INTRODUCTION:
Osteoporosis is a significant disease in developed countries. Not only the expenditure of medical care for osteoporosis is a socioeconomic burden for a society but also altering the patient’s life quality. A nutritional approach to prevent bone loss would be a future goal to achieve an inexpensive way for managing osteoporosis. Recent studies have suggested that green tea polyphenols (GTP) are promising agents for preventing bone loss in women. Tea drinking also reported to lower the risk of bone fracture. However, the effective components and the action mechanisms of green tea on bone remodeling remain unclear. Our previous study found that green tea catechin EGCG[(-)-epigallocatechin-3-gallate] increased the osteogenic function in mesenchymal stem cells. On the other hand, the higher rate of bone resorption than that of formation is a common feature of osteoporosis. Accordingly, to investigate the influence of green tea polyphenols on osteoclasts would help to understand the beneficial effect of tea on osteoporosis. In this study, we examined the effect of 14 green tea polyphenols on the potential of osteoclastogenesis in pre-osteoclasts.

METHODS
The murine macrophage cell line RAW 264.7 (ATCC, Rockville, MD) was maintained in Modified Eagle Medium (MEM) containing 10% FBS. Cells were induced to differentiate into osteoclasts by supplement of 100 ng/ml RANKL. Cultures were treated with green tea polyphenols (20 μM) for 5 days. Osteoclastogenesis was examined by tartrate resistant acid phosphatase (TRAP) solution assay. Cytotoxicity of EGCG on RAW 264.7 cells was measured by MTT and LDH assay according to a previous established protocol.

RESULTS:
Cells were induced to osteoclastogenesis by RANKL supplemented in medium. The result of MTT and LDH assay showed that the 6 polyphenols (20 μM) did not affect cell proliferation. This study showed that a 5-day treatment of 6 green tea polyphenols (1. (-)-Catechin gallate, 2. (-)-Epigallocatechin, 3. (-)-Epigallocatechin gallate, 4. (-)-Gallocatechin, 5. (-)-Gallocatechin gallate, and 6. Gallic acid 20 μM) significantly suppressed the RANKL induced osteoclastogenesis in RAW 264.7 cell cultures (Fig. 1). At the concentrations, these compounds did not affect RAW cell proliferation using MTT (Fig. 3) and LDH (Fig. 4) assay.

DISCUSSION:
Our previous experiment also showed that among 4 major green tea polyphenols, EGCG had the strongest effect on the osteogenesis in a mesenchymal stem cell line. In this study, we further found that a 5-day treatment of (1CG, 2EGC, 3EGCG, 4GC, 5GCG and 6.Gallic acid 20 μM) significantly suppressed the RANKL induced osteoclast differentiation in RAW 264.7 cells. We also found 6 biologically active compounds (Fig. 2) from green tea decreased the TRAP activity more than 60% in comparison to the control cells. From these results, we suggest that green tea polyphenols may be useful agent to prevent osteoporosis through both enhancing bone formation and suppressing osteoclast activity.

REFERENCES: