Synthesis of cobalt ferrite nanoparticles using spermine and their effect on
death in human breast cancer cells under an alternating magnetic field

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Abstract

To compare heat generation under an AC magnetic field for inducing cancer cell death, CoFe_2O_4 and Fe_3O_4 nanoparticles with a diameter of ~10 nm were synthesized by hydrolysis in an aqueous solution containing the metal chlorides iron(III) chloride and cobalt(II) or iron(II) chloride, with spermine or *N,N'*-bis(3-aminopropyl)butane-1,4-diamine, as a base and a protective reagent. Both CoFe_2O_4 and Fe_3O_4 nanoparticles were incorporated into human breast cancer MCF-7 cells; the nanoparticles exhibited little toxicity on the cells. When the cells containing nanoparticles were subjected to an AC magnetic field, MCF-7 cell death was induced by heat generated from ~10-nm CoFe_2O_4 nanoparticles but not from ~10-nm Fe_3O_4 nanoparticles. An increase in coercivity derived from the substitution of Fe^{2+} with Co^{2+} facilitated the ability of the nanoparticles to induce cell death in human breast cancer cells under an AC magnetic field.

Keywords: CoFe_2O_4 nanoparticles, Fe_3O_4 nanoparticles, Cellular uptake, Magnetic hyperthermia

1. Introduction

Hyperthermia (thermotherapy), in which cancer cells are treated with heat of approximately 43°C [1-3], has recently received attention as a fourth line of cancer therapy following surgical resection, chemotherapy, and radiation therapy. There are three very important advantages to hyperthermia: (1) it can be effectively applied to all types of cancer cells, (2) it is non-invasive, and (3) it has very few side effects. For example, the Thermotron RF-8 system (Yamamoto Vinita Co., Ltd., Osaka, Japan) has been clinically applied to kill cancer cells using heat derived from 8-MHz radio frequency (RF) irradiation [4,5]. However, achieving cancer cell-specific death is difficult in RF-induced hyperthermia because normal tissue is heated along with the cancerous tissue.

Recent investigations have focused on the biomedical applications of magnetic nanoparticles such as in drug delivery systems, magnetic resonance imaging, and magnetic hyperthermia [6-16]. In hyperthermia using magnetic nanoparticles and lower frequency (a few hundred kHz) magnetic fields, the samples generate heat derived just from their magnetic property. Superparamagnetic nanoparticles produce heat that is dependent on the amount of magnetic relaxation, consistent with the Néel and Brown relaxation theory, under an AC magnetic field [17-19]. In contrast, when the

ferromagnetic particles are placed under an external magnetic field, heat is generated by hysteresis loss [18,19]. Hence, if the magnetic nanoparticles were to specifically accumulate within a tumor, cancer cells could be exclusively heated under an alternating magnetic field; this process is referred to as magnetic hyperthermia [6-8,12-16]. Iron oxides such as Fe_3O_4 or $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles are generally used as heat sources as they have the advantages of high biocompatibility and relatively large magnetization. We demonstrated the synthesis of Fe_3O_4 nanoparticles, with the size controlled in the range of ~10 to ~40 nm in mean diameter, by hydrolysis in an aqueous solution containing ferrous and ferric salts at various ratios using an organic amine as a base and protective reagent [20]. We investigated their cellular uptake and their effect on cell viability with or without exposure to AC magnetic fields in various cells [21-24]. Especially with human breast cancer MCF-7 cells, we demonstrated that ~40-nm Fe_3O_4 nanoparticles with positive surface charge, attributable to the protonated spermine molecules adsorbed on the surface, tend to be incorporated into MCF-7 cells effectively [22], and that ~40-nm Fe_3O_4 nanoparticles showed a higher heating efficiency than did ~10-nm Fe_3O_4 nanoparticles when MCF-7 cells containing nanoparticles were subjected to the AC magnetic field [23]. We focused on breast cancer because magnetic hyperthermia can, in principle, be easily applied to tumors located on/under the human skin.

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3 In this study, we applied the synthetic method that we developed for Fe_3O_4
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6 nanoparticles [20] to the synthesis of CoFe_2O_4 nanoparticles owing to the potential of
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9 cobalt-ferrite nanoparticles to have greater magnetic anisotropy than iron-oxide
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12 nanoparticles [25,26]. High coercivity is expected from their large magneto-crystalline
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15 anisotropy, which should produce sufficient heat to induce cancer cell death under an
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18 AC magnetic field. Additionally, the amount of energy converted into heat per unit time
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21 and mass has been reported to be greater for CoFe_2O_4 nanoparticles than for other spinel
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24 ferrites of the same size, such as iron oxide [26]. The CoFe_2O_4 nanoparticles also
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27 maintain excellent chemical stability [27]. We synthesized CoFe_2O_4 nanoparticles using
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30 a procedure similar to that used for Fe_3O_4 nanoparticles with slight modification. Then
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33 we investigated cellular uptake and cytotoxicity of nanoparticles in human breast cancer
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36 MCF-7 cells, and cell death induced by heat generated from nanoparticles under an AC
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39 magnetic field for both CoFe_2O_4 and Fe_3O_4 nanoparticles.
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47 **2. Experimental**

