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Effect of ferrite thermoseeds on destruction of carcinoma cells under alternating magnetic field

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High iron-containing silicate glasses were prepared using a conventional melting and quenching process, and ferrimagnetic crystallites were precipitated via a nucleation and crystal growth mechanism. This study attempted to use these ferrite-containing glass-ceramics as thermoseeds for a hyperthermic cancer treatment under an alternating magnetic field. KB and L929 cells were used in the cell lysis experiments, as carcinoma and normal cells, respectively. These cells were mixed with an agar medium and stained prior to the annihilation test. After exposing the cells to the alternative magnetic field for 9 min, the number of carcinoma cells in the vicinity of the ferromagnetic specimen decreased with increasing time and almost all cancer cells were dead after 9 min while they were still alive in a region of 5 cm away from the specimen. When Sprague-Dawley rats imbedded the samples were exposed to a magnetic field, tumor cells disappeared after only 4 treatments of 15 min each. This amazing reduction in the tumor was not observed in any rats without the imbedded sample. It is expected that the prepared ferrite-containing glass-ceramics will be helpful in hyperthermic cancer treatment. Long-term research is needed to confirm this result.

1. Introduction

Gilchrist *et al.* reported localized magnetic hyperthermia using fine magnetic particles more than 40 years ago [1–3]. The heating of tumor tissue as a possible therapeutic modality in cancer treatment has received increasing interest in recent years because it is well accepted that temperatures in the range of 42–45°C are cytotoxic. That is, increasing the temperature of tumor cells from normal body temperature to 42–45°C for certain time periods, approximately one hour, results in the destruction of a very high percentage of tumor cells. When the cells were kept at 37°C, virtually all the cells survived. However, when the cells were kept at 41.5°C for 5 h, only 10% of the cells survived. An increase of temperature by only 1°C to 42.5°C dramatically increased the rate cell death so that only 0.01% of the cells survived [4–6]. Furthermore, a tumor is more easily heated than the surrounding normal tissues, since the blood vessels and nervous systems are poorly developed. Therefore, the oxygen supplied by the blood vessels in the tumor is not sufficient. Thus, the goal of hyperthermia treatments for cancer therapy

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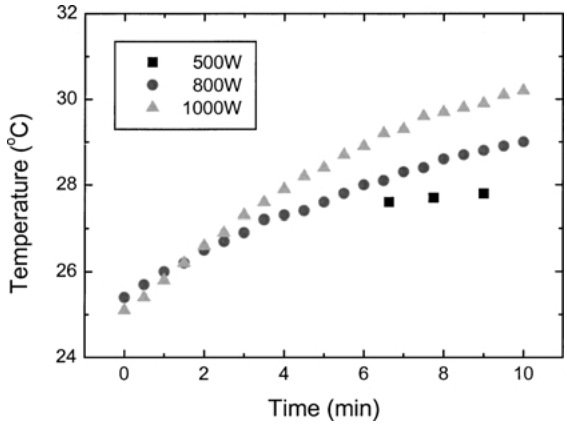
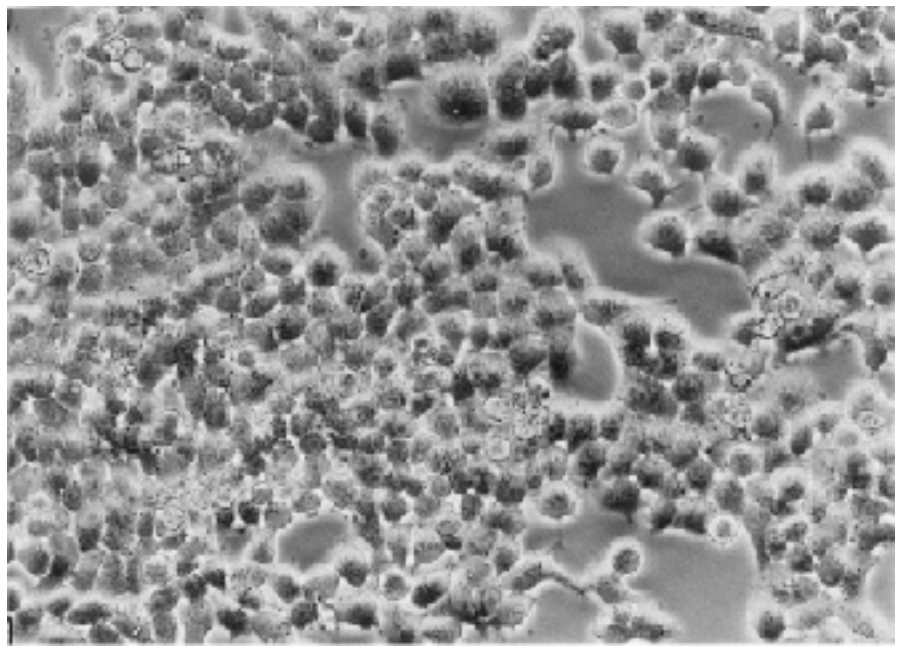
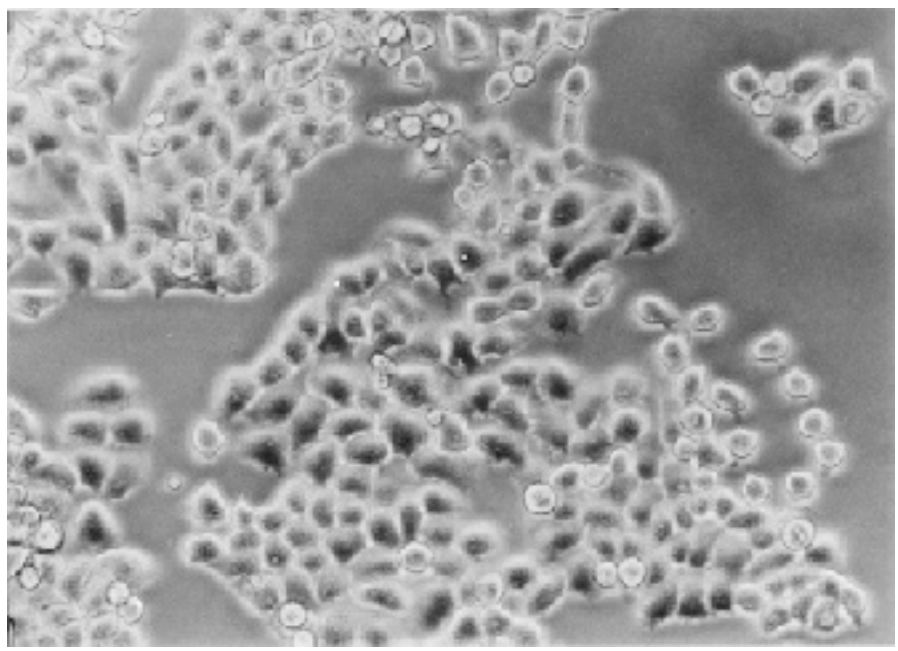


Figure 1 Temperature change of ferrimagnetic sample with various power of alternative magnetic field with 13.56 MHz *in vitro*.

is to raise the temperature of most of the tumor to above 42–43°C, while maintaining the temperatures in the surrounding healthy tissue below a certain safe level [7–10]. Superficial tumors can be heated with external electrodes, microwaves and ultrasound [11]. However, such techniques may pose problems in heating deep-seated tumors, e.g., prostate, bladder, or cervix cancers. The main problem is the overheating of healthy tissues because human tissue can easily absorb these external energies [12]. Therefore, in certain situations, some invasive method of heating might be preferred [13]. One of them, magnetic induction hyperthermia was first developed by Stauffer *et al*., who reported that in alternating magnetic fields, ferrimagnetic materials might act as a localized heat source at certain target regions inside the human body due to their hysteresis

