

Static Magnetic Fields Promote Osteoblast-Like Cells Differentiation Via Increasing the Membrane Rigidity

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Abstract—The aim of this study was to test the differentiative effects of osteoblasts after treatment with a static magnetic field (SMF). MG63 osteoblast-like cells were exposed to a 0.4-T SMF. The differentiation markers were assessed by observing the changes in alkaline phosphatase activity and electron microscopy images. Membrane fluidity was used to evaluate alterations in the biophysical properties of the cellular membranes after the SMF simulation. Our results show that SMF exposure increases alkaline phosphatase activity and extracellular matrix release in MG63 cells. On the other hand, MG63 cells exposed to a 0.4-T SMF exhibited a significant increase in fluorescence anisotropy at 6 h, with a significant reduction in the proliferation effects of growth factors noted at 24 h. Based on these findings, the authors suggest that one of the possible mechanisms that SMF affects osteoblastic maturation is by increasing the membrane rigidity and reducing the proliferation-promoting effects of growth factors at the membrane domain.

Keywords—Mechanobiology, Growth factor, Fluorescence anisotropy, SMF.

INTRODUCTION

Pulsed electromagnetic fields (PEMF) generated by electromagnets have been used extensively in clinical treatment of nonunited bone fractures for over two decades. Clinical evidence shows that, regardless of fracture location, therapeutic effects can be achieved for union-delayed and nonunion bone fractures using PEMF treatment.^{2,12–13,28} Furthermore, PEMF simulation provides marked improvement at the hydroxyapatite/bone interface.¹¹ Cellular study has demonstrated that PEMF

treatment of osteoblasts results in a more-differentiated and mature osteoblastic phenotype.²¹

The permanent magnet is a treatment variant also used in clinical practice.⁸ The physical response of tissue exposed to a static magnetic field (SMF) generated by a magnet differs from that exposed to a PEMF; for example, the SMF does not induce an electrical field in tissue.^{3,4} However, animal experiments have been to demonstrate that an SMF created by permanent magnets also increases bone strength, prevents bone mineral density decreases, and accelerates orthodontic tooth movement.^{8,25,38} Recently, cell culture studies have demonstrated that, like PEMFs, SMFs induce osteoblastic cell differentiation in the early maturation stage.^{29,37}

Osteoblasts synthesize bone-matrix macromolecules and induce calcification of the matrix. They are continuously replaced from pools of differentiating pre-osteoblasts that arise from osteoprogenitor cells. It is well known that when the gene associated with osteoblastic differentiation is initiated, proliferation of the osteoblastic cell is down-regulated.³² Recently, a number of scholars have proposed that the mechanisms of magnetic fields, in terms of their effects on bone, can be explained by osteoblastic cell-growth arrest and precursor-cell maturation.^{9,21}

In cells exposed to an SMF, the cellular membrane is assumed to be the target for the SMF interactions. It has been proposed that phospholipids can be oriented by external magnetic fields to reach an equilibrium state with a minimum free energy when the flux density of magnetic field exceeds a certain threshold.^{1,6,18,33} Alternatively,³⁴ discovered that gradient magnetic fields can unbalance the hydrostatic pressure across the bilayer lipid membrane.³⁴ Both reorientation of the molecules in the lipid bilayer and hydrostatic pressure unbalance across the membrane can possibly result in overdeformation of the cellular

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membrane, modification of the biological properties of imbedded receptors in the membrane and, thereby, altering the proliferation kinetics of the cells.^{7,31} However, although experimental evidence has been generated that suggests that static magnetic fields induce phospholipid orientation in the liposome.¹⁹ none of the associated study data was provided to permit validation of the hypothesis that an SMF affects the function of proteins embedded in the bone-cell membrane.

For this study, we have hypothesized that SMF effects on the properties of growth factor receptors embedded in the cellular membrane are reflected in growth factor mediation of the proliferation and differentiation properties of osteoblasts. Therefore, the effects of SMFs, in terms of the interactions between various growth factors and osteoblastic cells, were examined *in vitro*. In addition, changes in the physical properties of the cellular membrane post SMF exposure were assessed initially by analyzing cellular-membrane fluidity.

MATERIALS AND METHODS

Cell Cultures and SMF Exposure

In this study, MG63 osteoblast-like cells [ATCC CRL-1427] were utilized for all *in vitro* tests. The cells were maintained in Dulbecco's modified Eagle's medium, supplemented with L-glutamine (4 mM), 10% fetal bovine serum, and 1% penicillin-streptomycin (HyClone, Utah, USA). Cells were incubated in 5% CO₂ at 37 °C and 100% humidity. The cells were divided into control and SMF-exposed groups, and were separately incubated inside two identical incubators. Neodymium (Nd₂Fe₁₄B) magnets with a diameter of 10 cm were used to produce the SMF. The rare earth magnets were isolated from the surrounding environment by a thin layer of nickel. For all experiments, four 3.5 cm culture dishes were placed directly on the north surfaces of the permanent magnets. As demonstrated in Fig. 1, magnetic field intensity over the upper surfaces of the magnets and plastic dishes were monitored by a handheld Gauss meter (Model 5070, FW BELL, Orlando, FL, USA), and then labeled the magnets with 0.1, 0.25, and 0.4 T accordingly. The tested cells were placed on the north surfaces of permanent magnets, while controls were placed on non-magnetic neodymium disks in the second matched incubator at the same time as the experimental analogues. For all experiments, the cells were first incubated in an unexposed environment for 24 h. The SMF-exposed cells were then placed onto the magnets, with this defined as time point 0 h for all tests.

