Effect of Low Load Vibration on the Morphology and Structure of Osteoblast Cytoskeleton

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Abstract: Mechanical vibration is a common physical phenomenon, and mechanical vibration therapy has a long history and is currently widely used in the treatment of clinical diseases. In recent years, research on promoting fracture healing and preventing osteoporosis through vibration has been continuously deepened. From cell experiments to animal experiments and human experiments, it has been fully confirmed that low load vibration can promote bone formation, inhibit bone resorption, and have significant osteogenic effects. Low load vibration (LLV) has become a promising non-invasive treatment method in the fields of bone biology and regenerative medicine. But little is known about the cellular biology underlying this physiological phenomenon. For instance, the morphology of cells is crucial for cellular function, but there is no comprehensive description of how these morphological features are affected by vibration, what signals the cytoskeleton transmits inward after low load vibration, and what molecular effects it causes. This review aims to summarize the existing research on the effects of low load vibration on the morphology and structure of osteoblast cytoskeleton, as well as the intracellular signal transduction caused by it. It is expected to establish the connection between cell morphology and molecular mechanisms in low load vibration treatment of osteoblasts experiments.

***Keywords*:** low load vibration; osteoblast; cytoskeleton

1. Introduction

In recent years, the field of bone biology has garnered significant attention due to the escalating incidence of bone-related disorders, particularly osteoporosis and fractures, which pose a substantial burden on public health worldwide. These conditions are often accompanied by altered bone microstructure and reduced bone formation, underscoring the need for effective therapeutic interventions[1]. Among various non-invasive treatments, low-load vibration (LLV) has emerged as a promising modality, demonstrating its potential to stimulate bone growth and enhance fracture healing processes.

The cellular response to LLV is complex and multifaceted, involving intricate interactions between mechanical stimuli and biological systems. Of particular interest is the impact of LLV on osteoblasts, the primary bone-forming cells, and their cytoskeleton, which serves as a dynamic scaffold for cellular function and organization. The cytoskeleton, consisting of actin filaments, microtubules, and intermediate filaments, plays a pivotal role in transmitting mechanical signals to the nucleus, thereby modulating gene expression and cell behavior[2, 3].

Studies have shown that LLV, typically administered at frequencies ranging from 30 to 50 Hz and with peak accelerations of 0.2 to 0.3g, can stimulate osteoblast proliferation and differentiation, leading to increased bone formation[4, 5]. However, the specific effects of LLV on the morphology and structure of the osteoblast cytoskeleton remain largely unexplored. Understanding these effects is crucial for developing optimized therapeutic protocols that can maximize bone healing and regeneration.

The present review aims to provide a comprehensive overview of the current literature on the impact of LLV on the osteoblast cytoskeleton and its underlying molecular mechanisms. By synthesizing findings from both in vitro and in vivo studies, we will discuss how LLV modulates osteoblast cytoskeletal architecture and how the cytoskeleton serves as a signal relay station to transmit relevant signals into the cell. This review seeks to identify gaps in knowledge and suggest directions for future research, ultimately contributing to the development of more effective therapeutic strategies for bone-related disorders.

2. The biomechanical characteristics of low load vibration

Bones are composed of cells, extracellular matrix, and inorganic substances, and bone cells include osteoblasts, osteoblasts, osteoblasts, and osteoclasts. Osteoblasts are the main effector cells of mechanical stress stimulation for bone

formation and remodeling, and appropriate vibration frequency and intensity can promote osteoblast proliferation and differentiation[6].

Zha et al. [7] used a self-made composite vibration instrument to vibrate osteoblasts cultured in vitro, dividing them into a non vibration group and five experimental groups subjected to different vibration frequencies (3-10Hz, 15-30Hz, 25-45Hz, 50-80Hz, 80-100Hz). After 6 days of vibration stress loading, changes in cell cycle, cell proliferation ability, and alkaline phosphatase activity were detected. The conclusion is that the cell proliferation ability and ALP activity in the vibration group are significantly higher than those in the non vibration group ($p<0.01$). Different frequencies of vibration strain have an impact on the biological characteristics of osteoblasts, promoting cell proliferation and differentiation, with the optimal frequency being 15-45Hz[8]. The International Vibration Conference held in Hong Kong in 2007 unanimously concluded that mechanical vibration stimulation with a vibration frequency of 32-37Hz and an acceleration of 0.2-0.3g had the best effect in preventing and treating osteoporosis.