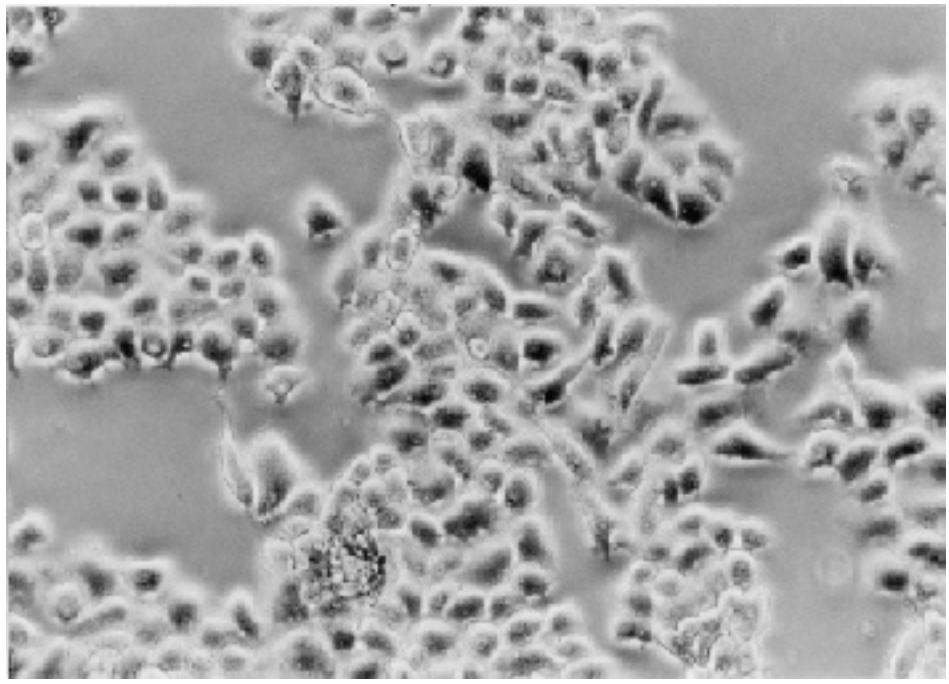


(a)



(b)

Figure 2 Carcinoma cells, KB, without ferrimagnetic sample under alternative magnetic field with 13.56 MHz after exposure for (a) 0, (b) 7, (c) 9, and (d) 11 min. (*Continued*)



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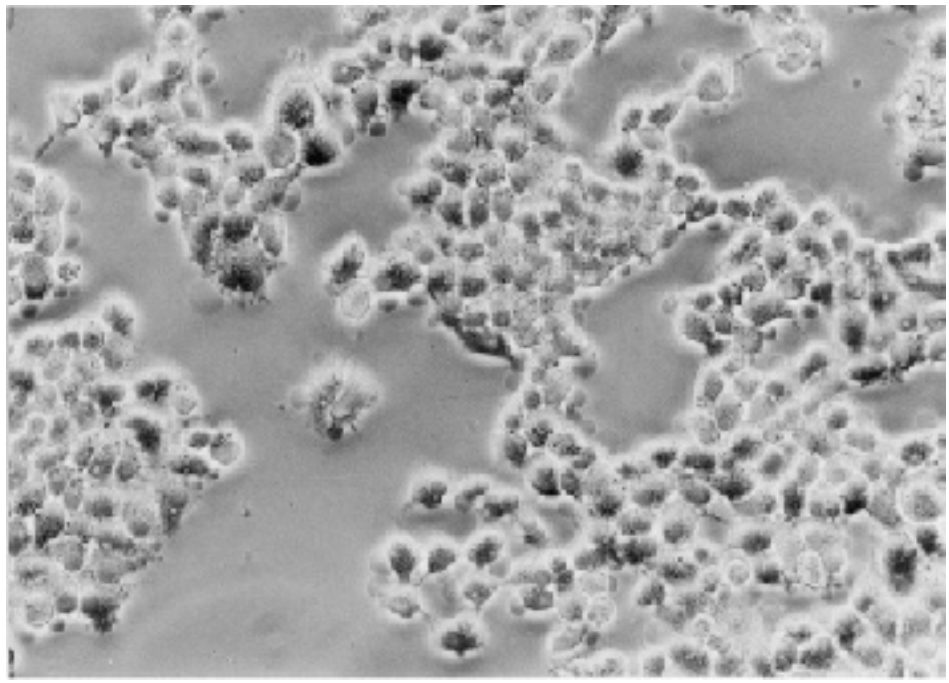


(d)

Figure 2 (*Continued*).

loss [14, 15]. The significant advantages of hysteresis heating are the convenient high localization such as via an injection and no attenuation by normal tissues as well as biocompatibility. Many recent laboratory and clinical trials already have demonstrated the efficacy of combining interstitial hyperthermia with interstitial radiation therapy [15–20].

Glass or glass-ceramics play an important role in magnetic induction hyperthermia. That is, the harmful leaching of metal ions into human body fluid by these materials can be avoided due to the encapsulation of each ferromagnetic particle by the glass matrix. Additionally, ferrimagnetic glass and glass-ceramics are bioactive while ferrimagnetic metals are bioinert. These ferrimagnetic glass-ceramics permit a much greater control over their physical, chemical, and magnetic properties by virtue of the glass composition and heat-treatment [21]. The magnetic and chemical properties as well as the preparation procedures of the ferrimagnetic glass-ceramics based on the Fe_2O_3-CaO-SiO_2 system have already been reported [22–25].

In order to reveal the possibility of applying ferromagnetic glass-ceramics in the Fe_2O_3-CaO-SiO_2 system for hyperthermic cancer treatment, the annihilation of cancer cells were examined under exposure to an alternative magnetic field both *in vitro* and *in vivo*.

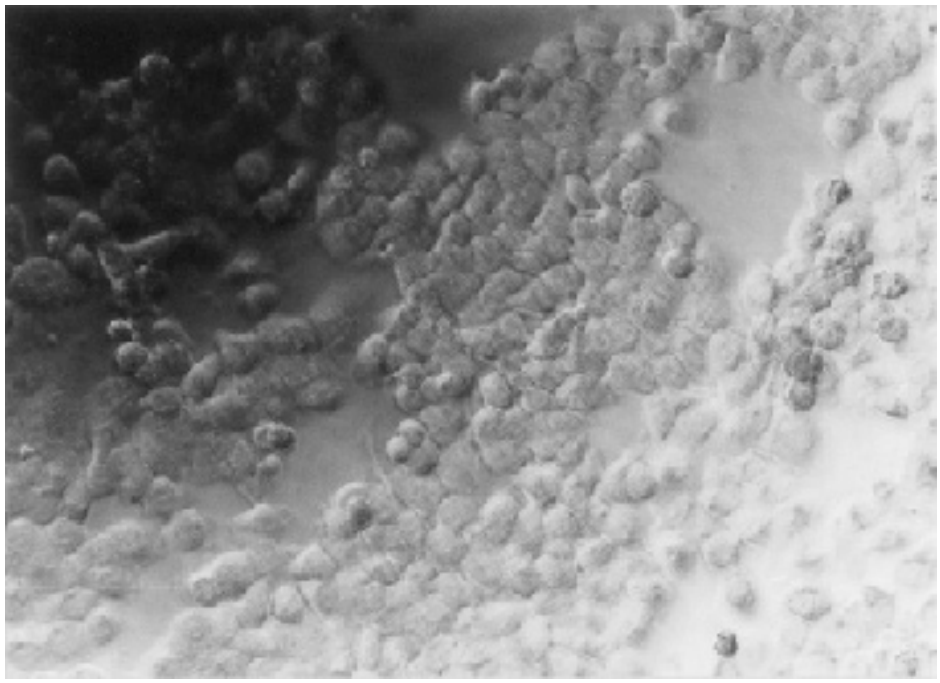
2. Materials and methods

Glass preparation and heat-treatment procedures were same as previously reported [22–25].

2.1. Temperature measurement under alternative magnetic field

Temperature changes in the heat-treated samples were determined using a fiberoptic thermometer (3100, Luxtron, Santa Clara, CA, USA) and automatically saved on a connected computer in every 30 s. The advantages of the fiberoptic thermometer are the multi-measurement of four temperatures simultaneously as well as not being influenced by the electromagnetic field. Hysteresis heating was induced by an alternating magnetic field up to 13.56 MHz generated by a radio-frequency power generator (CPS 500, Comdel Inc., Gloucester, MA, USA). The system consists of an adjustable (up to 1.25 kW), three-phase, 220 V unit with a working coil consisting of a four-turn, and 13.15 cm water-cooled copper tube with a 3.3 μm henry inductance. The generated power was controlled precisely using an automatic tuning network (Match-Pro CPMX 2500, Comdel Inc., Gloucester, MA, USA).

Rectangular samples with $10 \times 10 \times 1$ mm were used to measure the change in temperature under an



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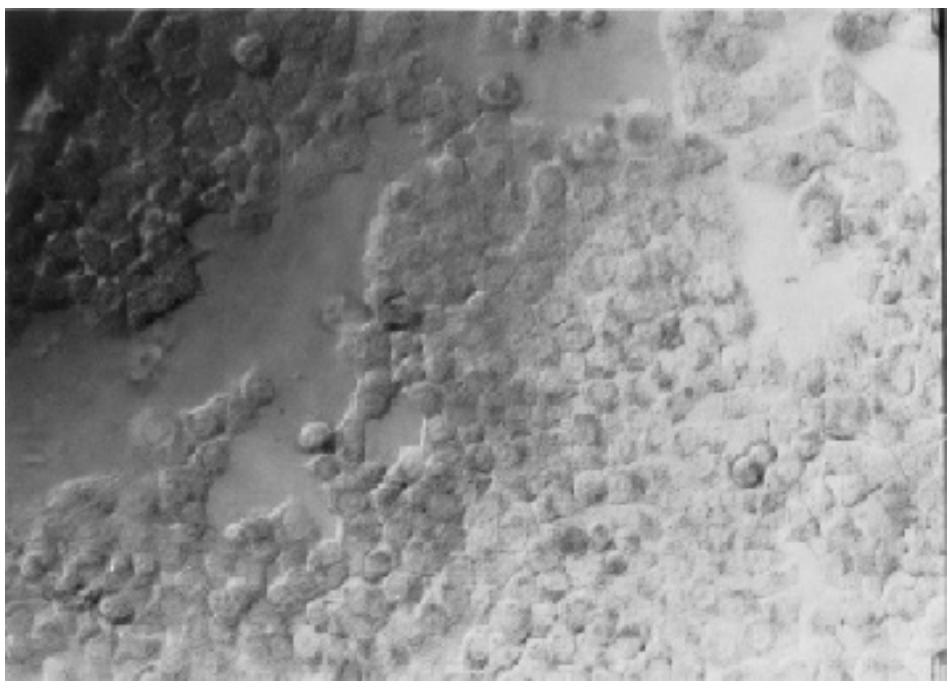
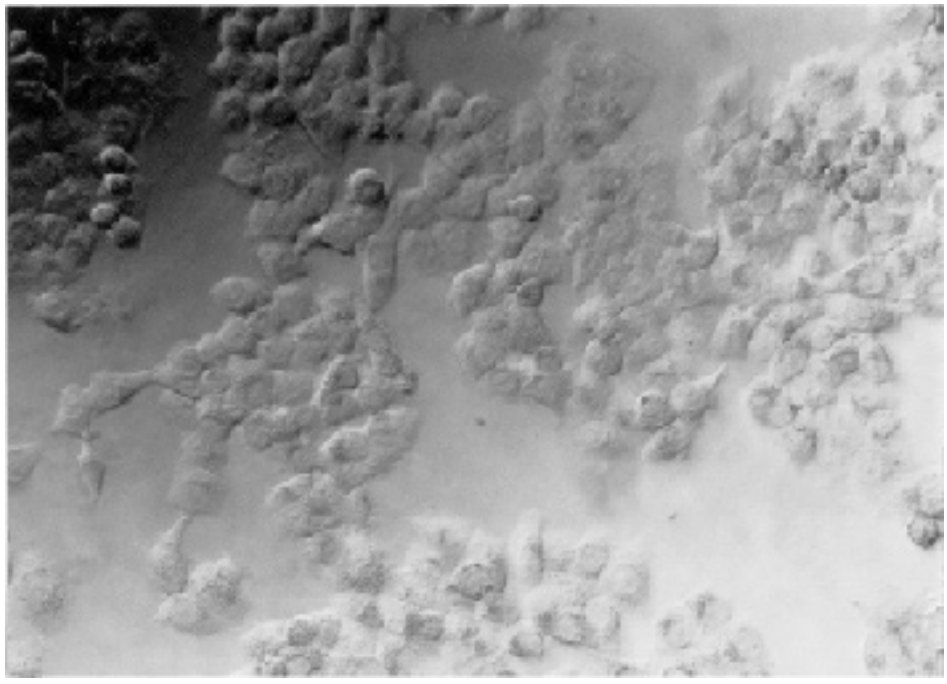


(b)

Figure 3 Carcinoma cells, KB, around ferrimagnetic sample under alternative magnetic field with 13.56 MHz after exposure for (a) 0, (b) 1, (c) 3, (d) 5, (e) 7, and (f) 9 min. (*Continued*)



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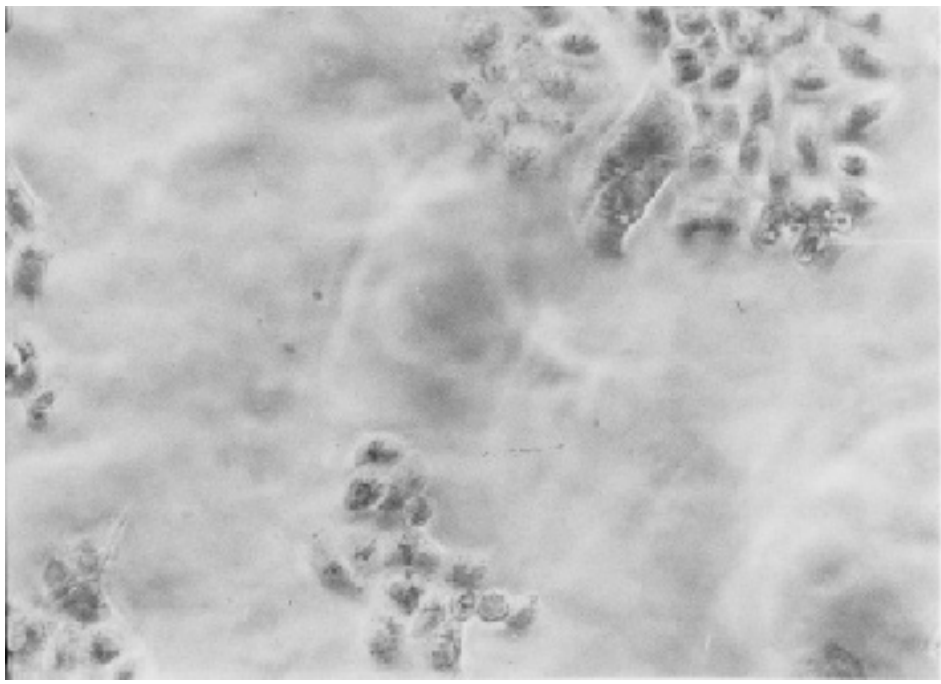


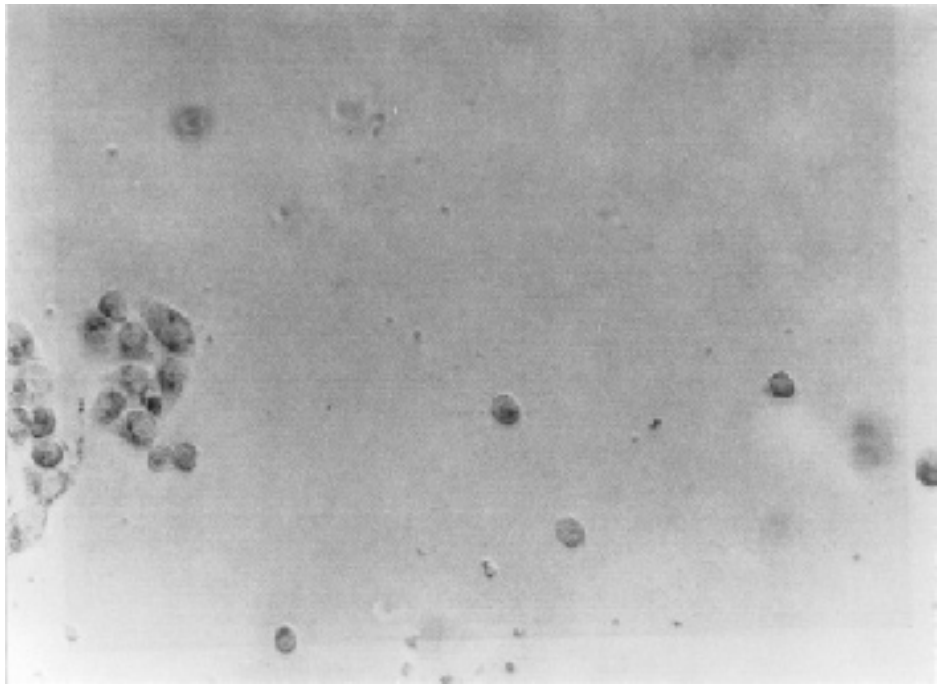
(d)

Figure 3 (*Continued*).

alternative magnetic field *in vitro*. Various powers applied to observe the temperature change *in vitro*. The *in vivo* temperature change was determined after loading the $2 \times 2 \times 1$ mm rectangular samples into the tumor site of the Sprague-Dawley rats. Each sample was placed in each 30 mm^2 sized tumor. As a control group, the temperature changes in mouth and the anus were examined without sample loading. The difference between the mouth and the anus was their location. The anus was located inside the coil, while the mouth was outside.

2.2. Cell culture

Established cell lines from the American Type Culture Collection (Rockville, MD, USA). L-929 (normal cell; fibroblast connective tissue of 100 days-old male mouse) was used to test the cytotoxicity of the samples. They were purchased in a frozen state and stored in a liquid nitrogen freezer until needed. Each cell line was defrosted prior to use. Prior to cell cultivation, dimethylsulfoxide (DMSO) containing a cryoprotectant, was removed. Each 1 ml of the cell line was mixed with 20 ml of Eagle's minimum essential medium (MEM) containing 5% fetal bovine serum (FBS). They were plated onto a 90 mm petri-dish and incubated to mistosis up to 3×10^5/ml at 37°C, 5% CO_2 for 1 week. The culture media were discarded and replaced by fresh culture media each day. When the concentration of cells exceed 3×10^5/ml cells, were subcultured and incubated again. They were distributed to 10 ml evenly over the



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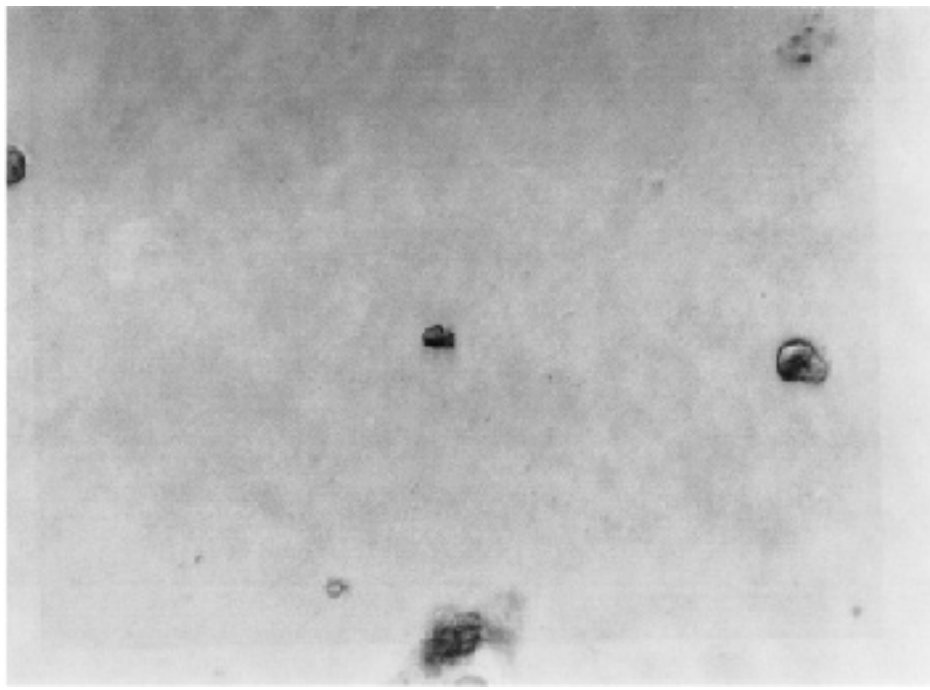


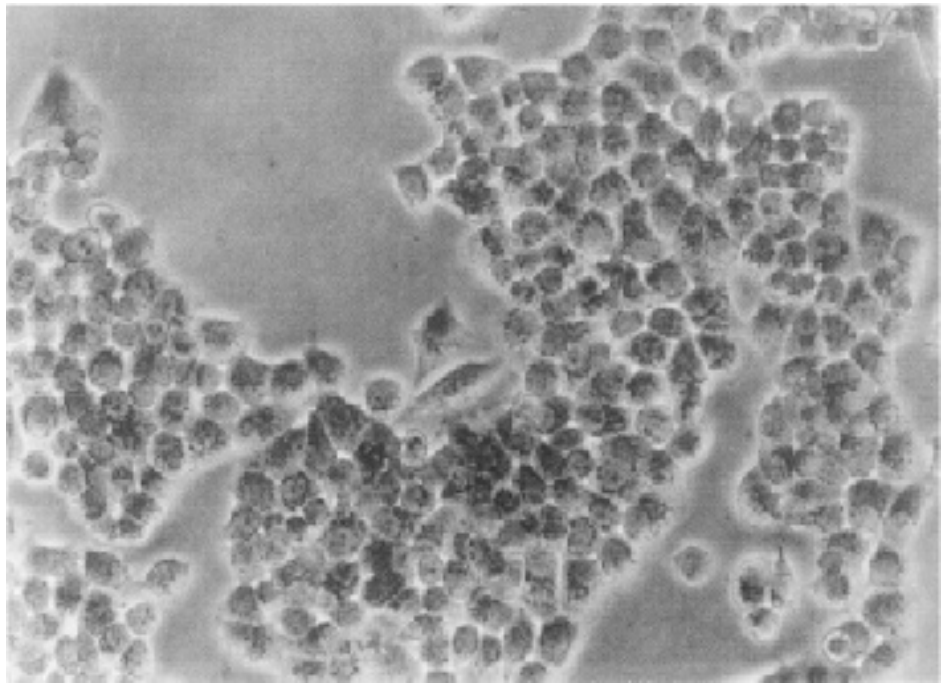
(f)

Figure 3 (*Continued*).

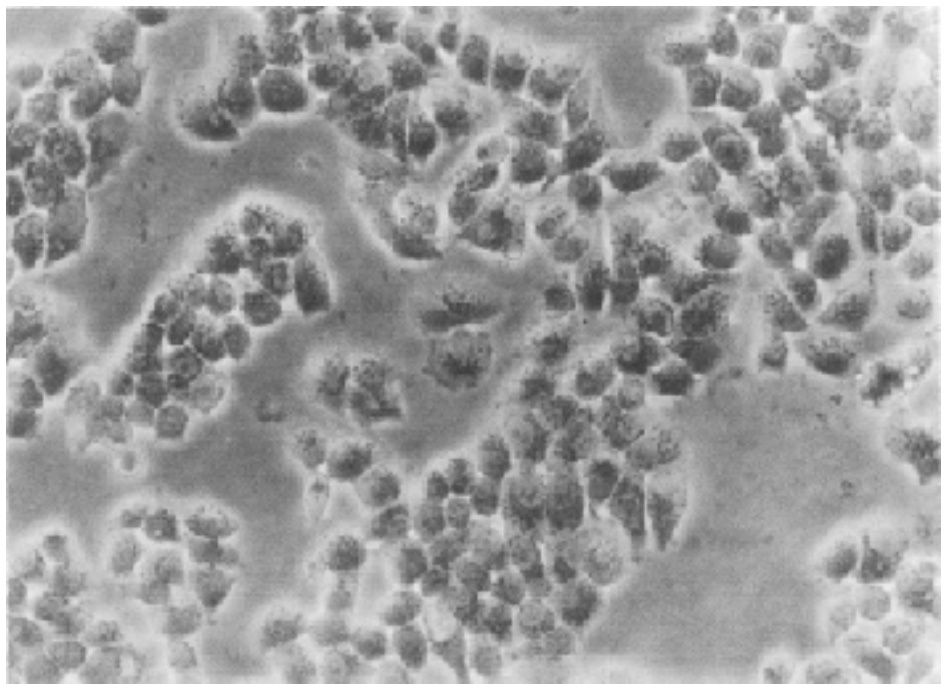
surface of each petri-dish by gentle rotation. The KB cells (carcinoma cell; epithelial mouth tissue of human) was cultured in the same as done for the L929 cells.

2.3. Cytotoxicity test

Prior to the cell annihilation test under the alternating magnetic field, the cytotoxicity of the prepared glasses and heat-treated samples was examined using an agar overlay method in accordance with ISO 10993-5. The Eagle's agar medium consisted of 50% agar and 50% Eagle's MEM with 5% FBS. Fresh agar media were replaced each Eagle's MEM containing FBS in the same volume and allowed to gel at room temperature for 30 min. Each agar medium was stained with a neutral red vital stain solution for 30 min in the same volume. A couple of $10 \times 10 \times 1$ mm rectangular specimens were overlaid onto each petri-dish filled with the agar medium with the negative and positive controls after removing the staining solution. The specimens were sterilized by ethylene-oxide gas prior to the agar overlay. Polyethylene was used as a negative control in order to demonstrate the background response of the cells. Stabilized polyvinylchloride was used as a positive control to prove the appropriate test system response.



(a)



(b)

Figure 4 Carcinoma cells, KB, apart 5 cm from the ferrimagnetic sample under alternative magnetic field with 13.56 MHz after exposure for (a) 7 and (b) 9 min.

Each petri-dish with four pieces on the agar medium were subsequently incubated at 37°C, 5% CO_2 for 24 h.

After 24 h, the cell lysis ratio in a decolorized area was determined. The decoloration zone was assessed around the samples using an inverted microscope with a calibrated screen and the decoloration index was determined for each sample according to ISO 10993 Part 5. The cell lysis ratio within the decolorized area was measured and converted to the lysis index also in accordance with ISO 10993 Part 5. The median decoloration index and lysis index were calculated for each specimen and the cell response is presented as follows:

$$\text{Cell response} = \text{Decoloration index/Lysis index}$$

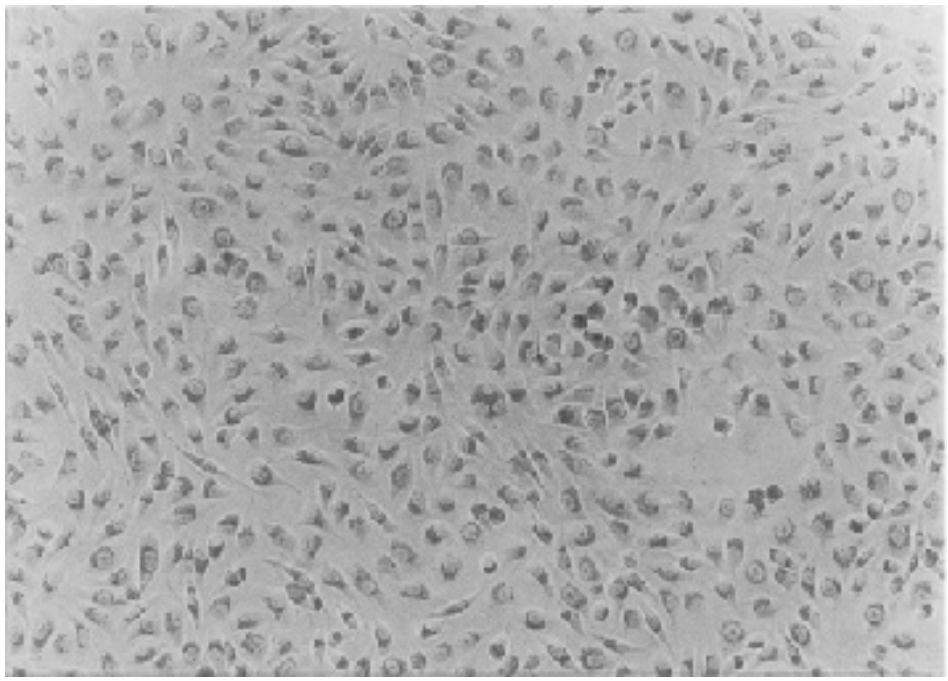
2.4. Tumor therapy *in vitro*

The KB cells was used to determine the effect of the prepared samples on the destruction of carcinoma cells *in vitro*. In order to reveal the intrinsic killing effect of the hysteretic heating of the prepared samples, cell lysis was also observed without the sample under an

alternative magnetic field using the same procedure. L929 cells were also used in order to compare the influence of the samples on normal and carcinoma cells. A total of four-groups (carcinoma cell w/ and w/o sample; normal cell w/ and w/o sample) were tested in a same experimental conditions. An agar overlay method was selected to determine the degree of cell annihilation after hysteretic heating under an alternating magnetic field. Four rectangular specimens sized $10 \times 10 \times 1$ mm overlaid onto each petri-dish were filled with the agar medium. Prior to plating onto the agar medium, the samples were sterilized by ethylene-oxide gas. After applying the alternating magnetic field in a certain time, cell lysis was observed at each time according to the ISO 10993 Part 5, as previously described.

2.5. Tumor therapy *in vivo*

The *in vivo* tumor therapy was examined utilizing 20 cases of female Sprague-Dawley rats aged 8 weeks. The carcinoma cells were induced using 9,10-dimethyl-1,2-benz(a) anthracene (DMBA, Sigma Chemical Co., USA) mixed with mineral oil at a 5% concentration.



