Development of new thermo-ablation therapy using cobalt contained ferromagnetic nanoparticles

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ABSTRACT

The purpose of our thermotherapy is making a cancer cell reach a thermo-ablation necrosis using magnetic particles. The heat generation capacity of conventional methods of magnetic fluid hyperthermia using superparamagnetic particles was insufficient for cure of multiple metastatic nodules and micro-disseminated lesions. Therefore, we improved the heat generation capacity using the ferromagnetic particles fluid. Our original ferromagnetic particle was 18-20 nm sized cobalt-contained iron oxide, and coated with SiO_2 in order to acquire decentralized stabilization, and adjust coercive force (90-550 Oe) without changing size. The maximum specific loss power of was 456 W/g at AMF (120 kHz, 450 Oe), this value was 10 times of conventional superparamagnetic particles. Thermotherapy for animal subcutaneous tumor by using our original high heat generation system made all the cases result in complete cure.

Keywords: magnetic fluid hyperthermia, thermo-ablation therapy, ferromagnetic nanoparticles

1 INTRODUCTION

Magnetic fluid hyperthermia (MFH) is a recent therapeutic approach for local targeting of hyperthermia and thermo-ablation, and is a promising treatment of malignant tumors in some institutions. MFH utilizing the heat generated by the magnetic particles exposed to external alternating magnetic field (AMF) has an edge over the other hyperthermia techniques, since the heat source can be localized in cancer cells with an external magnetic field and their position can be detected by utilizing their magnetic properties. Conventional MFH was a thermotherapy aiming at 43 degrees which introduces the apoptosis of a cancer cell. This system remains in the cell-growth suppression effect, and does not reach the cell necrosis effect [1].

The magnetic particles used for MFH is roughly divided into two materials with the diameter of a particle; superparamagnetic particles (SP) in a diameter of around 10 nm and ferromagnetic particles (FP) in a diameter of 20 nm or more. The mechanism of heat generation of MFH was there different types by character of magnetic particle itself; namely hysteresis, Ne´el and Brownian loss. SP do not show hysteresis loss, but exhibit Ne´el and Brownian loss, which are the causes for heat generation. Using these two losses, temperature around 42 degrees and above can be achieved. In order to acquire big heat generation by SP, it is necessary to enlarge frequency of alternating magnetic field (AMF). On the other hand, FP shows mainly the hysteresis loss. The hysteresis loss power is prescribed by properties of magnetic material itself (saturation magnetization and coercive force), and depend on the magnitude of an impression magnetic field. Historically, the magnetic particles which was used for MFH has changed from FP to SP, since FP aggregated after exposure to a magnetic field and cannot remove the inner body (blood vessel, heart, liver and so on) and was difficult to modify for biocompatibility. Therefore, SP was highlighted for MFH. The magnetic moments of SP are randomly reoriented by the thermal energy of their environment and do not display magnetism in the absence of a magnetic field. However, enlarging the heat generation capacity of SP has a limit for living body, since frequency of AMF is restricted in International Commission on Non-Ionizing Radiation Protection (ICNIRP) Guidelines at 1998; Exposure to electromagnetic fields at frequency above about 100 kHz causes significant increase in the temperature even in organ or tissues with no magnetic materials.

Therefore we were sure that a new big heat generation system needs to be established, in order to achieve the thermo-ablation of multiple metastatic nodules and/or micro-disseminated lesions. Concretely, we must create the polished up ferromagnetic fluid without aggregation and develop high heat generation system under the tolerance level to a living body.

In our present study, we developed the high heat generation MFH system using the new ferromagnetic nanoparticles fluid and AMF device under the tolerance level to a living body. And we demonstrated thermo-ablation therapy for animal subcutaneous model.

2 EXPERIMENTAL PROCEDURE

2.1 Materials

Ferromagnetic particle (NFMP): Cobalt-contained iron-oxide particles with spinel structure, (Co) $Fe_{3-\delta}O_4$, were produced by heating the coprecipitants containing Co^{2+}, Fe^{2+}, and Fe^{3+} ions in an alkaline solution at 130℃ for 5 h using conventional hydrothermal methods and coated with SiO_2 in order to acquire decentralized stabilization. Samples with coercive force (Hc) from 90 to 550 Oe were obtained by changing the contents of Co ions. Size of them ranged around 20 nm. The shape of the particles was observed with a transmission electron microscope (TEM) (Fig.1).

Superparamagnetic particles (SP): The core of particles was nano-sized iron oxide (γFe_2O_3) coated with carboxy dextran, using it as nuclear magnetic resonance imaging contrast agent (Resovist®: Bayer Schering Pharma, Berlin, Germany).

