# THE EFFECTS OF STRONG STATIC MAGNETIC FIELDS ON ECTPIC BONE FORMATION

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**Abstract**

We investigated the effects of a strong static magnetic field (SMF) on bone formation in both *in vivo* and *in vitro* system. After 60h of exposure to the SMF, cultured osteoblastic cells were transformed to rod-like shapes and were orientated in the direction parallel to the magnetic field. The SMF also stimulated ectopic bone formation in subcutaneously implanted bone morphogenetic protein-2 containing pellets in mice, in which the orientation of bone formation was parallel to the magnetic field. It is concluded that a strong SMF has the potency not only to stimulate bone formation, but also to regulate its orientation.

## 1. Introduction :

Physical factors such as mechanical, electrical, and magnetic stimulation induce anabolic actions specific to bone formation. Pulsed electromagnetic fields (PEMF) and static magnetic fields (SMF), for example, have reportedly aided in fracture healing, spinal fusion, ingrowth into ceramics or bone grafts. These magnetic fields, however, were of gauss (G) order, which was much less than the strength required to regulate the orientation of matrices and cells. The present study investigated the effects of strong SMF (8 T) on bone formation and orientation in both *in vivo* and *in vitro* systems.

## 2. Methods and Results :

A horizontal type superconducting magnet (Oxford, UK), 700 mm long with a bore of 100 mm in diameter, which produced 8 T at its center, was used. The field distribution of the exposure system adjusted the magnetic field strength from 8.0 to 7.96 T at z = 0 to z =±10 mm in the center of the bore. The ambient temperature in the magnet was maintained by circulating temperature-regulated water in a coiled tube, which was inserted into the bore. The maximum deviation was ± 0.1℃.

To determine the effects of strong SMF on bone formation *in vivo*, an ectopic bone formation model in and around a subcutaneously implanted BMP (bone morphogenetic protein) /collagen pellet in mice was used. After 21 days following 60 h of 8 T SMF exposure, BMP/collagen pellets were harvested, and radiological and histological analyses were performed. X-ray images revealed that the strong SMF stimulated bone formation in and around the BMP/collagen pellet. The newly formed bones extended parallel to the direction of the magnetic field in the exposed group, while only small spherical shaped ossicles were observed in the non-exposed group. The bone mineral content of the exposed group was approximately 4 times greater than the non-exposed group. Histological examinations revealed that the pellets were replaced by newly-formed bone tissues including bone marrow in both the exposed and non-exposed groups. Mature bone formation was observed mainly at the periphery of the induced tissue.

The effects of exposure to the strong 8 T SMF on the proliferation, orientation, and differentiation of cultured mouse osteoblastic MC3T3-E1 cells were also investigated. The proliferation rate of the MC3T3-E1 cells was not affected by strong SMF exposure for 24 h, as determined by BrdU uptake. In addition, there was no significant difference between the growth curves of the cultured MC3T3-E1 cells during the 6 days in culture for the SMF exposed and non-exposed groups for 60 h. These results indicate that strong SMF have no effect on the proliferation of osteoblastic cells. To determine the effect of SMF exposure on the differentiation of cultured MC3T3-E1 cells, the alkaline phosphatase (ALP) activity of the cells was examined. After 14 days in culture following the SMF exposure for 60 h, the cells exhibited a higher intensity of ALP staining as compared to the non-exposed cells. The Alizarin-red staining method showed an upregulation of matrix synthesis by SMF exposure after 21 days in culture. In addition, the ALP-positive cells and the Alizarin red-positive matrix maintained their parallel orientation to the magnetic field for 14 and 21 days, respectively, after SMF exposure for 60 hours.

The MC3T3-E1 cells were orientated parallel to the direction of the magnetic field after 8 T SMF exposure for 60 h, while they randomly orientated without magnetic field exposure. For the quantitative analysis of the cell orientation, the angle between the axis of each cell and the magnetic field was measured. The distribution of cell orientation was concentrated around 0 degrees in the exposed cells, whereas the control group was randomly orientated. The orientational order parameter for a 2-dimensional system ($f$2D) using the NIH image processing programs of both the exposed and control groups were 0.92 and 0.04 respectively. These results clearly show that the strong SMF controlled the orientation of cells to the magnetic field direction.

## 3. Conclution:

The strong SMF stimulated bone formation in both *in vitro* osteoblast cell cultures and *in vivo* ectopic bone formation models in the direction of the magnetic field. A combination of strong static magnetic fields and potent osteogenic agents such as BMP is a potentially viable clinical treatment for skeletal conditions such as osteoporosis and bone fractures.


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