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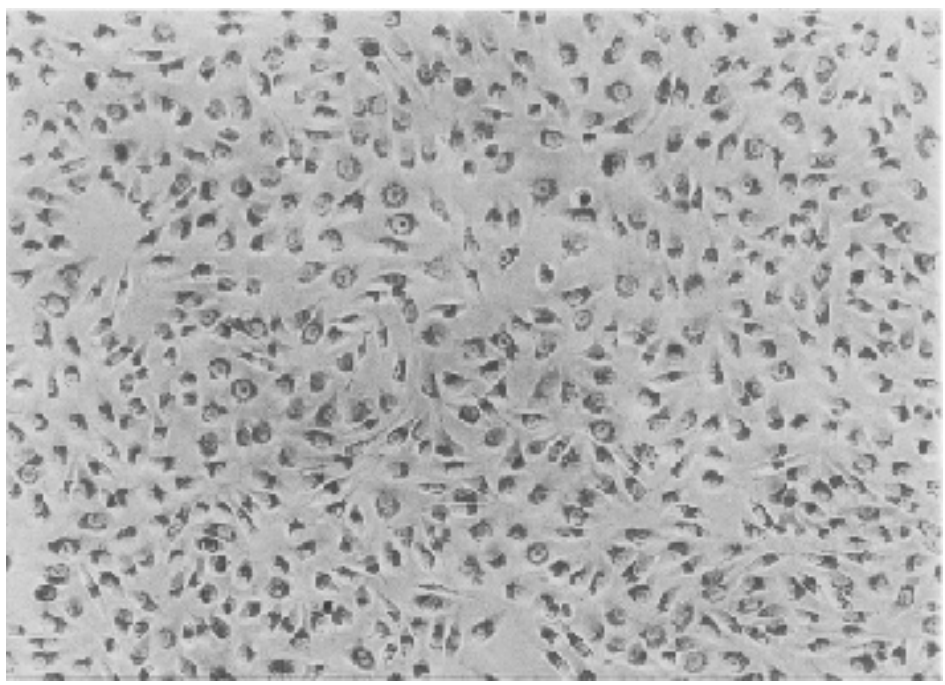
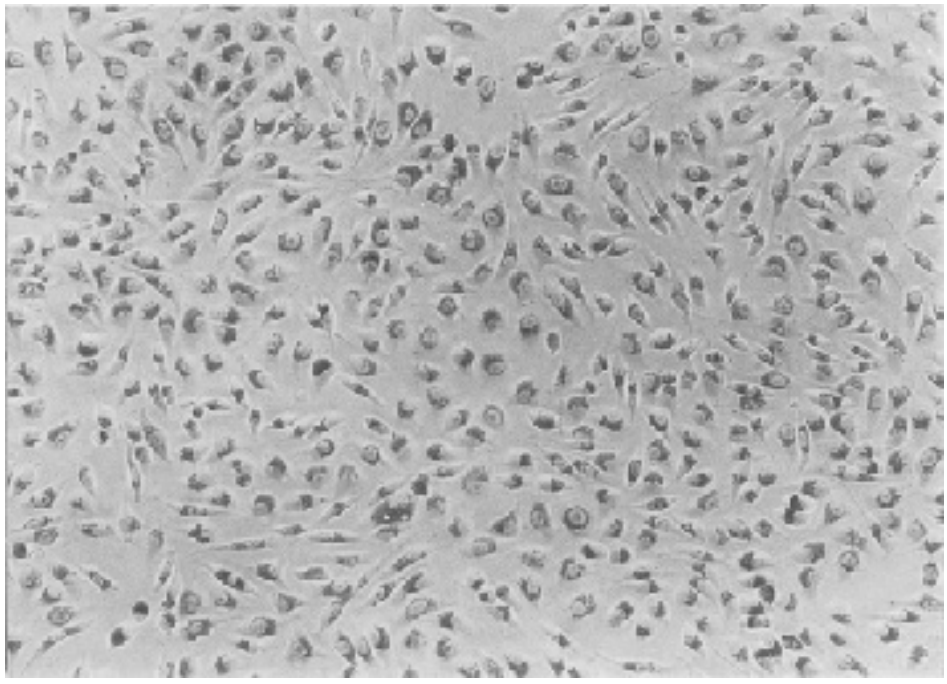


(b)

Figure 5 Normal cells, L929, without ferrimagnetic sample under alternative magnetic field with 13.56 MHz after exposure for (a) 0, (b) 7, (c) 9, and (d) 11 min. (*Continued*)



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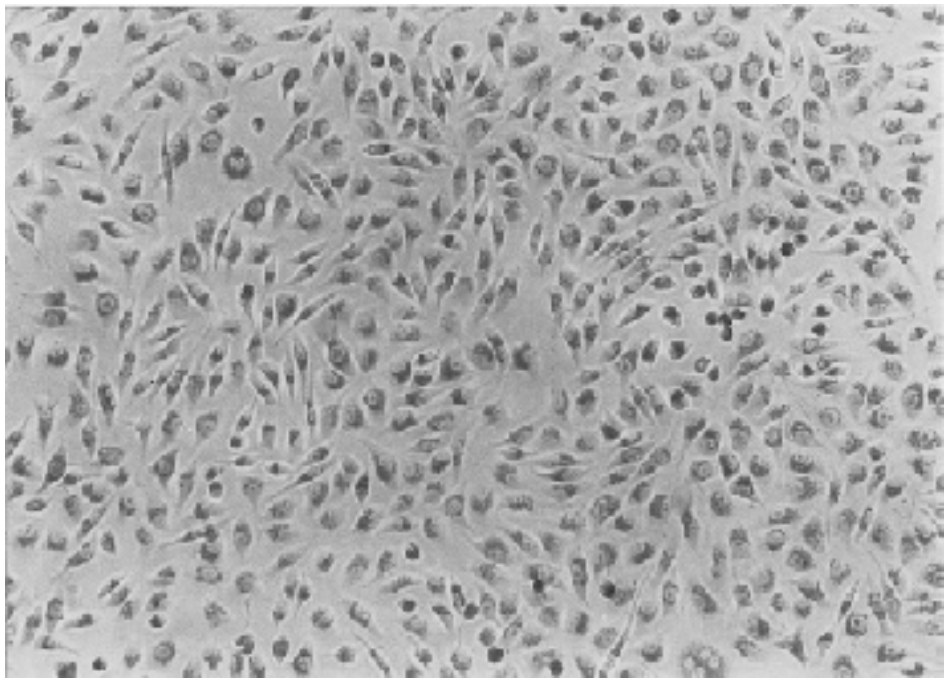


(d)

Figure 5 (*Continued*).

The rats were brushed with 0.5 ml 5% DMSO in the back region, which had been previously shaved in a 20×20 mm area using an electric clipper, 1 day prior. The rats were monitored and brushed daily for the development of the tumor. The tumor-bearing rats were treated on Day 0 when the palpable tumors averaged (8.3 ± 0.2) mm in diameter were observed. They were randomly assigned to experimental or control groups according to the sample loading. The rats in the experimental group received a couple of $2 \times 2 \times 1$ mm sized rectangular samples via an incision and subsequent suture in the immediate tumor region. The control groups were either treated with the magnetic field alone or underwent no treatment. The rats exposed to the alternating magnetic field were placed in an anesthetized condition and their bodies were exposed to the magnetic field except the head. They were exposed for 80 min during the 4 days. The experimental and control rats were followed-up daily.

3. Results and discussion

Prior to the cell annihilation test under the alternating magnetic field, it was confirmed that the specimens could not be killed by exposure to the magnetic field.

