Low-magnitude vertical vibration enhances myotube formation in C2C12 myoblasts

Chia-Hsin Chen1, Chau-Zen Wang2, Yan-Hsiung Wang2, Mei-Ling Ho2, Mao-Hsiung Huang2

1Department of Physical and Rehabilitation Medicine, Kaohsiung Medical University Hospital, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
2Orthopaedic Research Center, Kaohsiung Medical University, Kaohsiung, Taiwan

INTRODUCTION:
Mechanical load plays an important role in maintaining the cell physiology and leads to myoblast proliferation and differentiation. Vertical vibration (VV), a kind of mechanical load, can induce mechaotransduction in vitro. The signals from mechanical forces are transmitted to the nuclei from the extracellular matrix (ECM) and generate a complex sequence of the myoblast stage into myotube formation3. Collagen I, the primary collagen incorporated in muscle structure, acts as the muscle matrix formation and maintenance. Decorin is a well-studied proteoglycan for sustaining structure. Myogenin is able to actively fuse to form multinucleated myotubes3. Therefore, the aim of this study is to investigate the effects of increasing the collagen I, decorin, myogenin expressions in myoblasts and myotube formation after receiving VV.

METHODS:
A mouse myoblast cell line, C2C12, was cultures in this study. The cells were loaded under a 0.4 mm fixed amplitude VV along with 5Hz, 8Hz, and 10Hz respectively. All of the study groups were loaded 10 minutes per day for total 3 days. Cell proliferation was measured by MTS assay. Total mRNA expressions were assessed by real-time PCR. Myotubes were stained with MF-20 and the myotube length, number, and area were counted. The differences in cell proliferation, gene expression and myotube formation after VV were compared among the groups using ANOVA with Tukey Test. All differences were considered to be significant at p<0.05.

RESULTS:
After VV, the cell number was changed as shown in Fig. 1.

![Cell proliferation](image1)

Fig.1 Cell proliferation was assessed by MTS assay. The mRNA expressions of myoblasts were shown in Fig. 2–4.

![Effect of vertical vibration on collagen I gene expression](image2)

Fig.2 Effect of vertical vibration on collagen I gene expression. The VV with 10 Hz showed the most effective to promote the gene expression, especially on the third day.

![Effect of vertical vibration on decorin gene expression](image3)

Fig.3 Effect of vertical vibration on decorin expression. The VV with 10 Hz showed the most effective to promote the gene expression, especially on the third day.

![Effect of vertical vibration on myogenin expression](image4)

Fig.4. Effect of vertical vibration on myogenin expression. The VV with 8 Hz and 10 Hz showed the most effective to promote the gene expression, especially on the third day.

![Effect of vertical vibration on myotube formation](image5)

Fig.5. Effect of vertical vibration on myotube formation. The VV with 8 Hz and 10 Hz showed the most effective to promote myotube formation.

DISCUSSION:
This study demonstrates that myoblasts promote the mRNA expressions of collagen I, decorin with the application VV. Decorin can enhance the proliferation and differentiation of myoblasts. Collagen I, leading the major component in the ECM, plays an important role in maintaining the structure. Transcription factor, Myogenin, is responsible for coordinating muscle-specific gene expression. The myotube can sustain the external stress. Muscle development can be enhanced during different stages under VV. The findings might be related to the clinical treatment in patients with disused muscle atrophy.

REFERENCES: