Introduction: Mechanical stimulation to bones affects bone formation and bone cells have been reported to sense and respond to mechanical stimulation. However, osteoblast responses to mechanical simulation are less understood. By using a finite element (FE) method, the aim of this study was to investigate the responses of a cultured osteoblast to vibration of a broad range of frequencies. Firstly, the mode shapes were developed and natural frequencies were extracted. Subsequently, the base excitation acceleration 1 g and vibration frequency range 1−80 Hz were used to conduct harmonic analyses. Finally, the response of harmonic vibration was obtained as the curve of displacement versus frequency.

Subjects and Methods: According to the images of osteoblasts in vitro, an idealized FE model of an osteoblast was created using FE software ABAQUS. In this study, the osteoblast was comprised of three components, namely cell membrane, cytoplasm and nucleus. Based on parameters from previous studies, the whole volume of the osteoblast and nucleus volume were ~3000 μm³ and 105 μm³, respectively. In addition, the cell height was ~9.4 μm and the membrane thickness was ~6 nm. The cell was assumed as an elastic isotropic material. The natural frequencies were extracted by using ABAQUS software. The governing dynamic response equation of vibration of the osteoblast may be expressed by Eq (1):

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\text{F} = \text{M} \ddot{\text{x}} + \text{Ku} \text{x}.
\]

\[
\text{M} = \text{m} + \mu \text{m}.
\]

\[
\text{K} = \text{Ku} + \text{Ku} + \text{Ku}.
\]

Methods: Place all cultured cells were divided into three groups: control group (Ti-negative group), non-irradiated group (0 Gy group) and the irradiation group (0.5 Gy group). 0 Gy group and 0.5 Gy group stimulated by of Ti particles with LPS adhered. 0.5 Gy group received 0.5 Gy irradiation 1h after stimulation. The expression of IL-1β, IL-6 and TNF-α gene were measured by Real-time PCR method at 2, 4, 8, 12h after stimulation. The concentration of IL-1β, IL-6 and TNF-α in supernatant were measured by ELISA at 12, 24h after stimulation. The expression of Ph-p65, p65, Ph-perk, Ph-p38 in three groups were measured by Western Blot method at 4, 8, 12h after irradiation.

Results: Real-time PCR showed 0.5 Gy of LDI can reduce the increased expression of IL-1β, IL-6 and TNF-α in macrophage by stimulated by LPS Ti particles with LPS adhered. ELISA analysis showed 0.5 Gy of LDI could also inhibit the increased secretion of IL-1β, IL-6 and TNF-α. This inhibition is more obvious 12h after irradiation. The secretion of the most sensitive factor TNF-α is barely increased between 12~24h. Western Blot analysis showed 0.5 Gy of LDI can reduce the phosphorylation of p65 and ERK after, but had no effect on the phosphorylation of p38.

Conclusion: After playing a biological role, LDI not only effectively inhibited the inflammatory stimuli of the primary mouse peritoneal macrophage, which caused by Ti particles with LPS adhered, but also significantly reduce the formation of IL-1β, IL-6, TNF-α. This effect may be achieved by inhibition two pathways of NF-κB activation. A possible mechanism is that LDI would inhibit the expression of catabolic genes and related proteins under excessive mechanical stimulation duration (16 h) increased, yet the expression of anabolic genes and related proteins decreased. There were no obvious difference in cell morphology in the 0, 2, 4 and 8 h stimulation groups. CTFM measurements showed that the CTF of AFSCs gradually decreased as the mechanical stimulation duration for various durations. Changes in gene expression and related proteins were determined using RT-qPCR and ELISA analyses. The morphology of cells was visualized by cytoskeleton staining. The cell traction forces (CTFs) of cells were measured using cell traction force microscopy (CTFM).

Results: The expression of anabolic genes (type I collagen, type II collagen, aggrecan and related proteins in AFSCs undergoing moderate mechanical simulation duration (4 h) increased after CTS. The expression of catabolic genes (MMP-3, MMP-13, TIMP-1) and related proteins in AFSCs undergoing excessive mechanical simulation duration (16 h) increased, yet the expression of anabolic genes (aggrecan, type I collagen, and type II collagen) and related proteins decreased. There were no obvious difference in cell morphology in the 0, 2, 4 and 8 h stimulation groups. CTFM measurements showed that the CTF of AFSCs gradually decreased with the increase of CTS duration.

Discussion and Conclusion: The expression of anabolic genes and related proteins increased under moderate mechanical stimulation duration. On the other hand, the expression of catabolic genes and related proteins increased under excessive mechanical stimulation duration, yet the expression of anabolic genes and related proteins decreased. These findings may help understand the mechanism of AF.