TABLE I Cytotoxicity of the as-quenched and heat-treated samples with L-929 cell by the agar overlay method after 24 h

Specimen	Decoloration index	Lysis index	Response index	Cytotoxicity
Positive control	5	3	5/3	Severe
Negative control	0	0	0/0	None
As-quenched sample	1	1	1/1	None
Heat-treated sample	0	1	0/1	None

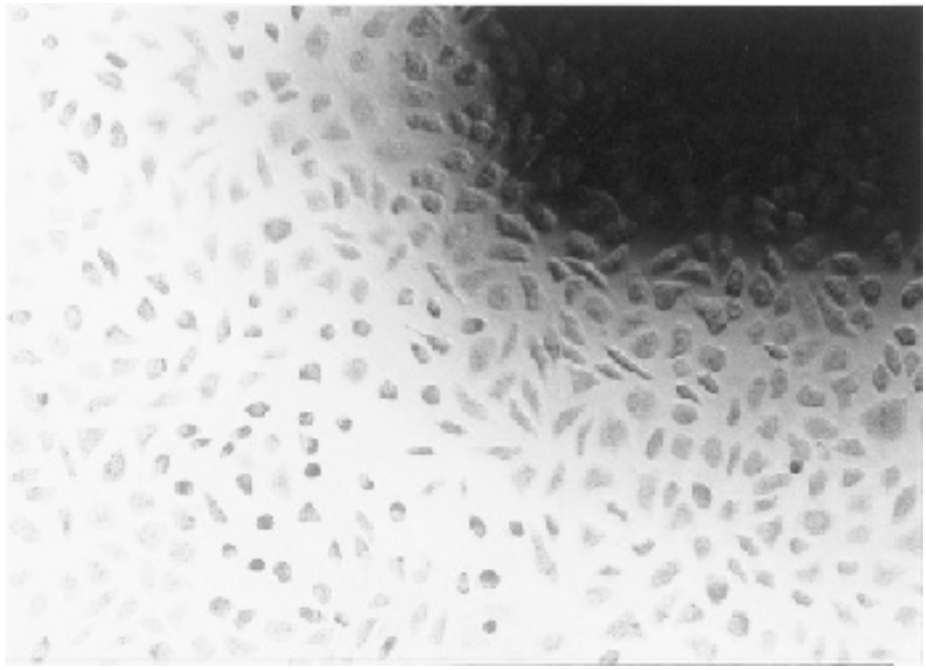
The result of the cytotoxicity test, as determined by the agar overlay method, was presented in Table I. Neither the heat-treated sample nor the parent glass exhibited any cytotoxicity.

TABLE II Response indices of carcinoma and normal cells after exposure to alternating magnetic field with the heat-treated samples

Exposure time	Carcinoma cell	Normal cell
1 min	0/0	0/0
3 min	0/0	0/0
5 min	1/2	0/0
7 min	2/3	1/3
9 min	2/5	1/4
11 min	3/5	1/5

The *i*carcinoma cells was attributed to the ferrimagnetic samples.

In order to verify the effect of the prepared sample on the normal cells, 100 days-old male mice. It



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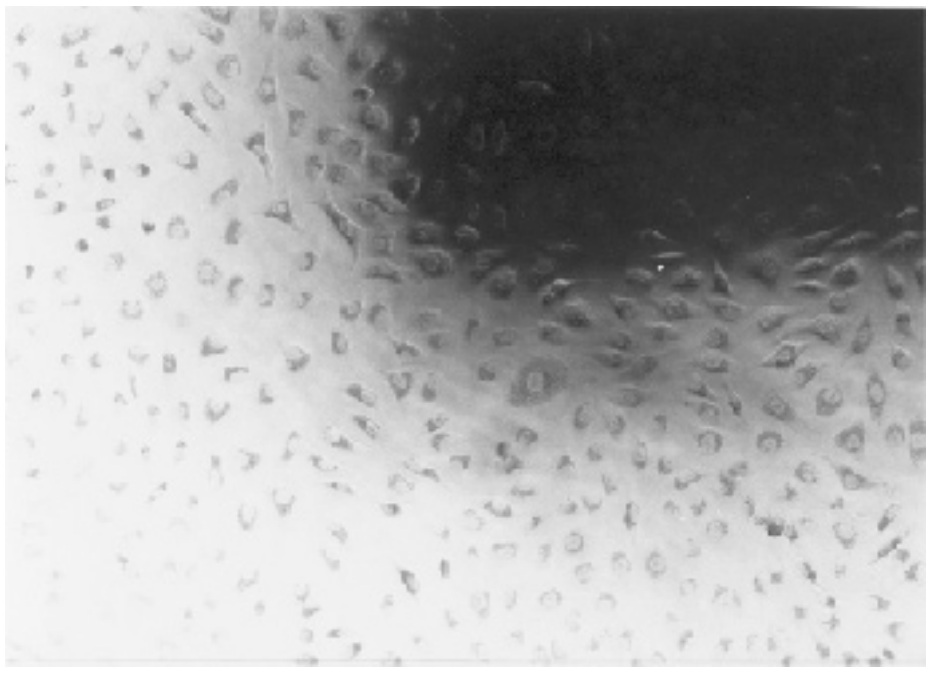
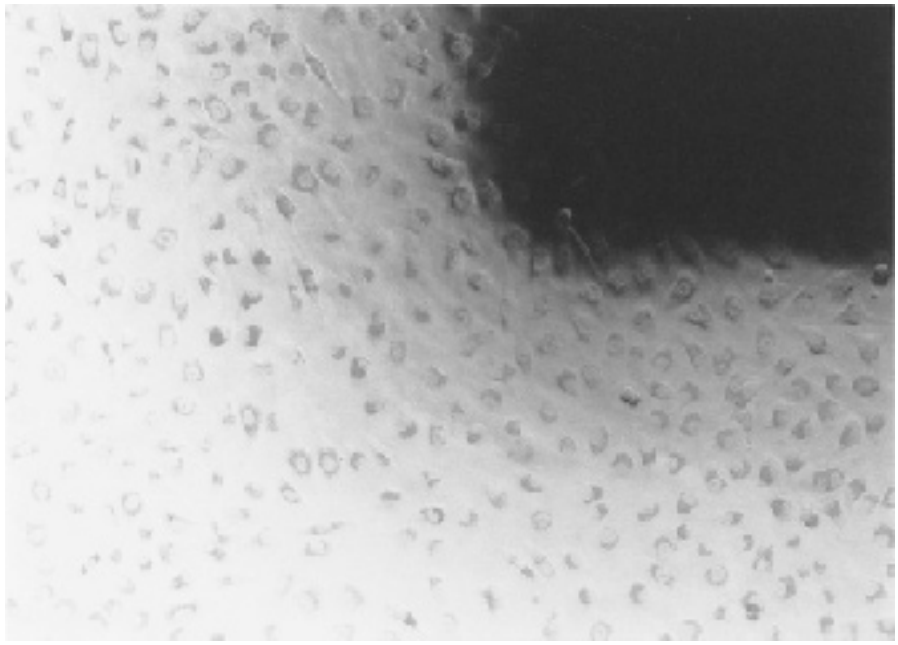


(b)

Figure 6 Normal cells, L929, in the vicinity of ferrimagnetic sample under alternative magnetic field with 13.56 MHz after exposure for (a) 0, (b) 7, (c) 9, and (d) 11 min. (*Continued*)



