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Electromagnetically controllable osteoclast activity

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ABSTRACT

The time-varying electromagnetic field (EMF) has been widely studied as one of the exogenous stimulation methods for improving bone healing. Our previous study showed that osteogenic differentiation of adipose-derived stem cells was accelerated by a 45-Hz EMF, whereas a 7.5-Hz EMF inhibited osteogenic marker expression. Accordingly, we hypothesized that each negative and positive condition for the osteogenic differentiation could inversely influence osteoclast formation and differentiation. Here, we demonstrated that osteoclast formation, differentiation, and activity can be regulated by altering the frequency of the electromagnetic stimulation, such as 7.5 (negative for osteogenic differentiation) and 45 Hz (positive for osteogenic differentiation). A 45 Hz EMF inhibited osteoclast formation whereas a 7.5-Hz EMF induced differentiation and activity. Osteoclastogenic markers, such as NFATc1, TRAP, CTSK, MMP9, and DC-STAMP were highly expressed under the 7.5-Hz EMF, while they were decreased at 45 Hz. We found that the 7.5-Hz EMF directly regulated osteoclast differentiation through ERK and p38 MAPK activation, whereas the EMF at 45 Hz suppressed RANKL-induced phosphorylation of IκB. Additionally, actin ring formation with tubules and bone resorptive activity were enhanced at 7.5 Hz through increased integrin β3 expression. However, these were inhibited at 45 Hz. Although many questions remain unanswered, our study indicates that osteoclast formation and differentiation were controllable using physical tools, such as an EMF. It will now be of great interest to study the ill-defined correlation between electromagnetic conditions and osteoclast activities, which eventually could lead to determining the therapeutic characteristics of an EMF that will treat bone-related diseases.

Introduction

The time-varying electromagnetic field (EMF) has been widely studied as one of the exogenous stimulation methods for improving bone healing. Notably, a low-frequency and low-intensity EMF showed effective acceleration and finalization of fracture healing in both delayed-union and non-union models [1,2]. Many papers have reported its effect on enhancement of the activities of osteoblastic cells and promotion of osteogenic differentiation *in vitro* by influencing DNA synthesis and being involved in mineralization, even though the underlying mechanism of the electromagnetic effect on cell activities remains unclear [3–5].

Our previous study showed that the osteogenic differentiation of adipose-derived stem cells and bone regeneration could be accelerated by EMF stimulation [6,7]. Based on the evaluation of osteogenic marker expression under various combinations of the frequency and the magnetic flux density, we found that osteogenic differentiation could be regulated by altering the frequency of the electromagnetic stimulation. In particular, osteogenesis was improved by EMF exposure at a frequency of 45 Hz, whereas a 7.5-Hz EMF yielded a lower osteogenic marker expression than that of the non-stimulated group. Accordingly, we hypothesized that each negative and positive condition for the osteogenic differentiation could inversely influence osteoclast activity and differentiation. In other words, we assumed that a 45-Hz electromagnetic frequency (*i.e.* positive for osteogenic differentiation) would inhibit osteoclast formation and differentiation, while those could be enhanced by a frequency of 7.5 Hz (*i.e.* negative for osteogenic differentiation).

Several studies have reported the effects of the EMF on osteoporosis and osteoclast activities. Shen and Zhao showed that an EMF (15 Hz) could suppress bone mass loss [8] and Tabrah et al. proved that bone density can be changed by EMF exposure (72 Hz) [9]. Additionally, an EMF inhibited the formation of osteoclast-like cells and bone resorption in bone marrow culture (7.5/1.5 Hz) [10,11]. These EMFs with different intensities regulated osteoclastogenesis through osteoprotegerin (OPG), receptor activator of nuclear factor κβ ligand (RANKL), and macrophage colony stimulating factor (MCSF) concentration and calcitonin [1,12].

Despite a smaller number of publications than that of reports on the osteoclastic activity suppression, several papers have noted that osteoclast activities can be promoted by several factors. Molecules, such as ascorbic acid and ghrelin were reported to accelerate osteoclast

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formation or increase bone resorption [13,14]. Reduced mechanical stress can induce osteoclastic bone resorption and suppress osteoblastic bone formation in the presence of osteopontin [15]. Unexpectedly, titanium particles were shown to improve osteoclast generation and its activities, inducing bone resorption *in vitro* [16]. However, it has never been reported that osteoclastogenesis was accelerated using an EMF.

Here, we report that an EMF could enhance or inhibit osteoclastic differentiation by controlling the electromagnetic frequency. Although various frequencies have been used in the literature reporting the inhibition of osteoclastic differentiation using an EMF, we selected experimental frequencies based on our previous report to test our hypothesis as we mentioned earlier. We observed the regulated osteoclastic activities at the frequencies which influenced osteogenic differentiation [6,7]. This study reports that the osteoclastogenesis could be regulated by altering the frequency of the EMF, in just the same way as the osteogenic differentiation is modulated.

