Effects of Gradient Magnetic Force and Diamagnetic Torque on Formation of Osteoclast-like Giant Cell

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Abstract. In bone tissue, two kinds of cells, osteoblast (OB) and osteoclast (OC), contribute to remodeling of bone. In the present study, a co-culture system of bone-forming cell (OB) and -dissolving cell (OC) was incubated in static magnetic fields of horizontal 14 T and vertical gradient 10 T. Effect of two kinds of magnetic fields was an inhibition of OC formation. Three kinds of mechanisms, magnetic orientation of OB, diamagnetic torque force acting on OC, and possible reduction of earth’s gravity were discussed.

1. Introduction

Bone tissue, which is in a solid state in the living body, also contains both positive and negative control processes. Two kinds of cells, osteoblast (OB) and osteoclast (OC), contribute to the formation and deformation of bone, respectively. In the present study, a co-culture system of bone-forming and -dissolving cells was incubated in a static magnetic field at 14 Tesla (T), and effects of magnetic orientation of OBs on the increase of the OCs that erode bones were investigated.

Previous studies indicated that diamagnetism, which is an induced magnetism of all materials under magnetic fields, possibly affect biological cells [1-4]. It is expected that two kinds of magnetic forces, diamagnetic torque force and gradient magnetic force possibly affect a cell component in living cell [1-3,5,6].

The diamagnetic torque force has effects on the rotational motion of molecules, and potentially affects the morphology of cells. The utilized superconducting magnet has gradient magnetic forces in a direction of bore’s axis in the superconducting coil. The magnetic flux provides torque forces on the fibers that assemble to form the cells [1, 5, 6].

We utilized an experimental system to investigate the effects of magnetic fields on bone formation. The experimental system is the co-culture system with both OB and OC. The processes start with bone marrow cells, and the cells differentiate to precursor OC, OB, etc.. The OC precursors do cell fusions with an assistance of OB and other factors, such as receptor activator of NF-κB ligand (RANKL) and interleukin 1 (IL-1) [7-9]. Finally, they manifest a large sized multi-nucleated cell, OC-like cell.
Our purpose is to apply these two kinds of magnetic force to control of living cells. The diamagnetic control of living cells was investigated by using bone cells. Bone has a solid state, which is known as hydroxy-apatite, in living body. The hydroxy-apatite is degraded by OCs, and reconstructed by OB to exhibit a bone remodeling [10].

Our previous studies showed that the magnetic orientation of OBs occurred under the horizontal magnetic fields of up to 8 T [11], however, we have still not obtained any data about magnetic field effect on OCs. As a model for the magnetic field effects on bone remodeling in vitro, a co-culture system with both OB and OC was exposed to a strong static magnetic field of up to 14 T.

2. Methods

2.1. Cell culture system for co-culture with OB and OC

The culture of bone marrow cells was carried out by using a cell culture dish (100mm in diameter, CORNING, USA) and a slide flask (17920, NUNC Co., Ltd., USA).

The right photo of Fig. 1 shows an OC, which was observed after 5 days of bone marrow incubation (in the absence of magnetic field exposure). The OC was surrounded by fibroblastic cells, OBs. The observed OC-like cell had about ten nuclei.

2.2. Magnetic field generators

We utilized three kinds of superconducting magnets, with a horizontal bore (max. 14 T) and with a vertical bore (max. 12T). The temperature in magnet bore was thermally stabilized by a cylindrical type of water jacket. In case with the cell culture in magnet during several days, the incubation was carried out without CO₂ control.

![Figure 1](image-url)  
**Figure 1.** Experimental procedure for the differentiation of bone marrow cells to OC-like giant cell and OB. Right photo shows an example of multi-nucleated giant cell (OC), which is surrounded by OB.
3. Results and Discussion

3.1. Horizontal magnetic field effect

After three to five days of primal cell culture, the cells were moved to a flask for magnetic field exposure. Then the cells were cultured with and without magnetic fields of 14 T for 50 hours (about two days).

An effect of magnetic fields, 14 T, was observed in the bottom photo of Fig. 2. OBs oriented parallel to the applied magnetic field direction. Top photo shows an OC with dozens of nuclei. The OB orientation near the OC was random while the orientation of OBs apart from OC oriented parallel to the magnetic fields.

Concerning the effects of magnetic fields on morphologies of OC, we compared the size and density of OCs under magnetic fields with those of OCs under ambient fields. An example of effect of magnetic fields on the size of OC is shown in Fig. 3. After starting the co-culture of OB and OC, matrix under OB caused contractions. Then the area of OC was separated from OBs, and OCs were observed in OB-poor area.

A normal circle pattern of OC formed a rounded area where OBs were absent when the sample was incubated for two days without the magnetic field exposure (left panel of Fig. 3). In case with magnetic field exposure at 14 T for two days, it was frequently observed that the pond became ellipsoidal and the size of OC decreased, as shown in an example in Fig. 3 (right panel).

Figure 4 shows the size parameters, the length of long axis (L) and short axis (S), of OC cells with and without magnetic fields of 13~14 T. The results showed that the magnetic fields decreased the size of multi-nuclei-area of OC. It was suggested that the 14 T horizontal magnetic fields decelerated the formation of OC-like multi-nucleated cells when the OBs were exposed to the magnetic fields after the cell passage.

![Figure 2](image-url). Effects of horizontal magnetic fields on OB and OC. The cells were stained with Gimsa’s solution after fixation with methanol.
**Figure 3.** Effects of horizontal magnetic fields on OC and OB.

**Figure 4.** Effects of magnetic fields of 13~14 T on size of OC-like multi-nucleated giant cells.
3.2. **Vertical magnetic field effect**

In the next, we carried out the OC-OB co-culture under vertical gradient magnetic fields of 10 T which was generated at an upper region of the superconducting magnet’s bore. The co-culture procedure was same as the experiment with horizontal fields except exposure period. In the early stage of culture, the cells were exposed to the 10 T vertical fields for three days, and continuously incubated under environmental fields (~0 T) of inside CO2 incubator for more three days.

The observed effects of a vertical magnetic flux and a vertical gradient force in parallel to the direction of gravity, was an inhibition of multi-nucleation rate in the process of OC formation, as shown in Figure 5. The two top panels (photos) show that the 10 T magnetic field exposure exhibited an OC with a size smaller than the control. We measured both long and short axes of OC and obtained a histogram of mean radii OC (bottom panels). The 10 T gradient fields increased the number of small OCs with maximum mean radii of less than 100 microns while reduced the number of OCs in the mean size of more than 200 microns.

The data indicated a possibility that the magnetic field exposure condition inhibited a cell fusion of pre-cursor OC and consequently had a delay in forming a multi-nucleated giant cell.

*Figure 5. Effect of vertical magnetic fields on OC formation.*
Figure 6. An illustration of magnetically reduced gravity which was equivalently produced for diamagnetic molecules.

3.3. Possible mechanisms and application
The results suggested that the suppression of OC size occurred both under horizontal and vertical magnetic fields of 10T order. An optional parameter, reduced gravity by diamagnetic force, was added to the experiments in the vertical bore (12 T vertical magnet).

In general for the formation of a multi-nucleated giant cell via a cell fusion process, cell to cell interaction has an important effect on the cell fusion. Particularly in the formation of OC, precursor OC to OB interaction was also considered to assist OC growth [9]. We speculated that a cell to cell interaction between growing OC and magnetically orienting OB had an important role in forming OCs under horizontal magnetic fields. We observed that the OBs in an appropriate cell density caused magnetic orientation, and its consequence, cellular assembly pattern changes where no OC exists. However, the presence of OC provided an agitation or confliction to the OB cell assembly, and probably the diamagnetic torque forces acting on OBs were accumulated in a cell assembly, then the diamagnetic anisotropic energy was applied for a suppression of cell fusion. No extension of OC morphology was observed under the 14 T horizontal magnetic fields.

In case with the OC-OB co-culture under vertical gradient magnetic fields of 10 T, the earth’s gravity was reduced by about 40 %. As shown in Figure 6, the utilized superconducting magnet with a vertical bore generated a maximum magnetic field of 12 T at a center of bore, and the upper side of gradient fields provided a diamagnetic force which was anti-parallel to earth’s gravity force, and as a result, a diamagnetic object was exposed to a field equal to a reduced gravitational field. The mechanism was not expected in the experiments of horizontal magnet. Possibly the reduced gravity by gradient magnetic fields was one of the mechanism candidates. To clarify its contribution to the OC formation quantitatively, further study under an enhanced gravity mode, at the lower part of magnet’s bore should be carried out in the future.

In the cells being exposed to gradient magnetic fields, a diamagnetic torque force was superposed on gradient magnetic forces. Previous our study suggested that the vertical magnetic fields changed the alignment of lipid molecules in cell membrane and the cellular morphology became balloon-like. The mechanism also resulted in making the size of OC small.
Multiplicate mechanisms existed in the observed phenomenon and above mentioned three mechanisms can make a delay in OC formation; i.e. cellular fusion. Preventing OC formation in a bone tissue of living body causes an enhancement on calcification and hydroxy apatite generation in bone tissue. So it is considered that 10 T order magnetic fields are useful for controlling bone metabolism.

4. Conclusions
Horizontal magnetic fields of 14 T induced an inhibition OC formation by means of cellular fusion. A magnetic orientation of OBs was observed in an OB rich area. Vertical gradient magnetic fields at 10 T also induced an inhibition OC formation. Cell differentiation and cellular fusion process had a delay due to the magnetic field exposure.

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