overnight. The cells were washed with PBS before further incubation with Alexa Fluor 488- and Alexa Fluor 594-labeled antibodies (1:200; Invitrogen, California, United States) for one hour. Finally, medium containing DAPI was added to the mount cells after washing. Images were captured by an inverted confocal microscope (Olympus, Tokyo, Japan).

Rat model of OTM

Male SD rats (4 wk old) weighing 200 ± 20 g were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and housed under laboratory conditions (12 h light/dark cycle, 21 ± 2 °C, 50% humidity, *ad libitum* access to food and water). All animal protocols utilized in the present work were approved by the Ethics Committee for Animal Experiments at Peking University Health Science Center (No. LA2022288) and were designed to minimize pain or discomfort.

In total, 27 rats were randomized to the control, force or force + LIPUS group. Before establishing the experimental OTM model, each rat was administered pentobarbital sodium (40 mg/kg of body weight) for anesthesia. In those latter two groups, the OTM model was established as described in our previous procedures[19]. A stainless steel ligation wire (0.025 mm in diameter; Tomy International, Inc., Tokyo, Japan) was used

to ligate a closed-coil spring (Tomy International, Inc., Tokyo, Japan) to the maxillary first molar and incisor neck. The spring offers 50 g of force to move the maxillary first molar mesially. To enhance the retaining force and prevent the device from falling off, a 0.5 mm deep groove was made by a slow speed mill near the gingival margin of the maxillary incisor to accommodate the ligature wire, which was subsequently filled with flowing resin (3 M, Minnesota, United States). All animals were sacrificed by an overdose of pentobarbital sodium (150 mg/kg) for tissue collection.

Micro-computed tomography imaging and measurements

A microcomputed tomography scanner was used to scan maxillary samples at 8.82 μm resolution, 500 μA tube current, 60 kV tube voltage, and 1500 ms exposure time. Inveon Research Workplace software (Siemens, Munich, Germany) together with Mimics Research 21.0 software (Materialise, Leuven, Belgium) was used for raw data reconstruction. With respect to the reconstructed 3D model, the shortest distance from the first to second molar crown was considered the tooth movement distance (Figure 1B). A straight plane was made at the cementum-enamel boundary (CEJ) on the first molar's distal buccal side, and the farthest distance between it and the parallel line tangent to alveolar crest resorption was measured to assess the reduction in alveolar bone height. In addition, Inveon Research Workplace software was used to evaluate the BV/TV, Tb.Th, Tb.N, Tb.Sp and BS/BV in the chosen region of interests (ROIs) (Figure 1C) by the reviewer, who was blinded to the groupings. All the samples were analyzed thrice to obtain the means.

Tissue processing

We set the experimental period at 7 and 14 d following the establishment of the tooth movement device. The rats were sacrificed with an overdose of pentobarbital sodium, after which the maxilla was dissected and immersed in 4% PFA. For hematoxylin and eosin (HE), tartrate-resistant acid phosphatase (TRAP) and immunohistochemical staining, the trimmed tissues were decalcified with 5% ethylenediaminetetraacetic acid-

2Na for 15 d, followed by ethanol dehydration and paraffin embedding. Thereafter, the samples were cut into 5-μm vertical serial sections with a rotary microtome (RM2125RT, Leica, Heidelberger, Germany).

HE, TRAP and immunohistochemical staining

Sections were stained with an HE staining kit (Sigma, Missouri, United States) or a TRAP staining kit (Solarbio, Beijing, China) following specific protocols. Immunohistochemical staining was performed as follows. After xylene deparaffinization and ethanol rehydration, 0.125% trypsin and 20 μg/mL proteinase K solution were added to the sections, which were incubated at 37 °C for a 30-min period. Endogenous peroxidase activity was blocked by 3% H_2O_2 for a 30-min period at room temperature. The sections were subsequently washed with PBS, blocked with 5% BSA for an hour and incubated with polyclonal anti-rabbit COL1 and OCN antibodies (1:200; Abcam, Cambridge, Massachusetts, United States) overnight at 4 °C, followed by another 1-h incubation with HRP-labeled secondary goat anti-rabbit IgG (Zsgb-Bio, Beijing, China) at room temperature. Diaminobenzidine (Zsgb-Bio, Beijing, China) was used for visualization in accordance with specific protocols. After hematoxylin counterstaining, gradient ethanol and xylene dehydration, the sections were mounted using neutral resins.