3. The cytoskeleton structure of osteoblasts

The osteoblast cytoskeleton is a complex protein fiber network that plays a crucial role in bone formation and remodeling. It consists of three main components: microtubules, microfilaments, and intermediate filaments, which together maintain cell morphology and support intracellular structure[9, 10]. In addition, the cytoskeleton serves as a sensor, accumulator, and converter for mechanical load signals. It not only directly transmits mechanical stress, but also connects with other mechanical sensors such as NOX2, calcium channels, integrins, and focal adhesion. These connections enable the cytoskeleton to transmit changes in the extracellular matrix (ECM) to the interior of the cell, acting as signal centers in mechanical signal transduction pathways. The interaction between the cell skeleton and the nuclear skeleton and cell skeleton (LINC) complex on the nuclear membrane. This interaction enables the cytoskeleton, including microfilaments, microtubules, and intermediate fibers, to directly transmit mechanical signals to the nuclear membrane[11]. This direct transmission is facilitated by the "ECM-integrin-talin-F-actin" connection mode mentioned previously.

In addition, the cytoskeleton can affect the expression of nuclear genes by regulating the activity of transcription factors in the cytoplasm. These transcription factors are typically inactive in unstimulated cells and migrate to the nucleus when stimulated, where they affect gene expression[12]. The cytoskeleton affects this process by regulating the activity of these transcription factors, thereby affecting the expression of nuclear genes.

In summary, there are two ways in which the cytoskeleton transmits mechanical signals: one is to directly transmit mechanical stress to the nuclear membrane; Another method is to convert mechanical signals into chemical signals, which then enter the nucleus to exert their effects.

4. The effect of low load vibration on the cytoskeleton of osteoblasts

The cytoskeleton is a dynamic mechanical structure whose organization often adapts to the mechanical environment in which the cell is located[13]. Mechanical signals can be transmitted from the cell membrane to the nucleus through the linker of nucleus and cytoskeleton (LINC) complexes in the cytoskeleton[14]. The FEM results confirmed that the effect of LLV vibration on cells is not strain dependent as in other studies. Therefore, the classical mechanical pathway[15] from extracellular to intracellular may differ in LLV vibration.

Rosenberg[16] found in his study that after applying low load vibration to osteoblasts, the total DNA content of the experimental group was significantly higher than that of the static group. However, the addition of colchicine (the specific microtubule block) could relieve this enhancing effect, indicating that the mechanical enhancement of osteoblast proliferation activity is specifically mediated by the microtubule components of the cytoskeleton.

In the study by Gao et al.[4], Phalliodin-FITC cytoskeleton staining images showed that compared with the control group, osteoblasts in the low load mechanical vibration group exhibited well-developed cytoskeleton, higher fluorescence intensity, increased number of oriented microfilaments, and thicker stress fibers.

Low load vibration can promote the expression of osteogenic related genes and reduce the expression of osteoclasts. External biomechanical stimulation is related to regulating cytoskeleton remodeling. Haffner et al.[17] found that LLV can increase actin remodeling, increase the number of microfilaments, and thicken stress fibers in the vibration group. These studies suggest that the vibration induced osteoblast effect may depend on the rearrangement of the cytoskeleton.

5. Biochemical signals in osteoblasts induced by low load vibration

5.1 NO signal transduction

NO signaling is related to bone adaptation to mechanical loads. For example, eNOS gene knockout mice exhibit reduced

bone mass accumulation during development, leading to decreased osteoblast function. Mechanical loading increases the activity of iNOS and eNOS in the body, and inhibiting this activity can suppress load induced bone formation[18]. In vitro, it has been reported multiple times that bone cells release NO under mechanical stimulation, which is associated with MAPK signaling, cytoskeleton adaptation, and PGE2 signaling[19, 20]. Interestingly, it has recently been demonstrated that primary cilia containing polycystic lutein are involved in NO signaling[21].