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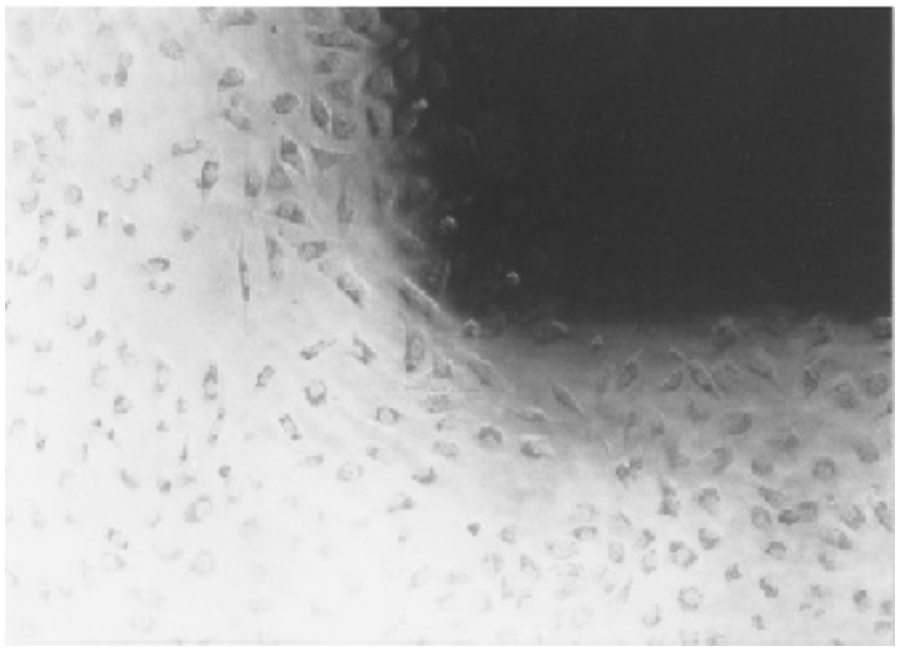

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Figure 6 (*Continued*).

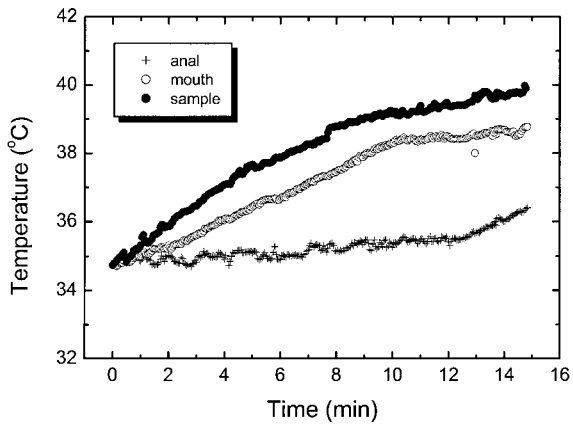

Figure 7 Temperature change of tumor site, mouth and anal when expose to the alternative magnetic field with 13.56 MHz *in vivo*.

was found that *in vitro* test results are summarized in Table II.

The *in vivo* cell annihilation test was is lower than that for the *in vitro* measurement. The temperature difference between the mouth and anus-might be due to the self-cooling system of the animal.

Photographs of Sprague-Dawley rats before and after exposure to the magnetic field are shown in Fig. 8. Big tumors around the breast disappeared after applying the alternating magnetic field for just 4 times of 15 min each after the sample loading. When the same procedure was repeated in the case without sample loading, the tumor did not disappear. This prepared sample can be used to destroy tumor cells *in vivo*.

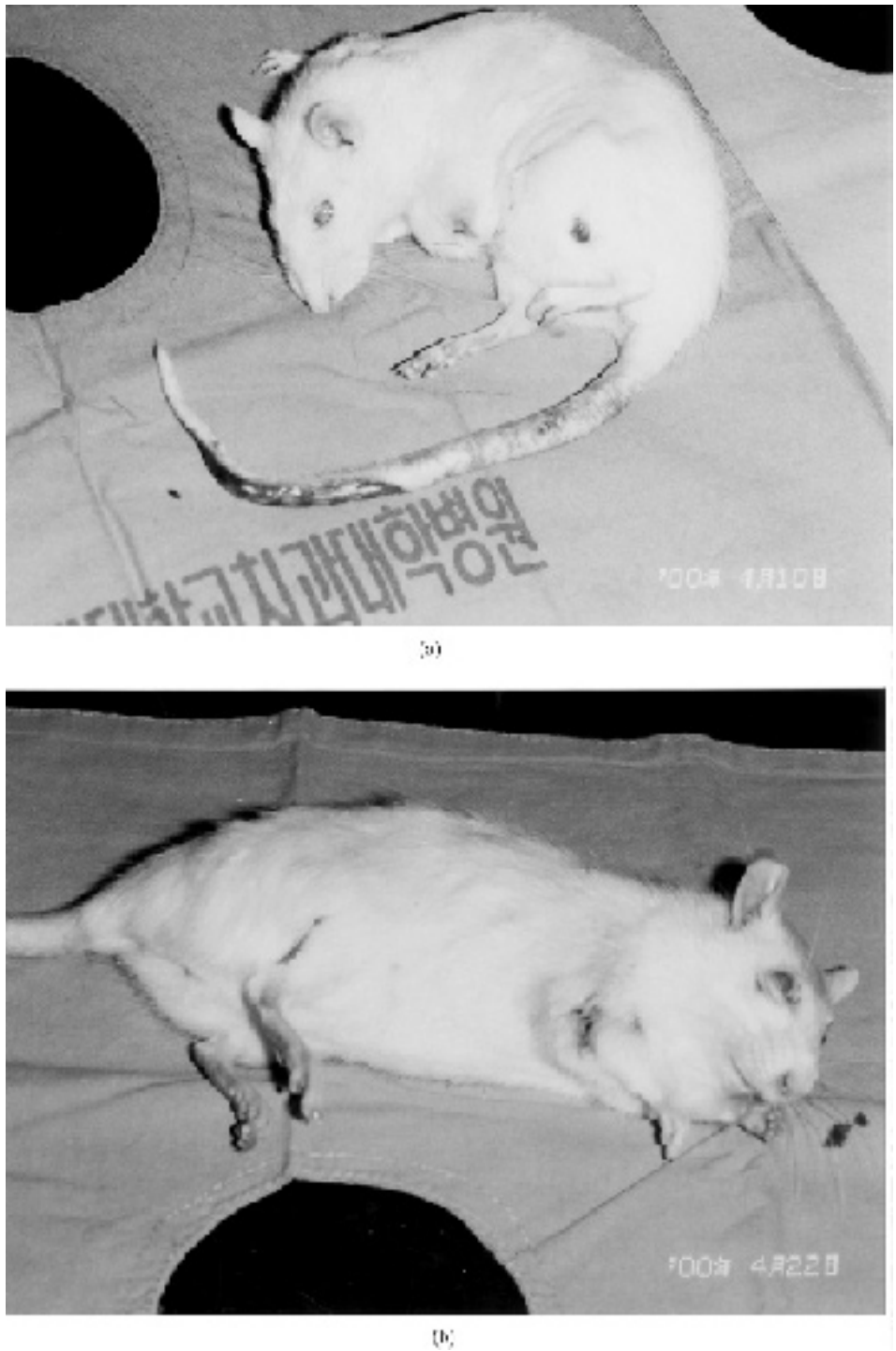

Figure 8 Photographs of Sprague-Dawley rats: (a) before magnetic field application and (b) after exposure to the magnetic field.

4. Conclusions

In order to reveal the suitability of ferrimagnetic glass-ceramics in the Fe_2O_3-CaO-SiO_2 system as thermoseeds of the hyperthermic cancer treatment, the lysis of carcinoma cells were examined after exposure to an alternative magnetic field both *in vitro* and *in vivo*. The malignant carcinoma cells were almost dead after 9 min exposure to the magnetic field application around the prepared sample, while they were still alive 5 cm away from the specimen. The normal cells were not annihilated even after 11 min. When the Sprague-Dawley rats imbedded with the samples were exposed to the magnetic field, the tumor cells disappeared after just 4 exposures of 15 min each. This amazing reduction of the tumor could not be observed in any of the rats not containing the sample. It is expected that the prepared ferrite-containing glass-ceramics will be helpful in hyperthermic cancer treatment. A long-term study as well as more disciplinary experiments are needed to confirm this result.

Acknowledgement

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