Feasibility of Induction Heating Using a Sintered $MgFe_2O_4$ Needle for Minimally Invasive Breast Cancer Therapy

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Abstract. *Background: This study investigated a novel approach for tumor ablation therapy using an alternating magnetic field combined with a sintered $MgFe_2O_4$ needle. This method differs from radiofrequency ablation (RFA) by dielectric heating with regard to the heating mechanism and improves some weak points of these conventional thermotherapies. Materials and Methods: Nude mice mimicking human breast cancer BT474 were treated using this method. The extent of tumor death was assessed after ablation. Results: Staining with hematoxylin and eosin showed gradual expansion of the pyknotic area until 48 h after ablation. Nicotinamide adenine dinucleotide diaphorase staining also showed complete tumor death by 48 h after treatment. The ablation area was well controlled and reablation was not necessary. The tumor could be completely controlled using this method without any risk of skin burn. Conclusion: This novel ablation therapy appeared to be more effective and less invasive for treatment of breast cancer treatment than RFA.*

Improvements in the performance of radiotherapy, chemotherapy and endocrine therapy have reduced the need for invasive surgical approaches in the treatment of breast cancer. Furthermore, requests for minimally invasive and cosmetically preferable approaches are growing among patients and medical personnel. Thermal therapy seems to represent one promising approach for breast cancer therapy that can satisfy this need.

Radiofrequency ablation (RFA) was introduced by Rossi *et al.* (1) and is now utilized to control liver cancer around the world. RFA has recently been applied to breast cancer and its efficacy and safety have been reported (2). RFA involves dielectric heating and electric current passing through the human body *via* an electrode and counter electrode. Friction produced by the movement of electrons around the needle produces heat. Although temperature elevations sufficient to destroy the target tissue are obtained rapidly, difficulties in handling the electrode, monitoring the ablated area during heating according to the generated bubble and the need to re-puncture when first treatment is insufficient have been identified as the weak points of this method.

Conversely, the inductive heating method in this study, which has not yet seen practical use, utilizes a heating mechanism within the magnetic material for thermotherapy or the electric conductor when a high-frequency electric current passes through the material for heating and thereby produces a magnetic flux across the electric conductor. In brief, the inductive heating method produces an increase in temperature of the magnetic material itself in comparison with the indefinite heating produced in surrounding tissue by dielectric heating.

In a previous study, we have investigated electromagnetic heating with various kinds of metals, identifying $MgFe_2O_4$ as offering the most effective heating (3-5), while $FeFe_2O_4$ has previously been chosen for electromagnetic heating (6-15). However, the later has a risk of iron oxidation, and long-term insertion in the body may cause adverse events, such as surrounding tissue injury, elution of materials into the circulation and finally reduced heating efficacy. In contrast, since $MgFe_2O_4$ has no risk of oxidization and is equally or more effective in induction heating even after long-term application, $MgFe_2O_4$ seems preferable for use. We also developed a new apparatus to pair with the sintered $MgFe_2O_4$ needle for this ablation therapy. Our treatment offers several benefits to patients, allowing free movement and the option of repeated treatments once needles are placed in the tumor. After checking the location of the needle, the patient only

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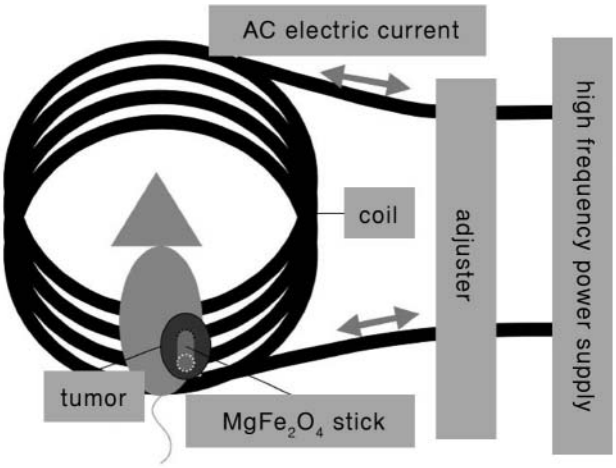