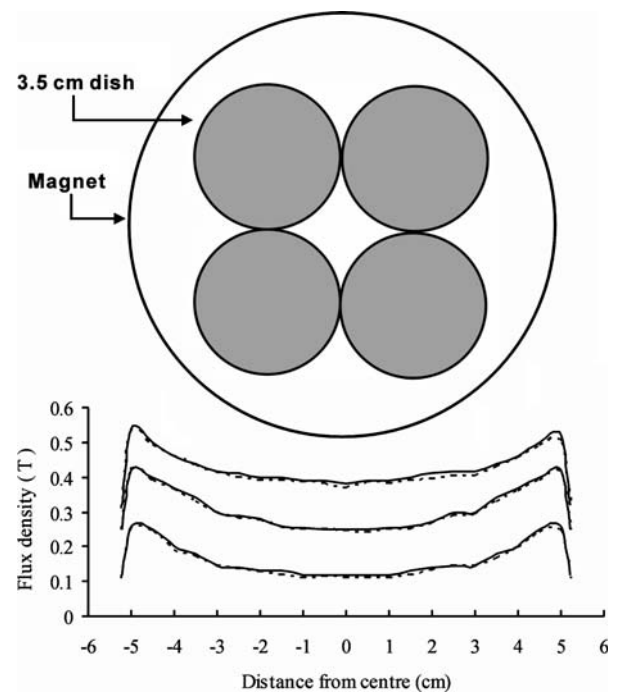


FIGURE 1. Top diagram demonstrates the geometrical size of the Petri dishes and the magnets used in this study. In the lower part, the solid and dashed lines show the distributions of magnetic field intensity over the upper surfaces of the magnets and culture dishes respectively.

Cell-Proliferation Assay

SMF effects on the proliferative activity of MG63 cells were evaluated using haemocytometers. The cells were exposed to an SMF, with observation times set at 0, 24, 48, and 72 h after the exposure. To compare the growth curves of the MG63 cells in the two identical incubators, simultaneous cell proliferation assays were performed before the SMF-exposure experiments. In addition, the number of cell doublings that occurred over consecutive days was calculated using the equation:²⁷

$$2^n = (P_2)/(P_1); \quad (1)$$

where n is number of cell doublings, P_1 and P_2 are the cell numbers measured on two consecutive days.

Cell Cycle Analysis

From flow cytometry analysis, it was determined that the SMF exposure conditions were identical to those for the cell number assay described above. At each observation time, the cells were fixed overnight in 70% ethanol at 4 °C. The cells were then treated with 0.25 mL 0.5% Triton X-100, followed by the addition of 1 mg/mL RNase (Sigma; St Louis, MO, USA) at 37 °C for 30 min. Afterwards the cellular DNA was

stained with 50- $\mu\text{g}/\text{mL}$ propidium iodide (Sigma) in PBS at 4 °C in the dark for 30 min. The cell suspension was then analyzed for DNA content by flow cytometry (FACSCalibur; BECTON DICKINSON, San Jose, CA, USA).

Alkaline Phosphatase Activity Assay

Alkaline phosphatase (ALPase) activity in the MG63 cell layer was determined from the rate of conversion of *p*-nitrophenyl phosphate to *p*-nitrophenol at a pH of 10.2.²¹ The treated cells were exposed to an SMF of 0.4-T flux density for 24 and 48 h. At each observation time, absorbance was measured at 405 nm with a multilabel plate reader (CHAMELEON; Hidex, Mustiokatu, Finland). Total cell lysate was also used for protein determination. Bicinchoninic acid (BCA Protein Assay Kit; Pierce, Rockford, IL, USA) was added to the cell lysate for 10 min, and then the absorbance was read at 590 nm. Specific enzyme activity was calculated using these two absorbance parameters.

Transmission Electron Microscopy

The SMF-exposed (0.4 T) and unexposed cells were cultured on chamber slides (Nunclon; Nunc, Roskilde, Denmark) for 48 h. At the specified observation time, the culture were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde (Sigma) for 30 min, and fixed with 1% osmium tetroxide in 0.1 M PBS for 30 min. Following the initial fixation, the samples were postfixed in osmium tetroxide. The samples were then dehydrated in graded ethanol solutions (concentration series 70, 80, 90, 95, 100%) and embedded in EPON. Thin sections were cut with a diamond knife and stained with lead citrate and uranyl acetate. The ultrastructure of the cells was then examined using a transmission electron microscope (TEM, H-600; Hitachi Ltd., Tokyo, Japan).

Growth Factor Incorporation Tests

Cell number assay was also conducted to test the effects of SMF on the proliferative activity of basic fibroblast growth factor (bFGF), insulin-like growth factor-I (IGF-I), transforming growth factor- β (TGF- β), and tumor necrosis factor- α (TNF- α). Before the incorporation assay, cells were kept serum free for starvation. The cells were then incubated with 5 ng/mL growth factors (Pepro Tech EC Ltd, London, UK) in serum free media. Additionally, cells cultured with 10% and 0% FBS were set as positive and negative controls, respectively. Cell number was enumerated in both the SMF (0.4 T) and sham exposures at 12 h.

Membrane Fluidity Measurement

The plasma membrane fluidity of the SMF-treated cells was determined by measurement of the fluorescence anisotropy of the hydrophobic fluorescent probe, 1-(4-(trimethylammonium)phenyl)-6-phenylhexa-1,3,5-triene (TMA-DPH; Molecular Probes, Eugene, OR, USA). Cells of the exposed group were placed on the 0.4-T neodymium magnet for 8 h. Labeling was performed by incubating the cells with 0.05 μM TMA-DPH at 37 °C for 15 min.³⁵ Fluorescence anisotropy was determined with the multilabel plate reader. Excitation and emission wavelengths were set at 360 nm and 430 nm, respectively. Fluorescence anisotropy (*r*) was calculated using the equation:²⁶

$$r = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2I_{\perp}) \quad (2)$$

where I_{\parallel} is fluorescence intensity measured with vertical excitation and vertical emission polarization filters and I_{\perp} is the analog measured with vertical excitation and horizontal emission polarization filters. An increase in fluorescence anisotropy reflects a decrease in probe mobility and an increase in membrane structural order or a decrease in membrane fluidity.³⁰

Statistical Analysis

Three independent experiments were performed for all tests, with data presented as mean \pm standard deviation (SD) from at least four samples for each of the three. For cell proliferation assay, Two-way ANOVA was used to test the differences from cell proliferation assay. In the other experiments, differences between the control and SMF-exposed cells were tested using the Student's *t*-test. For all tests, *p* value less than 0.05 was considered significant throughout the paper.

RESULTS

Statistically significant differences in cell numbers were not demonstrated for any of the observation intervals over the 72-h period comparing cells cultured in the two matched incubators. In Fig. 2a, cell numbers increased for both SMF-exposed and control groups throughout the entire experimental period. However, after 24 h, there were significant differences in cell number comparing the 0.4-T SMF exposed and control groups (*p* < 0.05). The number of cell doublings for the former was also significantly lower than for the controls (Fig. 2b), varying in a flux density-dependent manner in the first 24 h period (*p* < 0.05).

The total cell percentage differed for the three phases of the cell cycle at each observation time

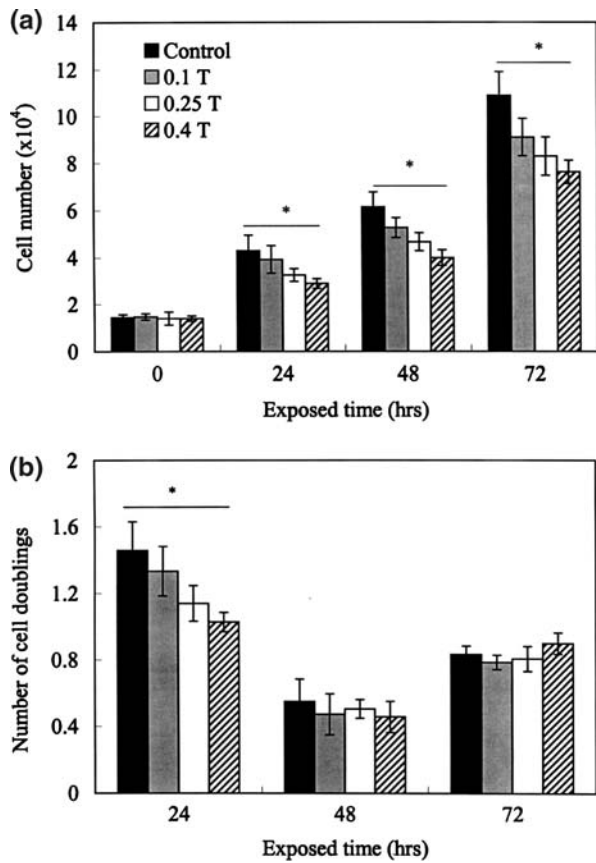


FIGURE 2. Cell proliferation assay for MG63 cells exposed to static magnetic fields of different flux densities. (a) Cellular exposure to SMF results in significant inhibition of proliferation rates in a flux density-dependent manner. (b) Number of cell-doubling assay demonstrates that growth rates of SMF-exposed cells were significantly lower relative to controls in the first 24 h period ($n = 4$; $*p < 0.05$).

(Figs. 3a–3c). During the 3-day tests, there was a tendency towards increased cell percentage in the G_0/G_1 phase (approximately 55, 55, 60, and 75% at 0, 24, 48, and 72 h, respectively), with fewer cells in the S and G_2/M phases (25, 26, 17, and 11% at 0, 24, 48, and 72 h, respectively). However, in each cell-cycle phase, significant differences were not demonstrated comparing the exposed and unexposed cells at each observation time. As the main alterations in proliferation rate were determined when cells were exposed to a 0.4-T SMF for 24 h, the following tests were performed to differentiate activity at this flux density.

When compared to the control group, the ALPase-specific activity in the SMF-stimulated cells was significantly greater at 48 h ($p < 0.05$; Fig. 4a). Using transmission electron microscopy, the greatest difference between the control (Fig. 4b) and SMF-exposed cells (Fig. 4c) was observed in the extracellular areas. Extracellular matrices, released as clusters from plasma membranes, were observed around the stimulated cells at 48 h.

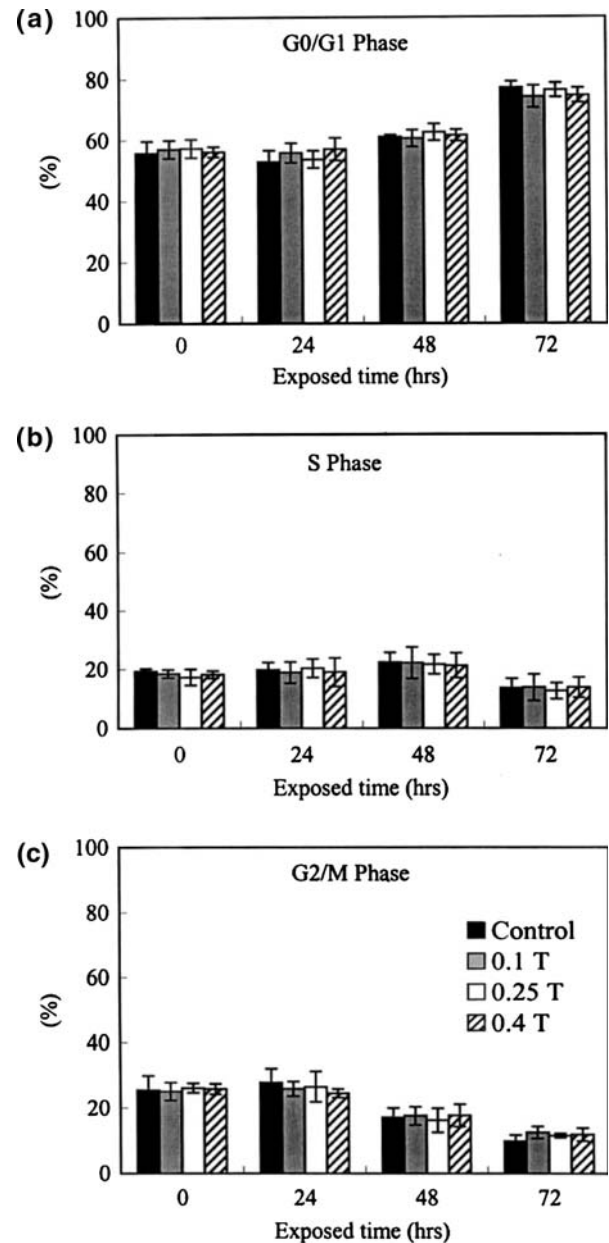


FIGURE 3. Cell cycle assay for MG63 cells exposed to static magnetic fields of different flux densities. However, comparisons of cell-cycle percentages at (a) G_0/G_1 , (b) S, and (c) G_2/M phases between SMF-exposed and control groups reveals no significant differences for at any flux density ($n = 4$; $*p < 0.05$).

The cell numbers for both the exposed and control cells remained at low levels, without significant change in any of the tests where cells were cultured for 12 h in a serum-free medium. When bFGF was added to the serum-free medium, the number of control cells was 162% higher than without it (Fig. 5a). However, this effect was significantly decreased (to 140%) when cells were exposed to a 0.4-T SMF ($p < 0.05$). Results were similar in the IGF-I (Fig. 5b) and TGF- β (Fig. 5c)

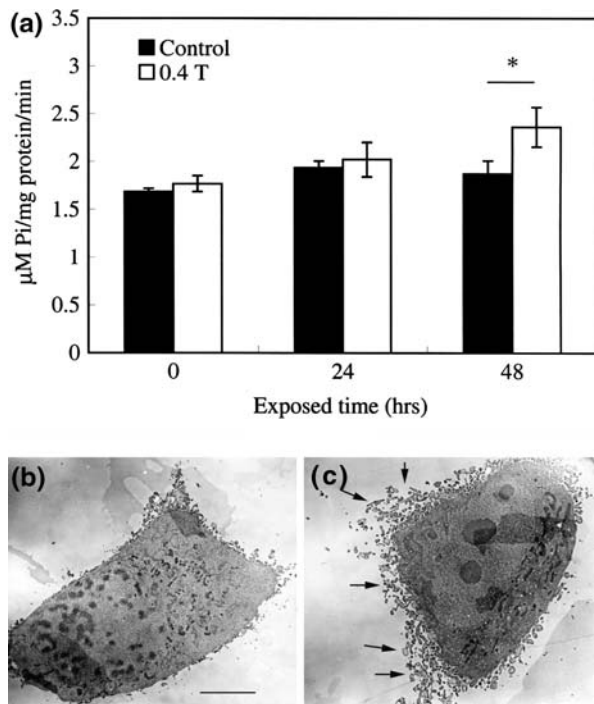


FIGURE 4. After 48 h of SMF exposure: (a) the alkaline phosphatase activity of the SMF-exposed cells was significantly greater than that of the controls ($n = 4$; $*p < 0.05$); the greatest difference between the untreated control (b) and SMF-exposed cells (c) was in the extracellular areas from TEM. Cells appear to release matrix vesicles (black arrows) around the SMF-exposed cells. Bar equals 12 μm .

tests. The cell proliferation effects were also significantly decreased from 200% and 159% to 181% and 142% in the IGF-I and TGF- β tests, respectively ($p < 0.05$). A contrasting result was obtained when TNF- α was added to the serum-free medium (Fig. 5d), with control-group cell number significantly lower (23%) than without it ($p < 0.05$). There was no statistical difference, however, comparing the two sets of cultures with SMF exposure.

Alterations in the membrane fluidity of the MG63 cells exposed to a 0.4-T SMF are illustrated in Fig. 6. A statistically significant increase in fluorescence anisotropy (from 0.13 to 0.16; $p < 0.05$) was demonstrated for the MG63 cells exposed to a 0.4 T SMF at 6 h, indicating that SMF exposure reduces the fluidity of the cell membrane in the hydrophilic region.

DISCUSSION

The activity of ALPase is an early marker of osteoblast phenotypic differentiation. In our study, the enzyme activity of SMF-exposed cells at 48 h was significantly greater than that of the controls (Fig. 4a). Furthermore, our TEM investigation indicated that

the release of extracellular matrix, known to be enriched with alkaline phosphatase was associated with the enzyme-activity changes (Fig. 4c). As extracellular matrix is a promoter of mineral deposition, the SMF-exposed cells progressed into the matrix development/maturation stage.

Down-regulation of osteoblastic cell proliferation is an early characteristic of osteoblastic differentiation.³² Although reduced proliferation rate of neuronal cells,^{24,27} renal cells,⁵ and fibroblasts²⁰ have been demonstrated in several studies of continuous cellular SMF simulation, this study is the first to show that SMF reduces the proliferation rate of osteoblastic cells *in vitro* in a flux density-dependent manner (Fig. 2a).

The major cell-proliferation effect of SMF occurred during the first 24-h experimental period. This result was supported by the findings of several previous studies, which reported that SMF affects the proliferative activity of osteoblastic cells in the early maturation stage.^{29,37} As the MG63 cells constitute a model of an early state of maturation in the osteoblastic lineage,²¹ they have the potential to process further differentiation. This explains the failure to demonstrate significant differences between the proliferation rates of the SMF-exposed and unexposed variants of previous incubations in line with other reports,^{22,37} possibly due to the fact that cells used in these studies have more-differentiated phenotypes. However, our results show that there is no association between SMF exposure and cell cycle progression (at any flux density) in any particular phase. These findings are consistent with the results of previous investigations of human neurons,²⁴ cancer cells²⁷ and fibroblasts.³⁶

It is well known that bFGF, IGF-I, and TGF- β are important for cell proliferation.^{14,19} However, SMF exposure reduces the proliferate effects of these growth factors. TNF- α is a cytokine that tends to inhibit the growth rate of osteoblastic cells. Interestingly, cytotoxic effects of TNF- α on the tested cells were also reduced with SMF exposure. Both bFGF and IGF-I receptors are tyrosine kinase receptors. The TGF- β and TNF- α receptors are serine/threonine kinase receptors and cytokine receptors, respectively. These results demonstrate that SMF-signaling effects are nonspecific in the signal transduction pathway.

