# EFFECTS OF LOW AMPLITUDE, HIGH FREQUENCY MECHANICAL STIMULI TO OSTEOBLASTS IN 3D POROUS MATRIX

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## ABSTRACT

Bone strain *in vivo* during locomotion includes not only high amplitude, low frequency periodic strain around 1-2 Hz, but also low amplitude, higher frequency strain components up to 50 Hz [1]. However, biological meaning of the strain components is not clear. In this study, we developed an *in vitro* loading system, in which a piezoelectric actuator applied strain waveforms to a porous collagen matrix. Cells in the matrix were exposed to applied strains as well as fluid flow induced inside the matrix pores. Using the loading system, we tested the hypothesis that low amplitude, higher frequency components in bone strain would alter mRNA expression of stress-sensitive genes in osteoblasts. Osteoblastic cells grown in the 3D matrix were given loading at three different frequency bands. Semi-quantitative RT-PCR demonstrated that mRNA levels of cyclooxygenase 2 and egr1 were elevated by strain including high frequency, low amplitude components, however, mRNA level of osteopontin was not affected by loading. The result suggests that low amplitude, higher frequency components in bone strain up to 5 Hz alter expression of selected stress-sensitive genes in osteoblasts.

## METHODS

### Osteoblast Cell Culture

MC3T3-E1 osteoblasts (1 x $10^6$ cells) at a passage number of 20 were seeded into a 3D porous collagen matrix with hydroxyapatite (HA) deposition (20 mm in width x 16 mm in length x 2 mm in thickness). The matrix populated by the cells were pre-cultured for 3 hours prior to loading in α-MEM medium with 10% fetal calf serum, and incubated at 37°C with 5% $CO_2$.

### HA-deposited porous collagen matrix

Prior to cell seeding, porous collagen matrix was soaked in a 500-mM $CaCl_2$ solution followed by a 500-mM $Na_2HPO_4$ solution for 15 min each. The matrix was rinsed in sterile water between immersions. This alternate immersion in the solutions was repeated twice. The two solutions react with each other and make hydroxyapatite deposition on collagen fibers [2]. Mean pore size measured after this hydroxyapatite deposition was ~ 56 μm in diameter.

### Mechanical Loading

Three strain waveforms were used in this study: (1) the strain consisting of 0-50 Hz frequency (recorded on a radius of dog during walking); (2) the filtered strain consisting of 0-5 Hz frequency; and (3) the filtered strain consisting of 0-2 Hz frequency (Fig. 1). The mechanical stimuli were applied just once to the cells for 3 min.

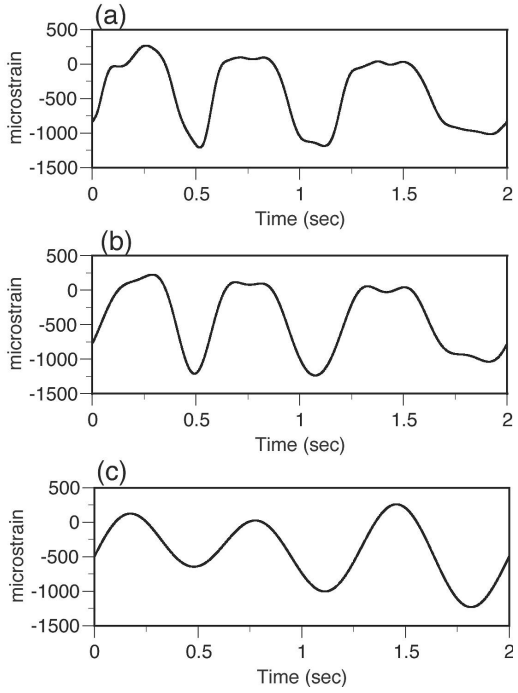


Figure 1. Three strain waveforms used in this study. (a) Original strain waveform recorded on a radius of dog during walking (0-50 Hz). (b) Filtered strain (0-5 Hz). (c) Filtered strain (0-2 Hz).

### Semi-quantitative RT-PCR

One hour after the mechanical treatment, the matrices were removed from the chamber of the mechanical stimulator and homogenized in lysis buffer. Total RNA was extracted and the isolated RNA was reverse-transcribed. Semi-quantitative RT-PCR was performed for 30 cycles using the following cycle (94°C for 1 min, 60°C for 30 sec, and 72°C for 30 sec) using a pair of primers specific to cyclooxygenase 2 (cox2), egr1, or osteopontin (opn). Glyceraldehyde-3-phosphate dehydrogenase (gapdh) was used as control.