Figure 1. *Schematic representation of the device for applying the electromagnetic field which comprised loops of copper pipe connected to a high-frequency power supply through an adjuster.*

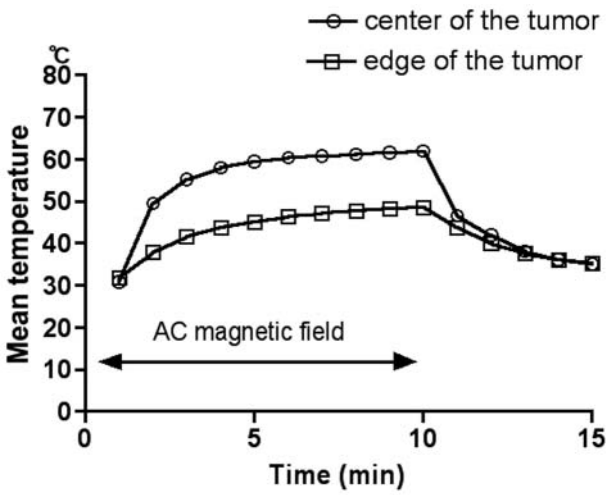

Figure 2. *Mean temperature elevation in stick-inserted tumors (n=9) during application of an AC magnetic field with amplitude 4.0 kA/m (2 kW, 540 kHz). At the tumor centre, the temperature plateaued within a few minutes and finally exceeded 60˚C (62.1±10.9˚C). Temperature elevation was milder at the edge than at the tumor centre, exceeding 45˚C within several minutes and finally reaching 48.7±9.8˚C.*

moves into the alternating magnetic field to receive a session. If the treatment later proves to have been insufficient, another session can be performed by again moving into the alternating magnetic field, with no need for an additional puncture. The objective of this study was to evaluate the efficacy of this new approach to breast cancer using xenograft animal models mimicking human breast cancer.

Materials and Methods

Animals. Female immunodeficient mice (4 to 6 weeks old) were obtained from Charles River Japan (Yokohama, Japan). All animals were treated in accordance with the guidelines for animal research of Ehime University School of Medicine. Mice were anesthetized using intraperitoneal administration of 50 μg/g pentobarbital (Nembutal; Dainippon Sumitomo Pharma, Osaka, Japan).

Human breast cancer cells from the BT474 line (JCRB Cell Bank, Osaka, Japan) were used. A suspension of $1.0x10^6$ BT474 cells in 0.1 ml of phosphate-buffered saline (PBS) was injected subcutaneously into the flank of a mouse using a 27-G needle. After 12 weeks, formation of a subcutaneous tumor 2 cm in diameter was confirmed. The tumor was resected and cut into 2-mm blocks. Blocks were inoculated into the right flank of 30 mice through small skin incisions. The study was started after 4-6 weeks, once the tumor exceeded 5 mm in diameter (mean, 8.7±1.5 mm). The mean weight of mice at this stage was 22.5±1.8 g.

$MgFe_2O_4$ needle and electromagnetic heating device. $MgFe_2O_4$ particles (mean particle size, approximately 1 μm) were purchased from Wako Pure Chemicals (Osaka, Japan). Particles were forced into a thin quartz tube before sintering at 1100˚C for 27 h. The completed $MgFe_2O_4$ needle is 1.0 mm in diameter and contains 2.21 mg $MgFe_2O_4$/mm. The entire manufacturing process was performed by the Department of Materials Science at the Faculty of Engineering at Ehime University.

The device for applying alternating current (AC) magnetic fields comprised loops of copper pipe connected to a T162-5712B high-frequency power supply (Thamway, Shizuoka, Japan) through an adjuster. Loops were cooled from within by flowing water in a CLU33 cooling unit (Iwaki, Tokyo, Japan) so that the coil could produce consistently reproducible magnetic fields (Figure 1).

Elevation of temperature at the centre and edge of the tumor was monitored using a fluorescence optic fiber thermometer (AMOTH FL 2000 sensor FS100-M; Anritsu Meter, Tokyo, Japan).

Induction heating. A total of 30 tumor-bearing mice were divided into 3 treatment groups and 3 sham treatment groups (n=5 each). $MgFe_2O_4$ needles were placed in the centre of the tumor. Animals were placed in the centre of the loops for 15 min. An AC magnetic field of amplitude 4.0 kA/m (2 kW, 540 kHz) was applied for 10 min only for treatment groups. Elevation of tumor temperature was monitored using a fluorescence optic fiber thermometer (AMOTH FL 2000 sensor FS100-M; Anritsu Meter). Animals from treatment and sham treatment groups were sacrificed at 0, 24 or 48 h after treatment (T0, T24, T48) or sham treatment (C0, C24, C48). Tumors were resected and cut in half perpendicular to the long axis of the implanted $MgFe_2O_4$ needles.

Microscopic observations. Hematoxylin and eosin (HE) staining and nicotinamide adenine dinucleotide (NADH) diaphorase staining were performed to evaluate the effects of thermotherapy. The NADH diaphorase stain is an accurate method for determining the extent of immediate cell death (16). This method is widely used in the determination of cell death following thermotherapy for breast cancer. Half of the tumor was fixed in 10% formalin and embedded in paraffin for 24 h. The paraffin-embedded section was cut at a thickness of 4 μm, dewaxed, dehydrated and stained using HE. The remaining half of the tumor was embedded in optimum cooling

