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A Newly Designed Experimental System for Exposure of Mammalian Cells to Extremely Low Frequency Magnetic Fields

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To examine the biological effects of extremely low frequency magnetic field (ELFMF), we have designed and manufactured a new equipment for long-term and high-density exposure of cells to ELFMF. The ELFMF exposure system consists of a generator of magnets with a bult-in CO_2 incubator, an alternating current (AC) power supply, a gas compressor and a thermocontroller for the incubator, and a cooling unit for the magnets. The CO_2 incubator made of acrylic resin is inserted into the inner-space of the silicon steel strip-cores. In this system, the temperature of the incubator is maintained at 37 ± 0.5°C. The maximum magnetic flux density on the exposure area of the incubator is 500 mT (T; tesla) at a current of 556 A*rms* (*rms*; root mean square) at 50 Hz. The long-term (up to 120 hr) exposure of 400 mT ELFMF did not affect the growth of both HL60RG and CCRF-CEM cells originated from human leukemia. The post-X-irradiation exposure of 400 mT ELFMF for 2 hr also did not affect the radiation sensitivity of GM0637 and TAT2SF cells originated from a normal human and an ataxia telangiectasia patient.

INTRODUCTION

Extremely low frequency magnetic fields (ELFMF) arise from a variety of sources such as a power distribution network, public transportation systems, elecrtical appliances with moters etc. Possible health effects due to exposure to ELFMF have become a subject of considerable public interest and concern. Several reports have claimed to link the exposure to ELFMF in the homes and workplaces to apparent elevation in cancer risk[1-5].

Effects of ELFMF by *in vitro* studies have been reported on cell proliferation[6,7], syntheses of DNA, RNA, and protein[8-12], chromosome aberrations[7,13], ornithine decarboxylase activity[14], and cation fluxes[15,16]. Even existance of the effects on ELFMF has been contradictory among the reports. The contradictions could be attributed, in most cases, to the quality and power of the experimental equipments. Most of the negative data might have not been reported.

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In the present study, we designed and manufactured a new equipment for long-term and high-density exposures of cultured cells to ELFMF and examined the effects of ELFMF on cell growth, survival and radiation sensitivity.

ELFMF EXPOSURE SYSTEM

Figure 1 shows a diagram of the ELFMF exposure system, which consists of a generator of magnets with a built-in CO_2 incubator, an alternating current (AC) power supply, a gas compressor for the incubator, a thermocontroler, and a cooling unit for the magnets. The electric power supply (Model IPM-70057002, IDX Corporation, Tokyo) is AC 200/220 V, 50/60 Hz, triphase, and 35 kVA. An 800 A peak current (an effective value for AC is 566 A*rms*) is supplied to AC magnets by the method of the parallel resonance with the inductance of the magnets and the condencer of the power supply. Mixed gas of 5% CO_2 and 95% air, analyzed by a gas analyzer (Model MGA-100, Tokyo Rikakikai Co., Ltd., Tokyo), is supplied by a gas compressor (Model GMU-1, Tokyo Rikakikai) to the incubator. The thermocontroller (Model UC-55N, Tokyo Rikakikai) supplies the warm water to the incubator to maintain the temperature of the incubator at 37 ± 0.5°C. The magnet cooling unit (Cool Ace, Model CA-210, Tokyo Rikakikai) supplies the cool water, 10 to 25°C, to the circumference of the magnet cores. The temperature in the incubator was monitored by a platinum resistance thermometer covered with quartz (Model R005-SP, CHINO Corporation, Tokyo) and an alcohol thermometer, and recorded by a Hybrid Recorder (Model EH-200, CHINO).

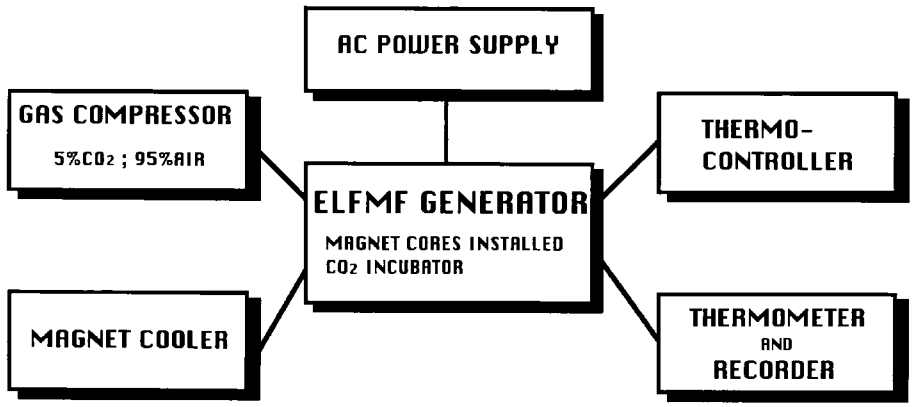

Fig. 1. Schematic diagram of the ELFMF exposure system consisting of a generator with a built-in CO_2 incubator, an AC power supply, a gas compressor and a thermocontroller for the CO_2 incubator, and a magnet cooler.

We measured the vibration of both the CO_2 incubator inserted into the generator and the conventional CO_2 incubator by using a vibration level meter (Model VM-14B, RION Co., Ltd.). The vibration values for the magnetic CO_2 incubator were as follows: x, 37 dB; y, 49 dB; z, 55 dB with the generator on (400 mT exposure) and x, 35 dB; y, 31 dB; and z, 40 dB with the

generator off, where these axes represent that x denotes axis front-to-back; y, axis left-to-right; and z, vertical axis. The vibration value for the conventional CO_2 incubator during operation were x, 38 dB; y, 37 dB; and z, 50 dB.

GENERATOR, INCUBATOR AND MAGNETIC FLUX DENSITY

Figure 2 shows the generator equipment including the AC magnets (IDX Corporation, Tokyo) and the CO_2 incubator (Sakuhana Seisakusho, Kyoto). Figure 3 shows (a) an ichnograph and (b) a side view of the silicon steel strip-core and the coil cupper. Six hundred silicon steel strips, each with 0.5 mm thickness, which were insulated each other, were piled up vertically by lap welding. The insulation of each silicon steel strip reduced the calorification induced by eddy currents and suppressed the reduction of the magnetic flux density. In this system, the magnetic field is directed vertically and both the magnet field for horizontal direction and the electric field are very weak. The weight of the core is approximately 1000 kg. As shown in Fig. 3, two copper coils penetrate into the silicon stell strip-core. The conductor for the coils was made of copper (10 mm$\times$20 mm, 2 lines) with 11 turns of the coil. The maximum magnetic flux density in the center of the space "G" shown in Fig. 3 is 500 mT at a current of 556 A*rms* with 50 Hz, which is measured by a Gauss Meter (Model 3251, Yokogawa Electrical Co., Ltd., Tokyo).

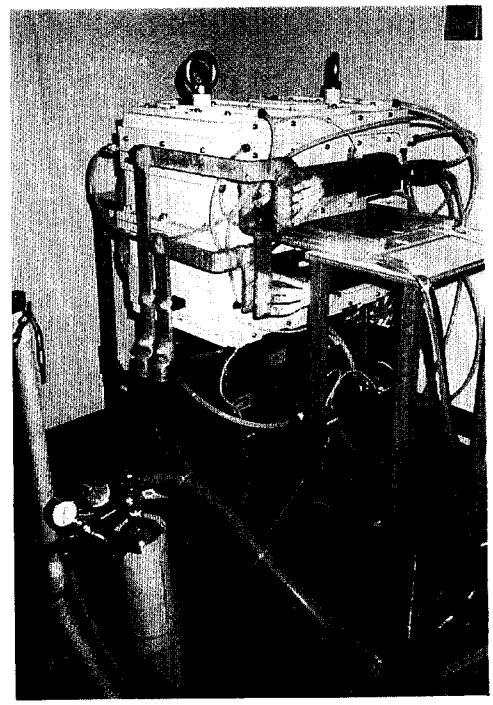

Fig. 2. ELFMF generator with a built-in CO_2 incubator.

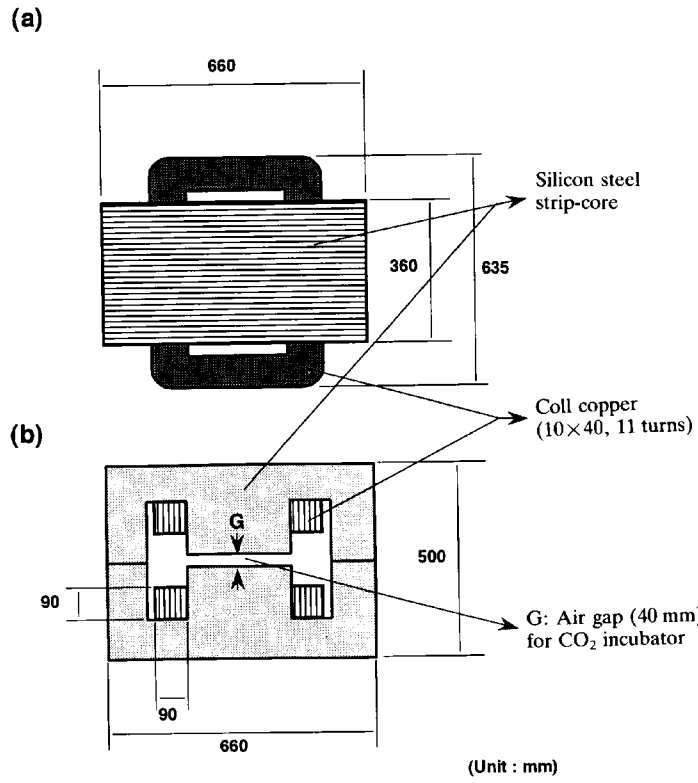

Fig. 3. (a) An ichnograph and (b) a cross-section of the silicon steel strip-core and the coil cupper. The CO_2 incubator is installed between the silicon steel strip-cores as shown in the space "G".

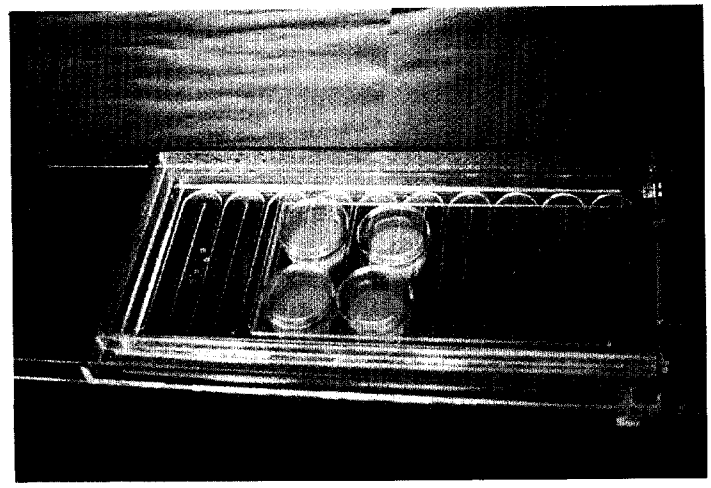

Fig. 4. The CO_2 incubator made of acrylic resin. Orientation of ELFMF is perpendicular to the base of the dish.

Figure 4 shows the CO_2 incubator made of acrylic resin. The CO_2 incubator was inserted into the inner-space of the silicon steel strip-core (space "G" as shown in Fig. 3). To keep the high humidity in the incubator, sterilized water was stored in the grooves surrounding the culture space. The warm water supplied from the thermocontroler flows in the space in the side-wall and bottom of the incubator. The mixed gas of 5% CO_2 and 95% air bubbling in the sterilized water at 37°C was supplied from the small holes at front lid to the incubator. The inner-space of the incubator for ELFMF exposure is 190 mm×220 mm×28 mm. Figure 5 shows the distribution of magnetic flux density at the exposure space "G" (Fig. 3) in the magnet cores. Density of the magnetic flux is homogeneous within 1% difference for the area housing four 10 cm or ten 6cm culture dishes.

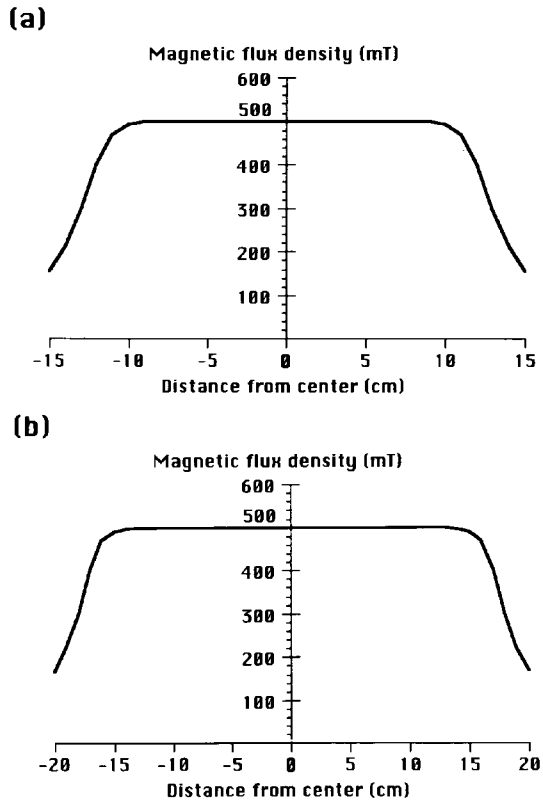

Fig. 5. Distribution of the magnet flux density at the exposure space "G" (Fig. 3) between the magnet cores. Upper panel (a) shows the distribution of a transverse section and lower panel (b) shows that of a longitudinal section.

CELL GROWTH AND SURVIVAL UNDER ELFMF EXPOSURE

Cells established from human leukemia, HL60RG and CCRF-CEM (obtained from Japanese Cancer Research Resources Bank, Tokyo), were suspended in the RPMI-1640 medium supplemented with 10% fetal bovine serum at a density of 5×10^4/ml for HL60RG and 4×10^4/ml for CCRF-CEM. They were exposed to 400 mT ELFMF in the exposure unit for up to 120 hr. Unexposed cells were incubated in a conventional CO_2 incubator placed in a separate room.

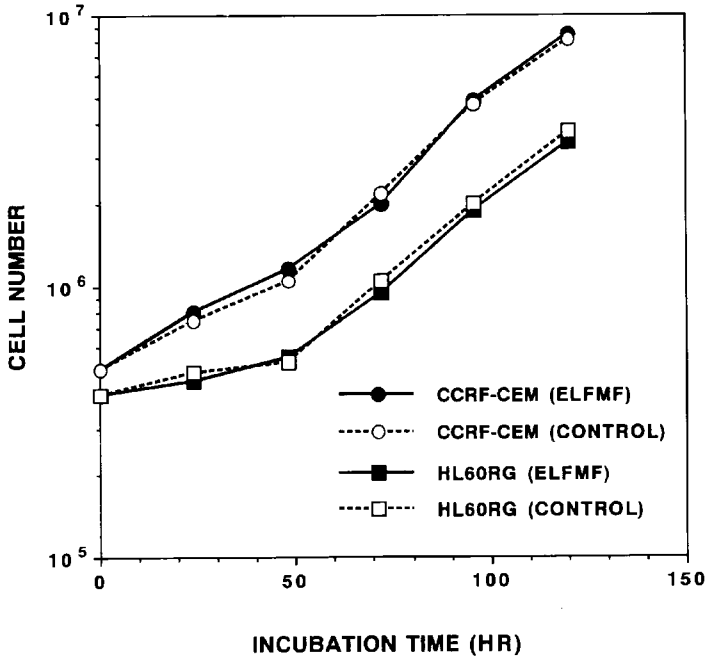

Fig. 6. Growth curves of CCRF-CEM and HL60RG cells under 400 mT ELFMF exposure with 50 Hz or in the conventional CO_2 incubator. The experiment was repeated two times. The data point represents the mean value of these expriments.

Figure 6 shows the growth curves of these cells, and no significant difference in the growth rate between the exposed and unexposed cells were noted. The 400 mT ELFMF exposure for up to 20 hr also did not affect the survival of human skin fibroblasts GM0637 and TAT2SF cells using colony formation method (data not shown). Livingston *et. al.*[7] reported that the growth rate and reproductive integrity of human lymphocytes and Chinese hamster ovary cells was unaffected by exposure to the 220 μT ELFLMF (60 Hz) for 96 hr. The magnetic flux density of our system is approximately 1800-fold higher than that of Livingston's report. From these results, we suggest that the long-term exposure of ELFMF may have no or very little effect on cellular control for the growth. However, several reports[8,9] demonstrated that DNA synthesis measured by the incorpolation of ^{3}H-thymidine was transiently stimulated by exposure to the pulsed electromagnetic field. Although the long-term exposures to ELFMF do not affect both growth and survival of cells tested, there may be a chance that some intracellular early responses to ELFMF are occured.

EFFECT OF ELFMF EXPOSURE ON RADIOSENSITIVITY OF NORMAL AND ATAXIA TELANGIECTASIA CELLS

SV40-transformed human cells, GM0637 and TAT2SF originated from a normal subject and an ataxia telangiectasia patient, respectively, were irradiated with X-rays at a dose rate of 1.2

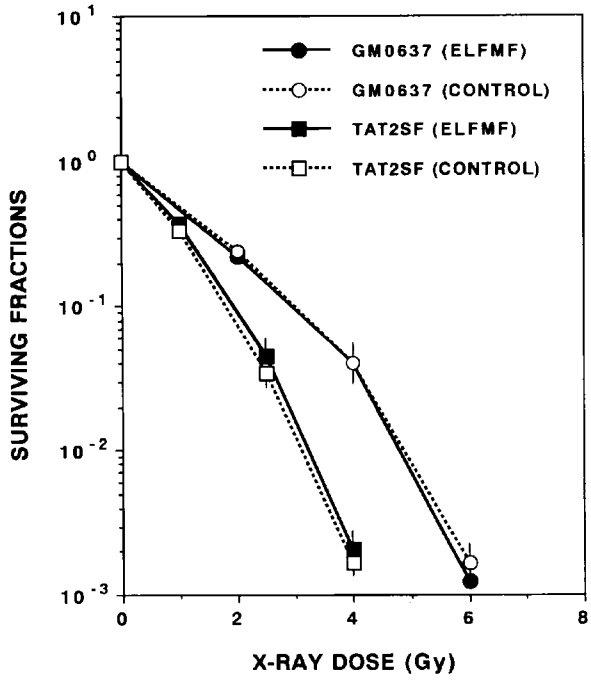

Fig. 7. X-ray dose-survival curves of GM0637 and TAT2SF cells exposed to X-irradiation followed by 400 mT ELFMF for 2 hr or to X-irradiation alone. Six plates were used for each data point and the error bar represents standard deviation.

Gy/min, and then incubated under the 400 mT ELFMF exposure for 2 hr. Figure 7 shows the X-ray dose-survival curves of GM0637 and TAT2SF cells with and without exposure to the ELFMF. TAT2SF cells were hyper-radiosensitive to X-rays. In both cell lines, the 400 mT ELFMF exposure for 2 hr did not significantly change the X-ray sensitivity of the cells. Nath *et al.*[17] reported that there was no significant change in survival when Chinese hamster cells were irradiated in the presence of an uniform magnetic feild at 2.05 T. Recently, Norimura *et al.*[18] demonstrated that T-lymphocytes previously exposed to a strong static magnetic field (6.3 T) for 24 hr were slightly more sensitive to X-rays than the non-exposed control cells. Although the modification of the radiation sensitivity by the magnetic field may depend on quality of the magnetic fields, period and sequence of the exposure, and cell types, remarkable changes in cellular radiation sensitivity may not be present by the exposure to the magnetic field.

CONCLUSION

We have designed and manufactured a new equipment for long-term and hihg-density exposures of cultured cells to ELFMF. The long-term (up to 120 hr) exposure of 400 mT ELFMF did not affect the growth of both HL60RG and CCRF-CEM cells. The post-irradiation exposure of 400 mT ELFMF for 2 hr also did not affect the radiation sensitivity of GM0637 and

TATK2SF cells.

At present, there is convincing evidence that ELFMF exposures do not produce DNA strand breaks or influence the repair of DNA damage caused by other agents[19,20]. The pericellular currents established by ELFMF may alter ion binding to membrane macromolecules and influence ligand-receptor interactions at the cellular surface. Cell membranes can play a key role in the transduction and amplification of ELFMF signals. In order to evaluate the biological effects of ELFMF, the investigation concerning the influence on signal transduction pathways by ELFMF is in progress by using our new system for ELFMF exposure.

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REFERENCES

1. Wertheimer, N. and Leeper, E. (1979) Electrical wiring configulations and childhood cancer. *Am. J. Epidemiol.*, **109**: 273–284.
2. Fulton, J. P., Cobb, S., Preble, L., Leone, L. and Foreman, E. (1980) Electrical wiring configulations and childhood leukemia in Rhode Island. *Am. J. Epidemiol.*, **111**: 292–296.
3. Bowman, J. D., Garabrant, D. H., Sobel, E. and Peter, J. M. (1988) Exposures to extremely low frequency (ELF) electromagnetic fields in occupations with elevated leukemia rates. *Appl. Ind. Hyg.*, **3**: 189–194.
4. Johnson, C. C. and Spitz, M. R. (1989) Childhood nervous system tumors: An assessment of risk associated with paternal occupations involving use, repair or manufacture of elecrtical and electronic equipment. *Int. J. Epidemiol.*, **18**: 756–762.
5. Savitz, D. A. Jow, E. M. and Kleckner, R. C. (1990) Magnet field exposure from electric appliances and childhood cancer. *Am. J. Epidemiol.*, **131**: 763–773.
6. Alvarez-Rodrigues, Y. Rivas, L., Rius, C. and Valladares, Y. (1985) White lightelectromagnetic field interaction causes a localized destruction of cultured HeLa cells. *Cancer Lett.*, **29**: 331–338.
7. Livingston, G. K., Witt, K. L., Gandhi, O. P., Chatterjee, I. and Roti, Roti, J. L. (1991) Reproductive integrity of mammalian cells exposed to power frequency electromagnetic fields. *Environ. Mol. Mutagen.*, **17**: 49–58.
8. Takahashi, K., Keneko, I., Date, M. and Fukada, E. (1986) Effect of pulsing electromagnetic fields on DNA synthesis in mammalian cells in culture. *Experientia*, **42**: 185–186.
9. Yen-patton, G. P. A., Patton, W. F., Beer, D. M. and Jacobson, B. S. (1988) Endothelial cell response to pulsed electromagnetic fields: Stimulation of growth rate and angiogenesis in vitro. *J. Cell. Physiol.*, **134**: 37–46.
10. Conti, P., Gigante, G. E., Cifone, M. G., Alesse, E. Ianni, G., Reale, M. and Angeletti, P. U. (1983) Reduced mitogenic stimulation of human lymphocyte by extremely low frequency electromagnetic fields. *FEBS Lett.*, **162**: 156–160.
11. Greene, J. J., Skowronski, W. J., Mullins, J. M. and Nardone, R. (1991) Delineation of electric and magnetic field effects of extemely low frequency electromagnetic radiation on transcription. *Biochem. Biophys. Res. Commun.*, **174**: 742–749.

12. Rodemann, H. P., Bayreuther, K. and Pfleiderer, G. (1989) The differentiation of normal and transformed human fibroblasts in vitro is influenced by electromagnetic fields. *Exptl. Cell Res.*, **182**: 610–621.
13. Khalil, A. M. and Qassem, W. (1991) Cytogenetic effects of pulsing electromagnetic field on human lymphocytes in vitro: Chromosome aberrations, sister-chromatid exchanges and cell kinetics. *Mutat. Res.*, **247**: 141–146.
14. Byus, C. V., Pieper, S. E. and Adey, W. R. (1987) The effects of low-energy 60-Hz environmental electromagnetic fields upon the growth-related enzyme ornithine decarboxylase. *Carcinogenesis*, **8**: 1385–1389.
15. Conti, P. Gigante, G. E., Alesse, E., Cifone, M. G., Fieshi, C., Reale, M. and Angeletti, P. U. (1985) A role for Ca^{2+} in the effect of very low frequency electromagnetic field on the blastogenesis of human lymphocytes. *FEBS Lett.*, **181**: 28–32.
16. Walleczek, J. and Liburdy, R. P. (1990) Nonthermal 60 Hz sinusoidal magnetic-field exposure enhances $^{45}Ca^{2+}$ uptake in rat thymocytes: Dependence on mitogen activation. *FEBS Lett.*, **271**: 157–160.
17. Nath, R., Schulz, R. J. and Bondiorni, P. (1980) Response of mammalian cells irradiated with 30 MV X-rays in the presence of a uniform 20-kilogauss magnetic field. *Int. J. Radiat. Biol.*, **38**: 285–292.
18. Norimura, T., Imada, H., Kunugita, N., Yoshida, N. and Nikaido, M. (1993) Effects of strong magnetic fields on cell growth and radiation response of human T-lymphocytes in culture. *J. UOEH*, **15**: 103–112.
19. Reese, J. A., Jostes, R. F. and Frazier, M. (1988) Exposure of mammalian cells to 60-Hz magnetic or electric fields: Analysis for DNA single-strand breaks. *Bioelectromagnetics*, **9**: 237–247.
20. Frazier, M. E., Reese, J. A., Morris, J. E., Jostes, R. F. and Miller, D. L. (1990) Exposure of mammalian cells to 60-Hz magnetic or electric fields: Analysis of DNA repair of induced single-strand breaks. *Bioelectromagnetics*, **11**: 229–234.