48 *2.1. Synthesis and characterization of nanoparticles*

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51 CoFe_2O_4 nanoparticles were synthesized using a procedure similar to that used for
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54 Fe_3O_4 nanoparticles, as previously described [20,22,23], but with slight modification. In
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synthetic process, we prepared a 50-mL aqueous solution containing 0.017 mol·L⁻¹ cobalt(II) chloride (CoCl₂·4H₂O) and 0.033 mol·L⁻¹ iron(III) chloride (FeCl₃·6H₂O), and a 50-mL aqueous solution containing 0.123 mol·L⁻¹ spermine, or *N,N'*-bis(3-aminopropyl)butane-1,4-diamine (pH 12.1) was also prepared as a base. After the aqueous solution containing spermine was heated at 90°C, the cobalt(II) and iron(III) chloride aqueous solution was added slowly to the aqueous spermine solution, which was followed by stirring for 12 h at 90°C. Finally, a black powder was obtained after washing the precipitate with water and ethanol several times and drying in a desiccator at room temperature. Fe₃O₄ nanoparticles with a diameter of ~10 nm were also synthesized according to our previous report [20,22,23] using iron(II) chloride (FeCl₂·4H₂O) and iron(III) chloride at a molar ratio of 2:1, and spermine as a base.

The shape and size of CoFe₂O₄ nanoparticles were observed by field-emission transmission electron microscopy (FE-TEM) with a Hitachi HF-2200 microscope (Tokyo, Japan) at 200 kV and their composition and elemental mapping were analyzed by energy dispersive X-ray (EDX) analysis in FE-TEM. The shape and size of Fe₃O₄ nanoparticles were observed by TEM with a JEOL JEM-1011 microscope (Tokyo, Japan) at 100 kV. The crystal structure of the products was characterized by X-ray diffraction (XRD) patterns recorded using a SmartLab diffractometer (Rigaku, Tokyo,

Japan) with Co K α radiation (1.7890 Å). The magnetic field dependence of the magnetization curves of samples was measured by an MPMS 3 or MPMS 7 superconducting quantum interference device (SQUID; Quantum Design, Ltd., San Diego, CA) at 300 K, in which the applied range was from –10 kOe to 10 kOe. The zeta potential was evaluated with an Otsuka Electronics ELS-8000 electrophoretic light-scattering spectrophotometer (Osaka, Japan).

2.2. Cell culture

Human breast cancer MCF-7 cells were cultured in Eagle's minimum essential medium (EMEM, Sigma-Aldrich, St. Louis, MO) with 10% fetal bovine serum (FBS, ThermoFisher Scientific, Waltham, MA), 5% L-glutamine (Invitrogen, Life Technologies, Grand Island, NY), 1% MEM non-essential amino acid (Gibco, Life Technologies), and 1% antibiotic-antimycotic (Gibco) under culture conditions of 37°C and 5% CO₂.

2.3. Cytotoxicity and cellular uptake study

MCF-7 cells were seeded in a 6-well dish at a density of 5×10^5 cells per 3 mL of medium in each well. CoFe₂O₄ or Fe₃O₄ nanoparticles in amounts of 0, 200, 400, 600,