Statistical analysis

The data are presented as the mean ± SD and were analyzed with GraphPad Prism 7 software (GraphPad, Inc., La Jolla, CA, United States). Every assay was carried out thrice. Student's *t* test was used to evaluate intergroup differences, while one-way analysis of variance (ANOVA) with multiple comparisons was used to evaluate differences among multiple groups. $P < 0.05$ indicated statistical significance.

RESULTS

Orthodontic force treatment decreased the alveolar bone height on the compression side

We detected the movement distance of the maxillary first molars on the 7th d and 14th d after force applied and found that the movement distance increased with time under the effect of 50 g of force ($P < 0.01$, $n = 3$) (Figure 1A), suggesting that the rat OTM model was successfully constructed. Moreover, the distance between the alveolar crest and CEJ significantly increased on day 7 and day 14, showing that orthodontic force led to vertical resorption of alveolar bone beginning on day 7 (Figure 1B). We selected the alveolar bone mesial to the medial 1/3 of the first molar's distal buccal root with a certain thickness as the ROI (Figure 1C). Micro-computed tomography results showed that at 7 and 14 d of tooth movement, the alveolar bone BV/TV and Tb.Th decreased after force applied, while the BS/BV and Tb.Sp increased after force applied (Figure 1C). This result showed that the bone density decreased on the compression side after orthodontic force treatment. Immunohistochemical staining revealed a decreased number of cells positive for the osteogenic markers COL1 and OCN in alveolar bone on the pressure side (Figure 1E), with a greater number of TRAP-positive cells on the pressure side near the alveolar bone edge (Figure 1F).

The osteogenic differentiation of BMSCs were inhibited by force treatment

Static pressure was used *in vitro* to simulate alveolar bone on the pressurized side. Compared to those in the control group, the osteogenic differentiation of the BMSCs in the force group was decreased, as evidenced by the ALP and alizarin red staining results (Figure 2A-D). Correspondingly, the expression levels of the osteogenesis-related markers ALP, COL1, runt-related transcription factor 2 (RUNX2) and OCN in the force group significantly decreased (Figure 2E and F) compared to the control ones, detected by qRT-PCR and western blot.

LIPUS the osteogenic differentiation of BMSCs

The force treatment decreased the osteogenic differentiation of BMSCs, while LIPUS could rescue the impaired osteogenic differentiation of BMSCs caused by force treatment (Figure 3A-D), analyzed by ALP and Alizarin Red staining. Moreover, the

expression of ALP, COL1, RUNX2 and OCN were accordingly increased after LIPUS treatment compared with the force group, as assessed by qRT-PCR (Figure 3E) and western blot (Figure 3F).

LIPUS decreased alveolar bone resorption in vivo

The movement distance of the first molar in the force + LIPUS group is greater than that in the force group on the 7th and 14th d after force applied, ($P < 0.05$) (Figure 4A-D). Moreover, the height of alveolar bone resorption was also reduced in the LIPUS treatment group ($P < 0.05$) (Figure 4E and F). ROI measurements of the corresponding sites indicated that the BV/TV and Tb.Th of the force + LIPUS group were higher than those of the force group, while the BS/BV and Tb.Sp were decreased ($P < 0.05$, $n = 3$) (Figure 4G-I), indicating that LIPUS promoted alveolar bone formation. Furthermore, the expression of COL1 and OCN, were increased on the compressed side in LIPUS treatment group compared with the force group (Figure 5A-D). However, no significant differences in TRAP-positive cell numbers were observed between the force group and the force + LIPUS group (Figure 5B and E).