5.2 Wnt signaling pathway

The typical Wnt signaling pathway, also known as the Wnt/β - catenin pathway, is associated with promoting osteoblast differentiation and proliferation, as well as inhibiting osteoblast apoptosis[22, 23]. The extracellular Wnt protein binds to the curled and low-density lipoprotein receptor associated protein (Lrp) 5/6 co receptors on the cell membrane, which subsequently leads to the stabilization of β - catenin in the cytoplasm and may further promote the transcription of Wnt targeted genes in the nucleus. After low load vibration treatment, the protein expression levels of typical Wnt signaling pathway members Wnt3a, Lrp6, and β - catenin in osteoblasts were significantly higher in the mechanical vibration group than in the control group. In addition, the expression of alkaline phosphatase (ALP), osteocalcin (OCN), Runx2, BMP2, and osteoprotegerin (OPG) mRNA also increased significantly. The changes in the levels of these cytokines suggest that a series of intracellular chain reactions can be induced through low load oscillations, promoting the generation and secretion of osteogenic related molecules[24, 25].

5.3 MAPK signaling pathway

The mitogen-activated protein kinase (MAPK) signaling pathway, known for its role in transducing extracellular signals into intracellular responses, has been implicated in the regulation of osteoblast proliferation, differentiation, and function. This pathway is intimately linked to cytoskeletal dynamics, as it governs the expression of genes involved in cytoskeletal organization and function[26].

Schmidt et al. found that due to mechanical stress, the phosphorylation of MAP kinase increases, and the activation of MAP kinase by integrins depends on the involvement of intact cytoskeleton and cytoskeleton related signaling molecules, such as focal adhesion kinase. This fact emphasizes the importance of controlled cytoskeletal anchoring of tyrosine phosphorylated proteins in influencing cellular behavior[27].

The cytoskeleton plays a crucial role in the physical anchoring of activated signaling molecules, thereby transforming physical forces into biochemical signaling events.

6. Future research directions and challenges

When delving into the future research directions and challenges surrounding the impact of Low load vibration (LLV) on osteoblast cytoskeleton morphology and structure, several pivotal avenues emerge. Firstly, it is crucial to further elucidate the precise molecular mechanisms underlying how LLV modulates osteoblast cytoskeleton dynamics. This involves identifying key signaling pathways and transcriptional regulators that are activated or repressed in response to LLV, leading to alterations in cytoskeletal organization and function.

Secondly, there is a need to investigate the long-term effects of LLV exposure on osteoblast health and bone remodeling processes. Current studies have primarily focused on acute or short-term responses; however, a comprehensive understanding of the chronic effects is essential for the development of effective therapeutic interventions.

A third direction for future research lies in exploring the potential synergy between LLV and other osteogenic stimuli, such as mechanical loading, growth factors, or pharmacological agents. By combining these approaches, it may be possible to enhance the osteogenic effects of LLV, leading to more efficient bone repair and regeneration strategies.

We also face some challenges in this field, including the complex interplay between genetic, epigenetic, and environmental factors that influence osteoblast response to LLV. Additionally, the heterogeneity of osteoblast populations within bone tissues adds another layer of complexity that must be taken into account[28]. Furthermore, the translation of preclinical findings into clinically applicable therapies remains a significant hurdle, requiring rigorous validation in animal models and human trials.

7. Discussion

We have reviewed the mechanical transduction of bone, tracking mechanical loads from macroscopic to molecular levels, outlining potential candidates for molecular transduction of low load vibrations as mechanical signals mediated by the cytoskeleton to biochemical signals, and then tracking biological responses as they are integrated at the organ level. By this way, we hope to have provided a solid foundation for interested researchers. We also hope that we have demonstrated

that the mechanical aspects of this system are much less understood than the biological aspects. Especially, there is little conclusive understanding of osteoblasts and extracellular mechanics. We believe that research in this field has the conditions to generate important new insights. We also suggest that the increasing use of in vitro cell models for insights will need to be translated into the biological level. If creative research results can be obtained in these areas, we believe it can promote the development of the treatment of bone related diseases.

In conclusion, the future of research on the effects of LLV on osteoblast cytoskeleton morphology and structure holds great promise but also poses numerous challenges. By addressing these challenges and pursuing innovative research directions, we can gain a deeper understanding of the mechanisms underlying bone remodeling and develop more effective strategies for promoting bone health.

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