Abstract—The aim of this study is to evaluate the effects of the laser and partial vibration stimulation on the mice tibia with morphological characteristics. Twenty female C57BL/6 mice (12 weeks old) were used for the experiment. The study was carried out on four groups of animals each consisting of five mice. Four groups of mice were ovariectomized. Animals were scanned at 0 and 2 weeks after ovariectomy by using micro computed tomography to estimate morphological characteristics of tibial trabecular bone. Morphological analysis showed that structural parameters of multi-stimuli group appear significantly better phase in BV/TV, BS/BV, Tb.Th, Tb.N, Tb.Sp, and Tb.pf than single stimulation groups. However, single stimulation groups didn’t show significant effect on tibia with Sham group. This study suggests that multi-stimuli may restrain the change as the degenerate phase on osteoporosis in the mice tibia.

Keywords—Laser, Partial Vibration, Osteoporosis, in vivo micro-CT, mice.

I. INTRODUCTION

A lot of researches have been made an attempt to study the pharmacological or non-pharmacological treatment for osteoporosis [1]-[3]. Osteoporosis is a systemic disease characterized as a diminution of bone mass and a deterioration of the bone microarchitecture [4] on-pharmacological treatment should be considered for an osteoporotic patient, because the pharmacological therapy over long periods can cause side effects [3]. Non-pharmacological therapy includes stimulation through physical and kinematics. It is well recognized that bone is one of the most sensitive tissues to external physical loadings, which can regulate bone homeostasis. Therefore, whole body vibration (WBV) was invited as non-pharmacological treatment and experimented as new alternative. WBV stimulation helped to prevent and treatment bone loss induced by post-menopause or space-flight [5]. However, long-term exposure to 15-20Hz frequency WBV appeared thoracodynia, gastrointestinal bleeding, and joint and bone neuropathy and high intensity WBV would increase possibility of nervous and musculoskeletal system disorder [6]. Accordingly, WBV was prohibited to the individual with health possibility of nervous and musculoskeletal system disorder [6].

Furthermore, WBV was hard to apply elders or patient who their activation was limited. Moreover, osteoporosis leads to increase of fracture risk and fragility fracture in femoral neck, vertebral, and distal radius. On this wise, the hallmark of osteoporotic fracture is occur on specific part of body [10]. Another alternative is partial vibration and laser. Up until now, each single therapy in non-pharmacological treatment has been researched [7], [8]. However, multiple therapies for osteoporosis were not researched. Thus, the aim of this study is to evaluate the laser and partial vibration stimulation effects of multiple therapies in the mice tibia with morphological characteristics.

II. METHODS

A. Animals

Twenty 12-week-old female C57BL/6 mice were used and allocated randomly into four groups; Sham (Sham, n=5), Vibration (Vib, n=5), Laser (Laser, n=5), and Laser plus Vibration (LV, n=5). All mice were ovariectomised (OVX) to induce osteoporosis for 2 weeks. Right tibiae of mice in Vib, Laser, and LV were stimulated for 2 weeks (3 days per week, Vib: 6Hz, 1500cycle, 2000 µstrain). All procedures were performed under a protocol approved by the Yonsei University Animal Care Committee (YWC-111221-1).

B. In vivo Micro-CT

Right tibiae in each mice were scanned by using in vivo micro-CT (Skyscan 1076, Bruker AXS, Germany) at 0 week (before to stimulation) and after stimulation of 2 weeks. From acquiring images, structural parameters (BV/TV, bone volume/total volume, %; BS/BV, Bone surface/volume ratio, mm⁻¹; Tb.Th, trabecular thickness, mm; Tb.Sp, trabecular separation, mm; Tb.N, trabecular number, mm⁻¹; Tb.Pf, Trabecular pattern factor, mm⁻¹) were measured and calculated by CT-AN (Bruker AXS, Germany). All data were presented as mean and standard error and t-test used to statistical analysis (p < 0.05).

III. RESULTS

All structural parameters of LV at 2 weeks were greater than others (Table I). The relative variations of structural parameters showed in Fig. 1 (1 at 0 week). The relative changes in the BV/TV, Tb.Th, and Tb.N in the LV group were significantly higher than those in Sham group (p < 0.05). In BV/TV and Tb.N, the Vib and Laser groups were higher than those in Sham group but not significant. In Tb.Th, Laser group was significantly higher than Sham group and Vib group was higher.
than Sham group but not significant. The relative changes in the BS/BV, Tb.Sp, and Tb.Pf in the LV group were significantly lower than those in the Sham, Vib, and Laser groups (except Tb.Pf of Sham group, \( p < 0.05 \)). There were differences in the relative changes in the BV/TV and Tb.Th between single stimulation groups and multi-stimulation group (\( p = 0.058 \) and 0.051, respectively). In addition, there were no significant changes in BV/TV, BS/BV, Tb.Sp, Tb.N, Tb.Pf between Sham and single stimulation groups. However, there were significantly changes in the BS/BV, Tb.Sp, Tb.N, and Tb.Pf between single stimulation groups and multi-stimulation group (\( p < 0.05 \)).

**TABLE I**

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<tbody>
<tr>
<td>Sham</td>
<td>3.3±0.47</td>
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<td>75.53±0.08</td>
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<tr>
<td>Vib</td>
<td>3.03±0.22</td>
<td>68.47±1.51</td>
<td>78.56±0.01</td>
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<tr>
<td>Laser</td>
<td>3.64±0.47</td>
<td>68.57±2.98</td>
<td>78.11±0.01</td>
<td>549.85±0.03</td>
<td>0.46±0.05</td>
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<tr>
<td>LV</td>
<td>5.92±1.41</td>
<td>62.05±2.48</td>
<td>81.29±0.01</td>
<td>514.68±0.03</td>
<td>0.71±0.15</td>
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**Fig. 1** Relative variations of structural parameters on the trabecular bone (Mean ± SE), * \( p < 0.05 \), (a) BV/TV, (b) BS/BV, (c) Tb.Th, (d) Tb.Sp, (e) Tb.N, (f) Tb.Pf
IV. DISCUSSION

The researches of mechanical stimulation have been progressed to treat bone and muscle diseases since 20 century. The effects of vibration to apply loading on bone and laser to stimulate the tissues have been reported. Therefore, we would like to evaluate the complex and synergy effects of vibration and laser stimulation. In our results, morphological characteristics are worsened in Sham group than single stimulation and multi-stimulation groups. This result suggests that the osteoporosis was induced by ovariectomy in all mice. Tibial trabecular bone quantity in single stimulation groups at 2 weeks was inclined to increase than that in sham group, but not significant (p > 0.05). We confirmed that single stimulation for 2 weeks might suppress a loss of bone quantity. Furthermore, laser and vibration stimulation during 2 weeks makes higher bone mass and better bone quality than in Sham and single stimulation. These results suggested that multi-stimulation for 2 weeks would be better bone quality even single stimulation and in further it could treat the osteoporotic patients. The laser and vibration stimulation are minimal invasive and noninvasive treat and almost have no side effects. Therefore, these stimulations should be critical means to treat osteoporosis. We would continue to investigate to treat or prevent the osteoporosis through multi-stimuli research. In addition, we also would apply mechanical stimulation to muscle diseases (muscle atrophy).

V. CONCLUSION

In this study, we evaluated the effects of multi-stimuli for treatment or prevention of osteoporosis. Taken together, the results showed that multi-stimuli may suppress the continuous progress of bone deterioration, thinning and disconnectivity. Therefore, multi-stimuli may be effective for treat and prevention of bone loss.

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REFERENCES