LIPUS activated the YAP1 signaling via Lamin A/C

To explore whether the role of LIPUS in regulating the cytoskeleton in BMSCs, we performed F-actin and Lamin A/C immunofluorescence co-staining *in vitro*. The results showed that the compressive force treatment downregulated the expression of F-actin and disrupted the cytoskeleton and inhibited the Lamin A/C ratio in BMSCs, while LIPUS effectively reversed these changes (Figure 6A). Lamin A/C was reported to mediate the YAP1 nuclear localization by regulating nuclear stiffness[20,21]. Next, we analyzed the expression of YAP1, the results showed that the expression of YAP1 was decreased by force treatment, whereas LIPUS restored the expression of YAP1 and increased the nuclear localization of YAP1, analyzed by immunofluorescence staining and western blot (Figure 6B and C). Consistently, the expression of YAP1 were

decreased in the force group, while its expression was increased in the LIPUS treatment group *in vivo* analyzed by immunochemical staining (Figure 6D).

Knockdown of YAP1 blocked the osteogenic differentiation of BMSC induced by LIPUS

To verify the role of YAP1 on the property of BMSCs, we used YAP1 siRNA to treatment BMSCs (Figure 7A) and the results showed that the increased expression of osteogenic differentiation-related marker ALP, COL1, RUNX2 and OCN increased by LIPUS treatment could be blocked by YAP1 siRNA treatment (Figure 7B and C). The osteogenic differentiation of BMSCs increased by LIPUS treatment could also be inhibited by YAP1 siRNA treatment as assessed by ALP and Alizarin red staining staining (Figure 7D and E). Our results demonstrated that LIPUS can accelerate tooth movement and reduce alveolar bone resorption by modulating the cytoskeleton-Lamin A/C-YAP axis (Figure 8).

DISCUSSION

The process of orthodontic treatment is the bone remodeling process. The force applied to drive tooth movement in some extent led to some adverse reactions, such as root resorption and bone mineral density decline. LIPUS has been reported alleviating chondrocyte damage in temporomandibular disorders[22], reducing root resorption[23] and enhancing bone remodeling[10]. However, there is lack of research on the effect of LIPUS on the aesthetic problem of gingival recession due to the loss of alveolar bone height during orthodontic treatment. Changes in alveolar bone morphology affect the aesthetic effect, safety, and stability[24] of orthodontic treatment. Clinical studies have shown that alveolar bone height loss often leads to insufficient periodontal supporting tissue, decreased dental stability, and aesthetic problems in the anterior teeth[25]. Our experiments showed that LIPUS could effectively ameliorate the aesthetic and health problems caused by alveolar bone height loss by reducing vertical alveolar bone resorption and improving the morphology of alveolar bone remodeling, and filled the research gap in the relevant field.

Clinical studies have used CBCT to assess changes in periodontal bone mineral density during orthodontic treatment. On average, the alveolar bone mineral density decreases by 24% after 7 months of orthodontic treatment, while the region with the largest reduction in bone mineral density seems to be related to the direction of tooth movement[26,27]. Consistently, in the present study, we found that bone mineral density decreased at 7 and 14 d after stress. Mechanical stress can modulate osteoclasts and osteoblasts, thereby promoting bone reconstruction[28,29]. Excessive compressive force can cause stem cell apoptosis[30]; aggravate mitochondrial dysfunction, ATP consumption and oxidative stress in stem cells[31]; and inhibit the proliferation, colony formation and migration of stem cells[32]. Bone mineral density is positively correlated with OCN protein level[33], In our study, static pressure on BMSCs inhibited the mRNA and protein expression of osteogenic differentiation markers such as COL1 and OCN, which partly explained the reduction in alveolar bone mineral density around moving teeth under orthodontic force.

Previous studies have shown that LIPUS plays a role in bone metabolism and remodeling by regulating osteogenic and osteoclast activity[34-36]. In our study, on day 7 and day 14, the number of osteoclasts in the force group was not significantly different from that in the force + LIPUS group, indicating that LIPUS may promote bone remodeling by promoting osteoclast activity but not by increasing the number of osteoclasts. As shown in numerous clinical trials and animal studies, LIPUS promotes osteogenesis, resulting in improved and quicker fracture healing[37,38]. It can also stimulate condylar growth and increase mandibular ramus height in rabbits[39,40]. LIPUS is effective in various cell processes, such as growth, differentiation[41,42], extracellular matrix formation, and mineralization of osteoblasts[43], and involves multiple signaling pathways, such as the hedgehog and TRPM7 pathways[44,45]. In addition to osteoblasts, LIPUS can promote adipose-derived stromal cells[46], mesenchymal stem cells[47], periodontal ligament stem cells[48], and rat osteosarcoma cell lines[49] and increase the expression of RUNX2 and COL1. LIPUS stimulation of BMSCs can enhance cell activity, promote osteogenic differentiation, and increase COL1 and OCN gene expression[50]. To

date, few reports have focused on the role of LIPUS in cellular functions under loading conditions. In our study, LIPUS increased COL1, RUNX2 and OCN expression at the mRNA and protein levels but rescued the inhibitory effect of compression force on the osteoblastic differentiation of BMSCs, demonstrating a positive effect on alveolar bone remodeling during orthodontic processing at the cellular level.

The way mechanical signals can be transmitted from the plasma membrane to the nucleus directly *via* the cytoskeleton is considered to be an important mechanical signal transduction pathway[51]. The nucleus is considered a key mechanoreceptor that can directly influence chromatin organization, epigenetic modifications, and gene expression[52]. Lamin A/C, as LMNA-encoded Lamin proteins, participate in nuclear mechanics[53] and the transduction of mechanical signals[54,55], thereby regulating the fate of stem cells. The lincRNAs (linkers of cytoskeleton and nucleoskeleton) complex links Lamin A/C to the cytoskeleton, thereby mediating the transmission of mechanical signals from the cytoskeleton to nuclei[56]. The Lamin A level is known to increase during osteogenic differentiation of MSCs but decrease during adipogenic differentiation[57,58]. In our study, Lamin A/C and F-actin were consistently downregulated after compression force application, and the cytoskeleton was more disordered under compression. To our knowledge, our study is the first to show that LIPUS increases Lamin A/C and F-actin expression and reorders the cytoskeleton under compression, thus reversing the decrease in osteogenesis of BMSCs induced by static force.

As a transcriptional coactivator protein, YAP1 is closely associated with changes in the mechanical state of cellular microenvironments[59]. YAP1 can relocate to the nucleus from the cytoplasm, interact with the TEA domain[60] and promote transcription. In mechanical transduction, it is also a downstream signal for the assembly and contraction of actin filaments[61]. Pressure can promote F-actin depolymerization and lead to cytoplasmic translocation of YAP1[62]. YAP1 was found to participate in multiple cellular activities. For example, YAP1 was reported to be activated during inflammation in endothelial cells induced by lipopolysaccharide[63] and was essential for epithelial cell

proliferation[64]. In the inflammatory microenvironment in periodontitis, YAP1 expression and nuclear translocation are decreased[65]. In the force group, the YAP1 level decreased, while in the force + LIPUS group, the YAP1 level increased both *in vitro* and *in vivo*, suggesting that YAP1 exerts a crucial effect on the regulation of BMSC osteogenesis by mechanical force and LIPUS and might be a downstream effector of the cytoskeleton and nuclear skeleton.

This study still has some limitations. Osteoblasts and osteoclasts jointly participate in the process of bone metabolism. This study mainly focused on the effect of LIPUS on the osteogenic function of stem cells, whether LIPUS could regulate the crosstalk of osteoclast and osteoblast is unclear, which need further investigation. In addition, the underlying mechanism of how LIPUS controls cytoskeleton changes remain unclear.

CONCLUSION

In summary, LIPUS can promote local alveolar bone remodeling, increase bone mineral density, reduce vertical alveolar bone resorption and consequent gingival recession by regulating the osteogenic ability of BMSCs. In terms of mechanism, LIPUS upregulated the expression and nuclear translocation of YAP, which was decreased by mechanical stress through effects on the cytoskeleton and nuclear skeleton, thereby affecting the osteogenic differentiation of BMSCs.

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