The spec of our AMF device: The frequency was set to 120 kHz, the magnetic field could control from 0 to 450 Oe. The inner diameter of solenoid coil was 55 mm, and wound up it inner 10 turn and outer 11 turn (Fig.2).

Animal: C57BL/6J mouse was used.

Cell Line: Mouse melanoma B16 cell line was maintained in a CO_2 incubator at 37 degrees, with Dullbecco's Modified Eagle's Medium (Sigma-Aldrich, Taufkirchen, Germany), containing 10 % fetal bovine serum (Sigma-Aldrich).

2.2 Methods

The first, specific loss power (SLP) of our original NFMP and SP was evaluated by temperature rise value under the AMF condition of 120 kHz and 0 - 450 Oe. The volume of 200μl of NFMP and SP controlled 3.7 Wt% magnetic particles were set into the plastic micro-tube and applied center of solenoid coil in the AMF device, and temperature were measured at different magnetic field from 0 to 450 Oe with optical thermo fiber. SLP is calculated as follows;

$$SLP\,(W/g) = \frac{\Delta T}{\Delta t}\,\frac{(mM \cdot CM + mW \cdot CW)}{mM}$$

(ΔT: temperature (℃), Δt: time(s), C: Calorific capacity (J/g/℃), m: mass (g), M: magnetic particle, W: water)

Secondary, a thermo-ablation therapy of a mouse subcutaneous tumor model was performed. A total of 1×10^6 B16 melanoma cell/ 100 μl were inoculated into the femoral region of the C57BL/6J mouse of 5 weeks. One week after the cell inoculation, animals were divided into following six groups; group A (n=5): NFMP injection + AMF, group B (n=5): SP injection + AMF, group C (n=3): only AMF, group D (n=3): only NEMP injection, group E (n=3): SP injection only, group F (n=3): none injection and AMF. NFMP and SP was injected 200 μl in tumor with infuser pomp for 30 minutes, and animal was set into the solenoid coil and started thermo-ablation therapy under the general anesthesia. The AMF device was set up in 120 kHz, 450 Oe and operated for 5 minutes. However, when a rectal temperature exceeded 42 degrees during a treatment, we controlled the magnetic field. The tumor temperature and rectal temperature were monitored during AMF irradiation with optical thermo fiber. The change of tumor volumes was measured with electronic slide calipers. Tumor volume was calculated as follows;

$$Tumor\ volume\,(mm^2) = \frac{\pi}{6}\left\{(short\ length)^2 \times (lomg\ length)\right\}$$

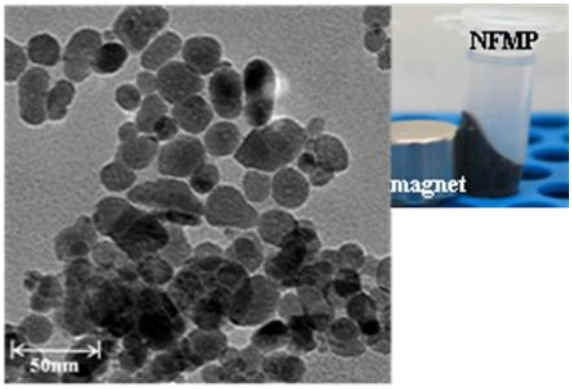

Figure.1 NFMP is a magnetic fluid which has decentralized stabilization. TEM photograph of resulting (Co) $Fe_{3-\delta}O_4$. The mean diameter is about 20 nm.

Magnetic nano-particle	Hc: Coercive force (Oe) at room temperature
Resovist®	0
NFMP1	90
NFMP2	180
NFMP3	260
NFMP4	440
NFMP5	550

Table.1 Characteristics of iron-oxide nanoparticles including cobalt.

Figure.2 (a) The AMF device: The size was 173×83×73 cm. Frequency was set to 120 kHz, the magnetic field could control from 0 to 450 Oe. (b) Solenoid coil was the structure which made the double roll the hose which knit the litz wire.

3 RESULTS

3.1 The value of Specific Loss Power

The samples of NFMP with different *Hc* ranging from 90 to 550 Oe were prepared (Table.1). The temperature of measurement limit was 100 degrees, since samples reached the boiling points. Fig.3 shows a SLP for several amplitude of H_0 for NFMP and SP, respectively. The SLP of NFMP becomes large in proportion to ac magnetic field H_0. At maximum ac magnetic field (= 450 Oe), the value of SLP was follows; NFMP1: 186, NFMP2: 327 NFMP3: 456, NFMP4: 285 NFMP5: 195, SP: 50.7 W/g. The largest SLP was observed for the NFMP3 sample at all of the amplitudes of ac magnetic fields H_0 (Fig.3).