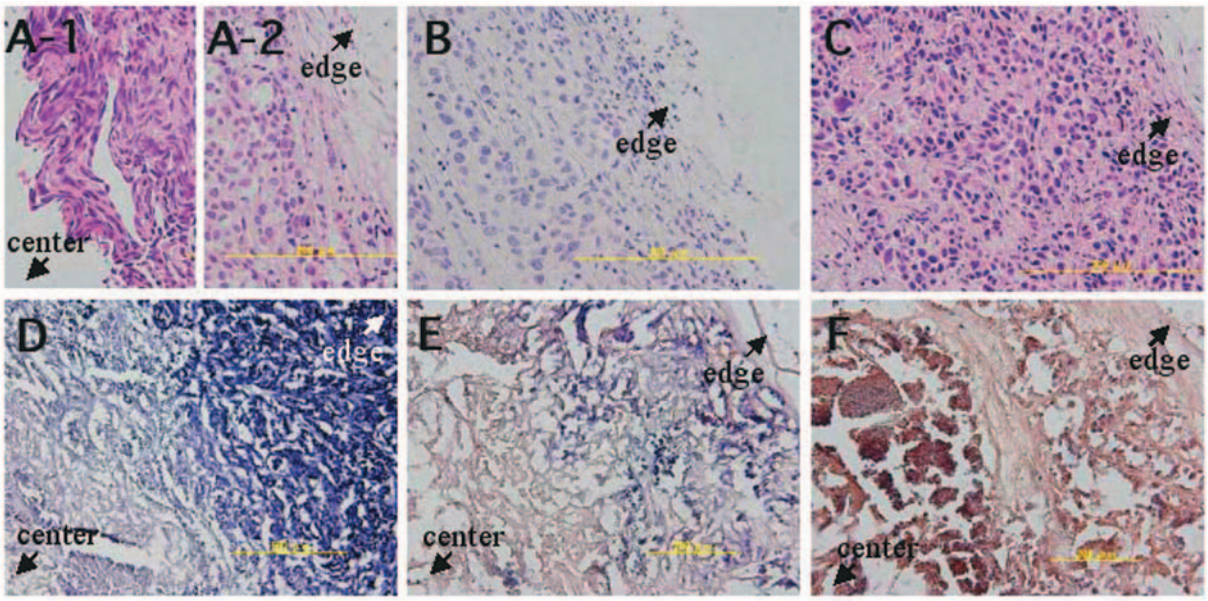

Figure 3. *HE staining of tumor tissue from the T0 (A1, A2), T24 (B) and T48 groups (C). HE staining of the T0 group show pyknosis only around the hole where the stick was inserted (A1). No histological changes are apparent at the tumor edge (A2). No marked changes are apparent between T0 and T24 groups (B). The area of pyknosis reaches the tumor edge in the T48 group (C). NADH diaphorase staining of T0 (D), T24 (E) and T48 (F) groups. A clear margin between stained and unstained areas is apparent in the T0 group (D). The stained area is not markedly smaller but is slightly lighter at 24 h (E). At 48 h after thermotherapy, the entire tumor is unstained (F).*

tissue (OCT) medium (Tissue-Tek, Sakura Finetechnical, Tokyo, Japan), snap frozen and stored at –80˚C until further processing. Cryostat sections (10 μm) were mounted onto glass slides. Cut sections were incubated for 15 min in a humidity chamber at room temperature with a test solution containing 1 ml of reduced NADH (2.5 mg/ml), 2.5 ml nitroblue tetrazolium chloride (Dojindo Laboratories, Kumamoto, Japan), 1 ml of PBS, and 0.5 ml of Ringer's solution. Sections were rinsed with distilled water, covered with Softmount aqueous mountant (Wako Pure Chemical Industries, Osaka, Japan), and dried at 70˚C for 20 min. Cells exhibiting mitochondrial activity stained blue.

Images of NADH diaphorase staining were uploaded to a personal computer, and necrotic areas were calculated using Image J software (National Institutes of Health, Bethesda, Maryland, USA).

Statistical analysis. Significant differences in areas of cell death at various time points were identified using the Kruskal-Wallis test with Dunn's multiple comparison test (GraphPad Prism 4.0c Macintosh Version; GraphPad Software, San Diego, California, USA).

Results

Elevation of temperature. Tumor temperature was measurable in 9 mice from the treatment groups (Figure 2). Before application of the AC magnetic field, the baseline temperature of animals was approximately 31˚C. At the centre of the tumor, temperature elevation plateaued within a few minutes. At the end of treatment, the mean temperature at the tumor centre was 62.1±10.9˚C and fell immediately to the baseline level after ending application. At the edge of the tumor, temperature elevation was somewhat mild, but exceeded 45˚C within several minutes and reached 48.7±9.8˚C by the end of treatment.

HE staining. Tumor from the T0 group showed pyknosis only around the centre of the tumor where the $MgFe_2O_4$ stick had been inserted (Figure 3A-1); this was not seen in sham treatment groups. The area showing pyknosis appeared wider in the T24 group (Figure 3B). At these time points, the margins of these changes were difficult to determine and the area affected by inductive heating was impossible to measure. All tumors in the T48 group showed pyknosis throughout the entire tumor (Figure 3C).

NADH diaphorase staining. The mean area unstained by NADH diaphorase staining in the C0, C24, C48, T0 (Figure 3D), T24 (Figure 3E) and T48 (Figure 3F) groups was 4.9±2.3 mm^2, 6.5±1.2 mm^2, 7.6±3.1 mm^2, 7.9±2.6 mm^2, 8.8±4.9 mm^2 and 12.3±3.3 mm^2, respectively (Figure 4). A significant difference was noted only between C0 and T48 ($p=0.0338$). The unstained area was significantly smaller in C0 than T48 ($p=0.0031$). No differences were apparent between other groups.

The percentage area unstained by NADH diaphorase in C0, T0, T24 and T48 groups was 23.6±15.0%, 47.8±16.0%, 45.4±12.4% and 100%, respectively (Figure 5).

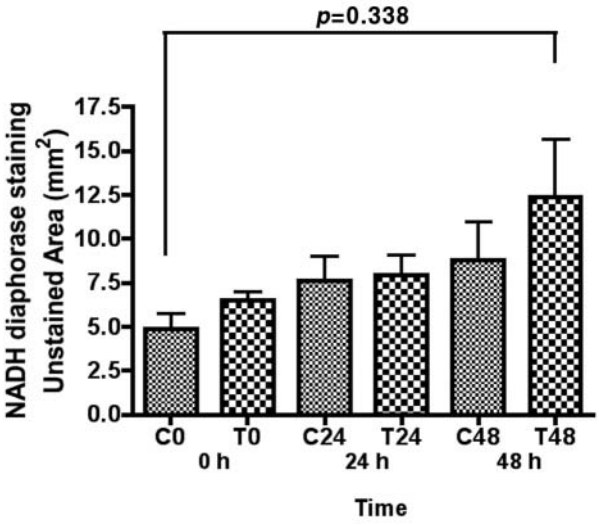

Figure 4. *Unstained area in NADH diaphorase staining. Groups with or without thermotherapy were sacrificed immediately (C0, T0), or at 24 h (C24, T24) or 48 h (C48, T48) after treatment or sham treatment and NADH diaphorase staining of tumor was performed. A significant difference was seen between C0 and T48 (p=0.0338).*

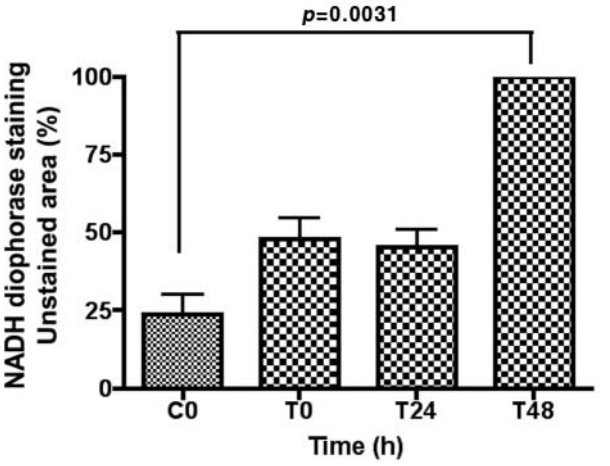

Figure 5. *The percentage of NADH diaphorase-unstained area was 23.6±15.0%, 47.8±16.0%, 45.4±12.4% and 100% in C0, T0, T24 and T48 groups, respectively.*

Discussion

Thermotherapy employing high-frequency AC electricity involves either dielectric or inductive heating. Dielectric heating includes RFA and microwave coagulation therapy (MCT), both of which are already in practical use. In these methods, the tumor must first be punctured with a needle-shaped applicator called an electrode (RFA) or antenna (MCT). Electromagnetic energy is applied through these applicators, then the friction associated with the movement of electrons around the needle produces heat and the body, including the tumor, produces heat. These methods are utilized to achieve local control in several types of malignancies because temperature elevation sufficient to destroy the target is obtained rapidly. Some inconveniences are encountered, *i.e.* the operator must hold the applicator during the heating session, hence there is a possibility it may escape the target. The effect at the ablation site is often evaluated on ultrasonography, but some studies found this difficult due to the development of an ill-defined hyperechoic zone (17). When ablation proves to have been insufficient, additional puncture is required several days later with further pain for the patient.

In comparison, the inductive heating used in this study achieves heating effects by placing a metallic magnetic material in a high-frequency magnetic field. Hysteresis loss is thought to be greatly involved, but the precise mechanisms remain largely unknown. Two advantages are seen with inductive heating in comparison with dielectric heating. The first is that there is no requirement to pass electric current directly to the body of the patient and repeated heating can be produced without subsequent contact with the body. The second is that the object itself (the sintered $MgFe_2O_4$ stick in this study) produces the heat, rather than the water molecules around the applicator. Thus, once a $MgFe_2O_4$ stick is placed in the tumor, the patient can move freely and the position of the needle is easily checked on radiography, sonography or other imaging modalities. Given these characteristics, $MgFe_2O_4$ particles may also be able to be administered *via* the blood stream to produce heat in tumors. This can only be achieved by inductive, not dielectric, heating.

After checking the location of the needle, patients only need to move into the alternating magnetic field to receive a session. If the treatment proves insufficient, patients can receive another session simply by again entering the alternating magnetic field, with no need for additional puncture. In our previous study, $MgFe_2O_4$ was identified as the most effective heating material for inductive heating (1-3). The first trial of inductive heating using iron oxide and a magnetic field was reported by Gilchrist in 1957 (18). Subsequent reports have described magnetic heating using magnetite ($FeFe_2O_4$) (6-15, 18). From the perspective of biocompatibility, $FeFe_2O_4$ carries a risk of iron oxidation and long-term placement may cause problems such as production of hydroxyl radicals in local tissues after tumor control. In contrast, $MgFe_2O_4$ has no possibility of further oxidization and equal or greater efficacy of heating can be expected even after long-term placement. For these reasons, $MgFe_2O_4$ appears optimal for inductive heating from the perspective of biocompatibility and heating efficacy, and might lead to clinical applications.

Progression of the ablated area or tissue injury area has yet to be clarified according to time course. In this study, HE staining showed kinetic spread of the pyknotic area, but seemed inappropriate for identifying the margin of changes. Immediately after thermotherapy, an area of pyknosis was observed just around the centre of the tumor where the $MgFe_2O_4$ needle was inserted. This area spread gradually and reached the margin of the tumor by 48 h after treatment.

In contrast, NADH diaphorase staining did not show complete tumor death immediately after treatment for 10 min. Application for 10 min was insufficient to immediately ablate the entire tumor, but NADH diaphorase staining 48 h later showed complete tumor death in all tumors. Absence of NADH diaphorase staining suggests irreversible cell death. Progressive cell death has been reported after application of MCT (19), RFA (20) and laser-induced thermotherapy (LITT) (21). Such progression has been attributed to several factors, such as microvascular damage, ischemia-reperfusion injury, induction of apoptosis, altered cytokine expression and modulation of immune response (21). In previous studies of thermotherapies, NADH diaphorase stain has been used as an early marker of cell death. In our study, the area of cell death seemed to expand beyond the unstained area of the early phase. In the early phase, evaluation of the area of cell death resulting from thermotherapy was inadequate. More sensitive methods must be established, as estimation of the area of cell death using this method may underestimate the margin of effective thermotherapy.

Tumor with insertion of the $MgFe_2O_4$ needle showed a temperature elevation of around 60˚C in the centre and 45˚C at the edge. Thermal injury to cells begins at 42˚C. In this range, 12-15 minutes are required to achieve a cell-destructive response. The exposure time needed for a cytodestructive response decreases as temperature increases, with 8 min required at 46˚C and 2 min at 51˚C (21). The mean temperature in our model plateaued within a few minutes even at the edge of the tumor and a 10-min application of the AC magnetic field might be sufficient to destroy the entire tumor. This result suggests that ablation at a comparatively low temperature is suitable to destroy breast cancer tissue. This may help to avoid skin burns in clinical use. At the beginning of this study, we examined the conditions for treating a small breast tumor. Under an AC magnetic field of amplitude 4 kA/m, all tumors were completely destroyed, despite their having a mean diameter of 8.7±1.5 mm (range, 6.6-11.3 mm). This result suggests that the present procedure may also be capable of destroying larger tumors.

In conclusion, magnetic heating using a $MgFe_2O_4$ needle combined with our inductive heating apparatus caused 100% cell death in BT474 xenografts mimicking human breast cancer. This new ablation method could become a feasible technique for breast cancer therapy.

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