### Histology

The matrix populated by osteoblasts was fixed 12 hours after seeding in a 100-mM cacodylate buffer containing 2% paraformaldehyde and 2% glutaraldehyde, and dehydrated in a series of graded ethyl alcohols. The fixed matrix with the cells was embedded in paraffin and sectioned at 5 μm in thickness. The section was stained with MacNeal's method for identification of the cells and mineralized matrix.

## RESULTS

### Osteoblasts in 3D Porous Matrix

Black-stained mineralized matrix was visualized in the collagen matrix with hydroxyapatite deposition (Fig. 2). Most cells were observed in the vicinity of mineralized matrix. The cells were apparently anchored and extended on the matrix surface.

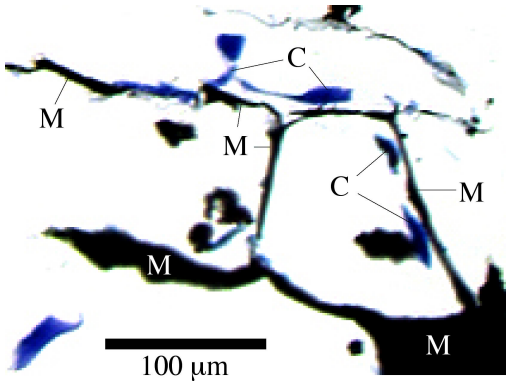


Figure 2. Osteoblast cells in 3D porous matrix. The letters, "C" and "M," indicate cells and mineralized matrix, respectively.

### Altered mRNA Expression Level by Mechanical Stimuli

The original strain (0-50 Hz) and the filtered strain consisting of 0-5 Hz induced a higher level of cox2 and egr1 mRNAs than the filtered strain consisting of 0-2 Hz (Fig. 3). No mRNA expression of cox2 and egr1 was detectable in control. Level of opn mRNA was not affected one hour after loading.

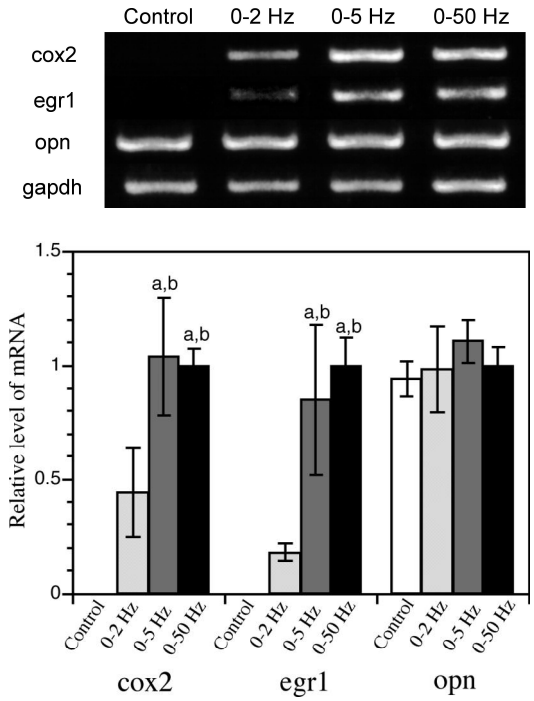


Figure 3. Messenger RNA levels in response to mechanical stimuli. The levels were normalized by gapdh mRNA level, and expressed as a ratio to the level in 0-50 Hz. Symbols: [a] $p<0.01$ vs. control; [b] $p<0.05$ vs. 0-2 Hz.

## DISCUSSION

The novel *in vitro* loading system is suitable for simulating 3D-force environment in bone. The loader driven by the piezoelectric actuator is capable of inducing physiologically relevant strain accompanied with fluid flow to osteoblasts at 0-50 Hz.

We examined for the first time the osteoblastic responses *in vitro* to bone strain waveform generated from *in vivo* experiment. Our RT-PCR result showed that cox2 and erg1 genes were responsive to low amplitude, higher frequency strain components included in the *in vivo* bone strain, but the opn gene was not. The result suggests that mRNA expression of some stress-sensitive genes is modulated by low amplitude, higher frequency components in bone strain up to 5 Hz.


## ACKNOWLEDGEMENT

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