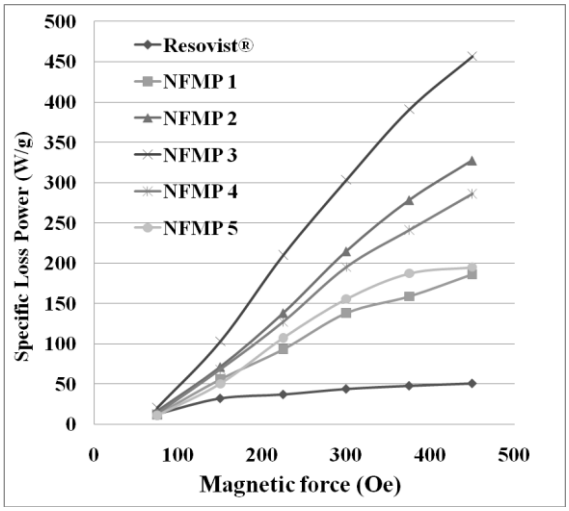

Figure.3 Specific loss power of NFMP and SP. The vertical axis of a graph is SLP (W/g) and a horizontal axis is ac magnetic field H_0.

3.2 Thermo-ablation therapy for animal model

According to the result of 3-1, we used the NFMP3 as treatment carriers for thermo-ablation therapy. Maximum intra-tumor temperature during thermo-ablation therapy was group A (NFMP3 + AMF): 65.3 ± 15.0, group B (SP + AMF): 49.9 ± 2.98, group C (AMF only without magnetic particles): 28.3 ± 0.77 degrees. All case of intra-tumor temperature of group A was over 50 degrees. Group C (AMF only without magnetic particles) was not observed the temperature rise during thermo-therapy (Fig.4). The average tumor size was a diameter of 8-9mm before thermo-ablation therapy, and the tumor volume on the 10th days after therapy was group A: 0, group B: 1275±1886, group C: 4249 ± 77.77, group D: 3113 ± 1086, group E: 2841 ± 1087 and group F: 3034 ± 942.9 mm^3 (Fig.5), and tumor doubling time were group B: 4.5 ± 17.8, group C: 3.2 ± 0.24, group D: 2.7 ± 0.59, group E: 2.0 ± 0.83 and group F: 2.1 ± 0.40 days, respectively (Fig.6).

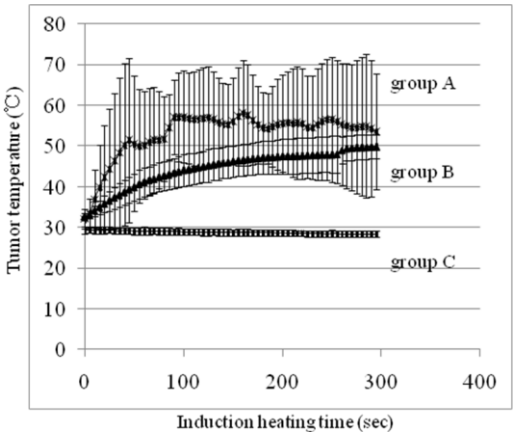

Figure.4 The intra-tumor temperature change during thermo-ablation therapy. The reason the error of group A is large, since magnetic field (H_0) must be controlled in order to do not exceed 42 degrees of rectal temperature.

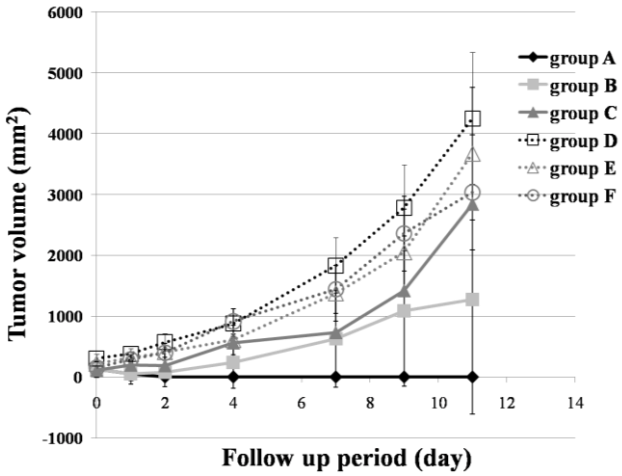

Figure.5 A change of a tumor volume after thermo-ablation therapy. As for the group A (NFMP3 + AMF), a tumor disappeared completely. On the other hand, the tumor of control group(C, D, E and F) increased remarkably.