Materials and methods

Osteoclast culture and differentiation

Bone marrow-derived macrophages (BMMs) were prepared as previously described [17]. In brief, whole bone marrow cells were isolated from 6- to 8-week old C57/BL6 mice and cultured in α-minimal essential medium (MEM) containing 10% FBS and 50 ng/ml MCSF for 3 days. For the osteoclastogenesis experiments, the cells were plated and cultured in α-MEM containing 10% FBS in the presence of 20 ng/ml of RANKL (R&D systems, Minneapolis, MN, USA) and 10 ng/ml of MCSF (R&D systems). The cells were fixed in 4% paraformaldehyde (PFA) for 10 min and the osteoclasts were stained for tartrate-resistant acid phosphatase (TRAP) with 0.1 M acetate solution (pH 5.0) containing 6.76 mM sodium tartrate, 0.12 mg/ml naphthol AS-MX phosphate, and 0.07 mg/ml fast red violet. After TRAP staining, the numbers of red-stained TRAP-positive cells that had more than three nuclei in the red background were counted under a light microscope. Cells were cultured in 96-well plates and 5 wells per plate were counted. The values were averaged. This counting was repeated five times.

EMF stimulation

The cells were exposed to a uniform EMF produced by a solenoid coil that was used in our previous studies [6,7]. We used 7.5 Hz, expecting a positive effect, and 45 Hz for a negative effect for osteoclastogenesis with an identical magnetic flux density (1 mT). The input signals were sinusoidal waveforms. Before the stimulation, we confirmed the uniform area inside the solenoid coil at each frequency by measuring the magnetic flux density using magnetic field sensors (MFS-3A, Ametes, CA, USA). Fig. 1 shows that the EMF was uniform at the center of the coil, while the magnetic flux density varied inside the coil at 1 mT. Based on this data, the experimental area was set for uniform exposure with a ±5% margin of error. The electromagnetic exposure was performed in a humidified incubator at 37 °C under 5% CO_2 for 8 h per day.

Cell viability

The cell viability was quantified by a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, which is a colorimetric method for measuring the number of viable cells in cytotoxicity. The cells were incubated with 100 μl of fresh medium, and 20 μl of CellTiter 96 AQueous One Solution (Promega, Madison, WI, USA) was added for 4 h at 37 °C. The absorbance was measured at 490 nm using a microplate reader (Wallac 1420 plate reader, PerkinElmer, Waltham, MA, USA).

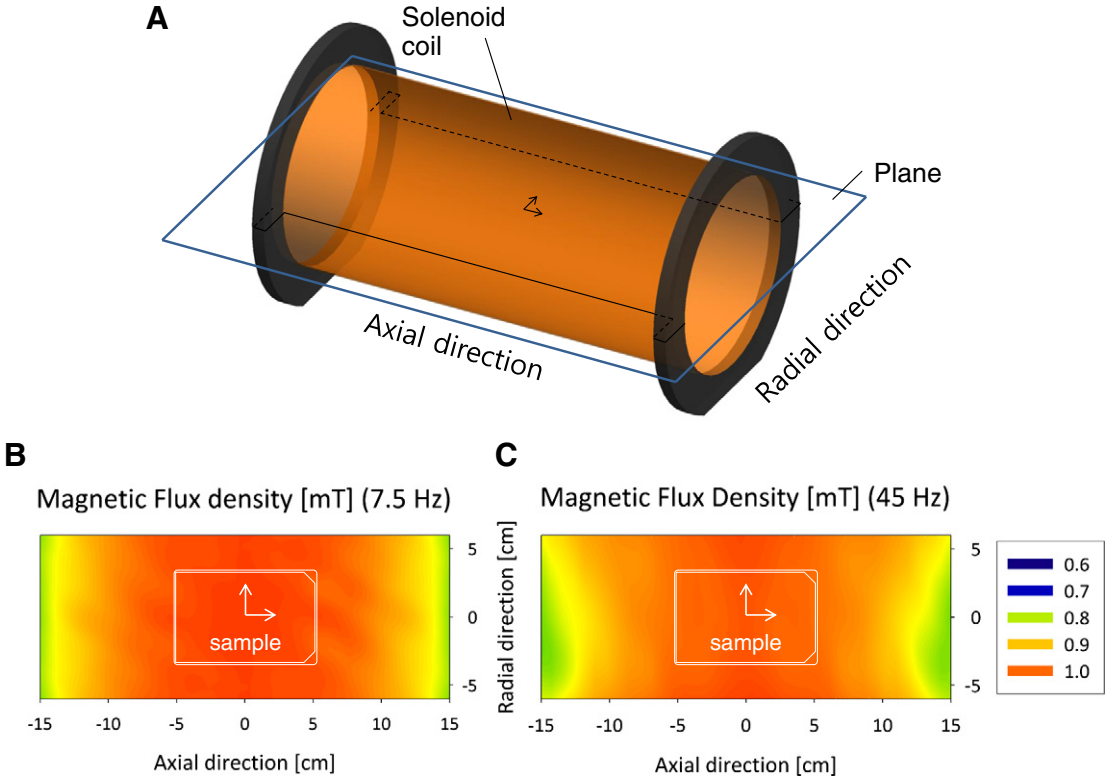

Fig. 1. Verification of magnetic flux density (MFD) inside a solenoid coil. (A) The MFD was measured inside the coil along the plane. The MFD was measured using a magnetic field sensor inside the coil at (B) 7.5 and (C) 45 Hz. These confirmed that every sample was uniformly exposed to the EMF in a range from 0.95 to 1.05 mT.