Membrane fluidity is an important biophysical property of cellular membranes.¹⁵ Previous study has found that electrical stimulation cause cells to temporarily halt proliferation by decreasing their membrane fluidity.¹⁷ Therefore, one possible result of these changes in membrane fluidity is linked to alterations in the physiological processes of the cell membrane, such as carrier-mediated transport, activity of membrane-bound enzyme, and membrane fluorescence anisotropy.^{16,17} This linkage was also revealed after cells

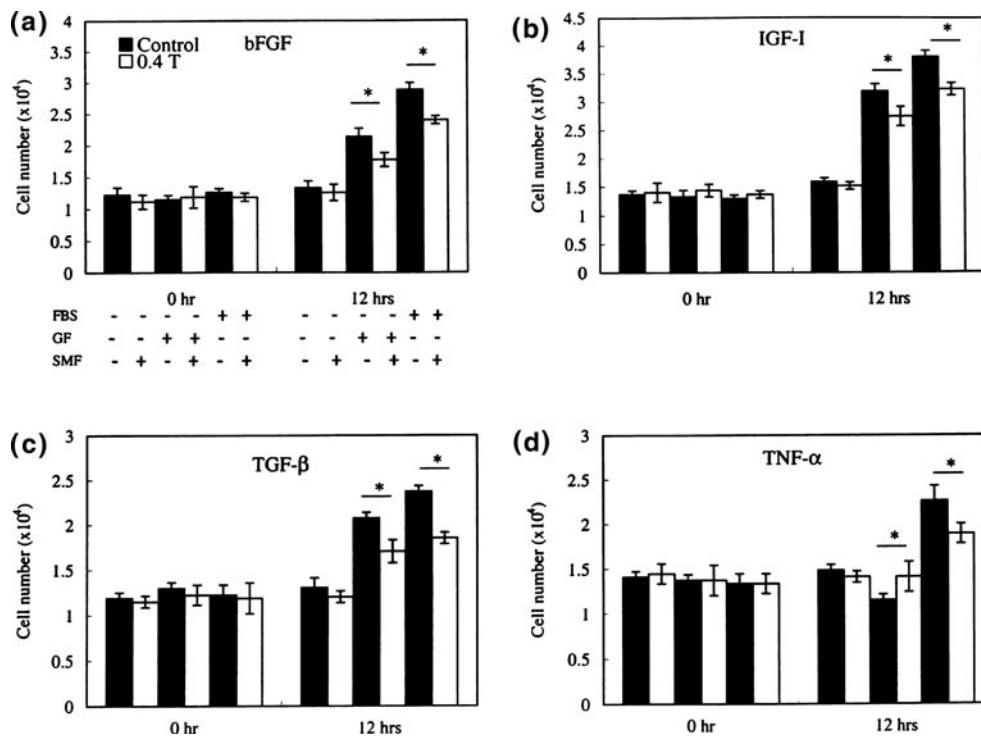


FIGURE 5. Growth factor incorporation-induced cell proliferation is affected by SMF: (a–c) All three growth factors demonstrated proliferation effects on MG63 cells, however, effects are reduced significantly with SMF exposure; (d) cytotoxicity effect of TNF-α which down-regulates the proliferation effects of the cells, is reduced significantly with SMF exposure ($n = 4$; $*p < 0.05$).

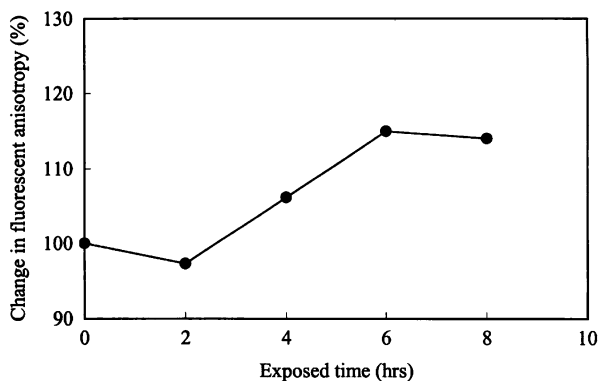


FIGURE 6. Time course of the change in mean fluorescent anisotropy of MG63 cells treated with 0.4-T SMF. $r\% = 100 (r/r_0)$, where r_0 is the mean fluorescent anisotropy at the beginning of SMF application.

were exposed to a 0.4-T SMF for 6 h (Fig. 6), suggesting an association between SMF exposure and membrane structural order. Phospholipids are rod-like molecules that exhibit liquid crystal properties in a host of biological systems. As the SMF can re-orientate liquid crystal molecules¹⁰ and change the hydrostatic pressures across the membrane,³⁴ the membrane should be affected by external SMFs. This effect may result in deformation of the lipid bilayer, which then affects proteins, such as growth factor receptors and

ion channels, embedded in the membrane.³¹ This mechanical effect may possibly account for the facts that the SMF inhibit the regulatory functions for each type of growth factor (Fig. 2a) and the rate of cell proliferation (Fig. 2a).

CONCLUSIONS

Based on these observations, it appears reasonable to suggest that one of the possible effects of SMF stimulation is that SMF reduces membrane fluidity and inhibits proliferate activity of MG63 cells through alteration of the signaling function of growth factors. Sequentially, when the proliferation of SMF-treated cells is arrested, an increase in ALPase activity is revealed with a more differentiated morphology observed. Based on our limited results, we would suggest that static magnetic fields are potential clinical tools in orthopedic rehabilitation, although more advanced *in vivo* studies are needed to substantiate this apparent potential.

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REFERENCES

- ¹Aoki, H., H. Yamazaki, T. Yoshino, and T. Akagi. Effects of static magnetic fields on membrane permeability of a cultured cell line. *Res. Comm. Chem. Pathol. Pharm.* 69:103–106, 1990.
- ²Bassett, C. A. L., S. N. Mitchell, and S. R. Gaston. Treatment of ununited tibial diaphyseal fractures with pulsing electromagnetic fields. *J. Bone Joint Surg.* 63A: 511–523, 1981.
- ³Berk, S. G., S. Srikanth, S. M. Mahajan, and C.A. Ventrice. Static uniform magnetic fields and amoebae. *Bioelectromagnetics* 18:81–84, 1997.
- ⁴Bruce, G. K., C. R. Howlett, and R. L. Huckstep. Effect of a static magnetic field on fracture healing in a rabbit radius: preliminary results. *Clin. Orthop. Rel. Res.* 222:300–306, 1987.
- ⁵Buemi, M., D. Marino, G. DiPasquale, F. Floccari, M. Senatore, C. Aloisi, F. Grasso, G. Mondio, P. Perillo, N. Frisina, and F. Corical. Cell proliferation/cell death balance in renal cell cultures after exposure to a static magnetic field. *Nephron* 187:269–273, 2001.
- ⁶Camilleri, S., F. McDonald, and M. O. MIBiol. Static magnetic field effects on the sagittal suture in *Rattus Norvegicus*. *Am. J. Orthod. Dentofac. Orthop.* 103:240–246, 1993.
- ⁷Coots, A., R. Shi, and A. D. Rosen. Effect of a 0.5-T static magnetic field on conduction in guinea pig spinal cord. *J. Neurol. Sci.* 222:55–57, 2004.
- ⁸Darendeliler, M. A., P. M. Sinclair, and R. P. Kusy. The effects of samarium-cobalt magnets and pulsed electromagnetic fields on tooth movement. *Am. J. Orthod. Dentofac. Orthop.* 107:578–588, 1995.
- ⁹Diniz, P., K. Shomura, K. Soejima, and G. Ito. Effects of pulsed electromagnetic field (PEMF) stimulation on bone tissue like formation are dependent on the maturation stages of the osteoblasts. *Bioelectromagnetics* 23:398–405, 2002.
- ¹⁰Feinendegen, L. E., and H. Muhlensiepen. *In vivo* enzyme control through a strong stationary magnetic field – the case of thymidine kinase in mouse bone marrow cells. *Int. J. Radiat. Biol.* 52:469–479, 1987.
- ¹¹Fini, M., G. Giavaresi, R. Giardino, F. Gavani, and R. Cadossi. Histomorphometric and mechanical analysis of the hydroxyapatite-bone interface after electromagnetic stimulation. *J. Bone Joint Surg.* 87B:123–128, 2006.
- ¹²Garland, D. E., B. Moses, and W. Salyer. Long-term follow-up of fracture nonunions treated with PEMFs. *Contemp. Orthop.* 22:295–302, 1991.
- ¹³Gossling, H. R. S., R. A. Bernstein, and J. Abbott. Treatment of ununited tibial fractures: a comparison of surgery and pulsed electromagnetic fields (PEMF). *Orthop.* 15:711–719, 1992.
- ¹⁴Hill, P. A., A. Tumber, and M. C. Meikle. Multiple extracellular signals promote osteoblast survival and apoptosis. *Endocrinol.* 138:3849–3858, 1997.
- ¹⁵Iwasaka, M., and S. Ueno. Detection of intracellular macromolecule behavior under strong magnetic fields by linearly polarized light. *Bioelectromagnetics* 24:564–570, 2003.
- ¹⁶Jedrzejczak, M., A. Koceva-Chyla, K. Gwozdziński, and Z. Jozwiak. Changes in plasma membrane fluidity of immortal rodent cells induced by anticancer drugs doxorubicin, aclarubicin and mitoxantrone. *Cell Biol. Int.* 23:497–506, 1999.
- ¹⁷Kojima, J., H. Shinohara, Y. Ikariyama, M. Aizawa, K. Nagaike, and S. Morioka. Electrically controlled proliferation of human carcinoma cells cultured on the surface of an electrode. *J. Biotechnol.* 18:129–139, 1991.
- ¹⁸Kotani, H., H. Kawaguchi, T. Shimoaka, M. Iwasaka, S. Ueno, H. Ozawa, K. M. Nakamura, and K. Hoshi. Strong static magnetic field stimulates bone formation to a definite orientation in vitro and in vivo. *J. Bone Miner. Res.* 17:1814–1821, 2002.
- ¹⁹Liburdy, R. P., T. S. Tenforde, and R. L. Magin. Magnetic field-induced drug permeability in liposome vesicles. *Radiat. Res.* 108:102–111, 1986.
- ²⁰Linder-Aronson, A., and S. Lindskog. Effects of static magnetic fields on human periodontal fibroblasts in vitro. *Swed. Dent. J.* 19:131–137, 1995.
- ²¹Lohmann Schwartz, C. H. Z., Y. Liu, H. Duerkov, and D. D. Dean. Pulsed electromagnetic field stimulation of MG63 osteoblast-like cells affects differentiation and local production. *J. Orthop. Res.* 18:637–646, 2000.
- ²²McDonald, F. Effect of static magnetic fields on osteoblasts and fibroblasts in vitro. *Bioelectromagnetics* 14:187–196, 1993.
- ²³Nohan, S., and D. Baylink. Bone growth factors. *Clin. Orthop. Rel. Res.* 263:30–48, 1991.
- ²⁴Pacini, S., G. B. Vannelli, T. Barni, M. Ruggiero, I. Sardi, P. Pacini, and M. Gulisano. Effects of 0.2 T static magnetic field on human neurons: remodeling and inhibition of signal transduction without genome instability. *Neurosci. Lett.* 267:185–188, 1999.
- ²⁵Parkinson, W. C. Comments on the use of electromagnetic fields in biological studies. *Calicif. Tissue Int.* 37:198–207, 1985.
- ²⁶Przybylska, M., A. Koceva-Chyla, B. Rozga, and Z. Jozwiak. Cytotoxicity of daunorubicin in trisomic (+21) human fibroblasts: relation to drug uptake and cell membrane fluidity. *Cell Biol. Int.* 25:157–170, 2001.
- ²⁷Raymond, R. R., A. C. Clavo, and R. L. Wahl. Exposure to strong static magnetic field slows the growth of human cancer cells in vitro. *Bioelectromagnetics* 17:358–363, 1996.
- ²⁸Sharrard, W. J., M. L. Sutcliffe, M. J. Robson, and A. G. Maceachern. The treatment of fibrous non-union of fractures by pulsing electromagnetic stimulation. *J. Bone Joint Surg.* 64B:189–193, 1982.
- ²⁹Shimizu, E., Y. Matsuda-Honjyo, H. Samoto, R. Saito, Y. Nakajima, Y. Nakayama, N. Kato, M. Yamazaki, and Y. Ogata. Static magnetic fields-induced bone sialoprotein (BSP) expression is mediated through FGF2 response element and pituitary-specific transcription factor-1 motif. *J. Cell Biochem.* 91:1183–1196, 2004.
- ³⁰Short, W. O., L. Goodwill, C. W. Taylor, C. Job, and M. E. Arthur. Alteration of human tumor cell adhesion by high-strength static magnetic fields. *Invest. Radiol.* 27:836–840, 1992.
- ³¹Silva, V. S., M. Cordeiro, M. J. Matos, C. R. Oliveira, and P. P. Concalves. Aluminum accumulation and membrane fluidity alteration in synaptosomes isolated from rat brain

- cortex following aluminum ingestion: effect of cholesterol. *Neurosci. Res.* 44:181–193, 2002.
- ³²Stein, G. S., and J. B. Lian. Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of the osteoblast phenotype. *Endo. Rev.* 14:424–442, 1993.
- ³³Suda, T., and S. Ueno. Magnetic orientation of red blood cell membranes. *I.E.E.E. Trans. Magn.* 30:4713–4715, 1994.
- ³⁴Suda, T., and S. Ueno. Effect of strong magnetic fields on the characteristics of bilayer lipid membranes. *J. Appl. Phys.* 81:4318–4320, 1997.
- ³⁵Toplak, H., V. Batchiulis, A. Hermetter, T. Hunziker, U. E. Honegger, and U. N. Wiesmann. Effects of culture and incubation conditions on membrane fluidity in monolayers of cultured cells measured as fluorescence anisotropy using trimethylammoniumdiphenylhexatriene (TMA-DPH). *Biochim. Biophys. Acta.* 1028:67–72, 1990.
- ³⁶Wiskirchen, J., E. F. Gronewaller, R. Kehlbach, F. Heinkelmann, M. Wittau, H. P. Rodemann, C. D. Claussen, and S. H. Duda. Long-term effects of repetitive exposure to a static magnetic field (1.5 T) on proliferation of human fetal lung fibroblasts. *Magnet. Reson. Med.* 41:464–468, 1999.
- ³⁷Yamamoto, Y., Y. Ohsaki, T. Goto, A. Nakasima, and T. Iijima. Effects of static magnetic fields on bone formation in rat osteoblast cultures. *J. Dent. Res.* 82:962–966, 2003.
- ³⁸Yan, Q. C., N. Tomita, and Y. Ikada. Effects of static magnetic field on bone formation of rat femurs. *Med. Eng. Phys.* 20:397–402, 1998.