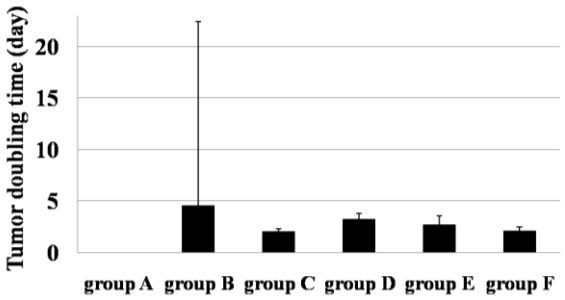

Figure.6 Tumor doubling time of group A-F. The tumor volumes of a control group (C-F) become doubled in two to three days.

4 DISCUSSIONS

Heat generation capacity of our original new NFMP (120 kHz, 450 Oe) was about 10 times higher than that of conventional SP using early stage of our development of thermo-ablation therapy. Our high heat generation system for thermo-ablation therapy was effective for the treatment of an animal subcutaneous tumor.

The newly ferromagnetic particles were good design for thermo-ablation therapy. The first emphatic point of NFMP is superior to decentralized stabilization compared with other ferromagnetic substances. The second point was that the diameter of a particle is uniformly produced by 20 nm, since a diameter of 10-100nm was suitable for drug delivery system of tumor by using blood flow. The next point was that heat generation capacity is controllable by controlling coercive force, without changing the diameter of a particle.

It is noted that the larger coercive force gave, the lower SLP bordering on NFMP3 (*Hc*: 260 Oe); the SLP of NFMP4 (*Hc*: 440 Oe), NFMP5 (*Hc*: 550 Oe) was lower than that of NFMP2 (*Hc*: 180 Oe) and NFMP3. It is thought that an impression magnetic field suitable for the coercive force of particles exists. If an impression magnetic field suitable for NFMP5 (*Hc*: 550 Oe) can be given, SLP exceeding 456 W/g will be obtained. According to other report, some magnetic particles with SLP exceeding 1000 W/g are introduced at 500 kHz or more [2]. However, the magnetic-field × frequency which may be given to a living body has restrictions in International Commission on Non-Ionizing Radiation Protection (ICNIRP) Guidelines at 1998. In the range which we get to know, 130 kHz and 1350 Oe is the maximum AMF in which an animal can survive [3]. As mentioned above, we succeeded in development of the high heat generation capacity system in the range which does not have a bad influence on a living body.

The results of thermo-ablation therapy for an animal subcutaneous tumor model are gave us big impact. The all case of group A (our original new ferromagnetic fluid and AMF device) become a complete cure. It is the result of the ability to supply sufficient energy to exceed 46 degrees to whole tumor, which leads to a tumor necrosis. On the other hand, the cure cases and the recurrence case are intermingled in group B. It is expected that there was a case to which the whole tumor does not reach 46 degrees. The recurrence cases observed a tumor reduction in a treatment early stage, and a rapid tumor enlargement is shown after a recurrence. From the above-mentioned reason, the error bar of group B is large (Fig.5 and Fig.6). In spite of a tumor mean temperature was over 46 degrees in group B, the thermo-diffusion effect is taken up for the reason a complete cure was not obtained; The value of $\Delta T/\Delta t$ was exceeding 3deg/sec in the experiment of *in vitro* by 200μl (Fig.3), but *in vivo* the value of $\Delta T/\Delta t$ was 0.4 – 0.5 degrees by 200μl. Our group reported the temperature distribution in the magnetic hyperthermia; a temperature is radiately cooled in a split second from the spot supplying heat in a living body and in order to rise of tumor temperature, the smaller a tumor volume, the larger the heat generation capacity required.

Magnetic particle injection only group (group D and E) has a tumor growth remarkable in this experiment, it is proved that there is no antitumor effect by the magnetic particle direct injection to a tumor. Furthermore, by only impressing a magnetic field (group C), an elevation of a rectal temperature is not recognized; it means that AMF of 450 Oe and 120 kHz was permitted to the animal model. However, we have to take care that a temperature rise too much, in fact, there is a case in which rectal temperature exceeded 42 degrees, and the whole body of mouse was cooled with a cool pad, and we could not control the impression magnetic filed during thermo-ablation therapy.

Finally, for achievement of our new anti-cancer thermotherapy, the following expression must achieve; (*Heat generation capacity of magnetic particles*) × (*Amount of tumor accumulation of particles*) − (*Thermal diffusion loss*) > (*Required heat power to cancer necrosis*). In this present study, we succeed in the (*Heat generation capacity of magnetic particles*). From now on, the accumulation technology of the magnetic particle to the tumor by intravascular medication needs to be improved.

High heat generation system using our original ferro magnetic particles fluid suggested the new potentially of MFH field.

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