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3 or 800 μg were added to each well and then incubated at 37°C at 5% CO_2 for 24 h. After
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6 removing excess nanoparticles by three washes with Dulbecco's phosphate buffered
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9 saline (DPBS; Gibco) followed by centrifugation at 1200 rpm for 5 min, the cells were
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12 dispersed in 1 mL of DPBS with 6% FBS. We used a BD Cell Viability Kit (BD
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15 Biosciences, Becton, Dickinson and Co., Franklin Lakes, NJ) to discriminate live cells,
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18 dead cells, and nanoparticles: 1 μL of $42\ \mu\text{mol}\cdot\text{L}^{-1}$ propidium iodide (PI) in dimethyl
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21 sulfoxide and 2 μL of $4.3\ \text{mmol}\cdot\text{L}^{-1}$ thiazole orange (TO) in water were added to 1 mL
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24 of cells suspended in DPBS, and then analyzed with a BD FACSCanto II flow
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27 cytometer. Live cells are PI negative and TO positive, dead cells are both PI and TO
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30 positive, and nanoparticles are both PI and TO negative.
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35 Cellular viability as well as cellular uptake of nanoparticles can be analyzed via
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38 flow cytometry because increased side scatter (SSC), which is indicative of intracellular
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41 complexity, can be measured when the laser light crosses the cell in the flow cytometer.
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44 Hence, the SSC intensity of a cell that has incorporated nanoparticles is greater than that
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47 of a cell that has not incorporated nanoparticles [22,24]. In the present study, the cellular
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50 uptake of CoFe_2O_4 and Fe_3O_4 nanoparticles was measured based on SSC intensity.
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54 The amount of nanoparticles contained per cell was also evaluated for both CoFe_2O_4
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57 and Fe_3O_4 nanoparticles as previously described [23,24]. Intracellular Fe was formed
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3 into a thiocyanate-iron(III) complex by using HCl, trichloroacetic acid solution, H₂O₂,
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6 and potassium thiocyanate solution, followed by measurement of absorbance at 480 nm
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9 using a JASCO V-550 spectrophotometer (JASCO International Co. Ltd., Tokyo,
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16 To observe cellular uptake of nanoparticles with an optical microscope, prussian
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18 blue staining was performed using an Iron Stain Kit (Sigma-Aldrich), by which
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20 iron-containing nanoparticles turned blue and cells were stained red. Iron stain solution
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22 including potassium ferrocyanide solution and hydrochloric acid solution at a ratio of
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28 1:1 was added to DPBS-washed cells at a particle dose of 200 µg in a 6-well dish. After
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31 10 min, the residues were rinsed away with DPBS. Then, pararosaniline solution was
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35 added for staining cells, and after 5 min, the residues were rinsed away with DPBS. The
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38 cells were then observed under a Nikon TE2000-U microscope (Tokyo, Japan).
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44 *2.4. Evaluation of the dissolution of Co²⁺ from CoFe₂O₄ nanoparticles in water*

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48 CoFe₂O₄ nanoparticles (1 mg) were dispersed in 3 mL of water in a polypropylene
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50 tube (pH ~7). After a 24-h or 72-h incubation at 37°C and 5% CO₂, the supernatants of
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53 both samples were collected by centrifugation, and the concentration of Co²⁺ in the
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56 sample was measured by a Thermo Scientific iCAP Qc inductively coupled
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3 plasma-mass spectrometer (ICP-MS). Before measurements, a calibration curve of Co^{2+}
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6 concentration was made using a cobalt standard solution (Co in 0.1 mol/L HNO_3 ; Kanto
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9 Chemical Co. Ltd., Tokyo, Japan). The concentration of Co^{2+} in the samples was
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19 *2.5. Evaluation of cell death with nanoparticles under an AC magnetic field*

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28 the cells three times with DPBS, the cells containing nanoparticles were collected by
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31 magnetic separation and suspended in 500 μL of medium. An AC magnetic field was
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34 applied for 20 min using both a 3-turn coil with an outer diameter of 40 mm and an
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37 EASYHEAT induction-heating device (Alonics, Ltd., Tokyo, Japan) operated at an
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40 output power of 4.3 kW, an electric current of 569.1 A, and a frequency of 325 kHz,
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43 from which the strength of the magnetic field was calculated to be 536 Oe. The change
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46 in temperature under the AC magnetic field was monitored with an Anritsu FL-2000
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49 fiber thermometer (Tokyo, Japan). Temperature curves were also obtained for 500 μL of
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52 water containing 1 mg of nanoparticles without cells. Cell death mediated by
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55 nanoparticles subjected to the AC magnetic field was analyzed by flow cytometry using
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PI and TO staining.

3. Results and discussion

First, the synthesis and characterization of CoFe_2O_4 and Fe_3O_4 nanoparticles were performed. Fig. 1A and 1B depict the TEM images of CoFe_2O_4 and Fe_3O_4 nanoparticles, respectively, and revealed that both particles were spherical or truncated cubic in shape, with a mean diameter of ~ 10 nm. For CoFe_2O_4 nanoparticles, the ratio of Co to Fe in an individual particle was analyzed by EDX. The ratio of oxygen could not be precisely determined due to the limitations of the measurement method. The EDX results suggest that the atomic ratio of Co to Fe is 1 to 2, and particles composed of only cobalt or iron were not observed. We also examined the elemental mapping of CoFe_2O_4 nanoparticles (see Fig. A.1 in Appendix). Fe and Co are found to be present uniformly along nanoparticles. Fig. 1C shows XRD patterns for the products synthesized with cobalt(II) chloride and iron(III) chloride (a) and with iron(II) chloride and iron(III) chloride (b). The pattern (a) was matched with the standard pattern of CoFe_2O_4 (JCPDS# 22-1086) and the pattern (b) was matched with the standard pattern of Fe_3O_4 (JCPDS# 19-0629). Although both patterns resembled each other, the positions of diffractions peaks were slightly but certainly different between the patterns (a) and (b). For example, the d

values of the lattice spacing of (311) was calculated to be 2.526 Å for pattern (a) and 2.529 Å for pattern (b) by using the Bragg equation for the peak observed at $2\theta \sim 41^\circ$, and the slight difference in these values was attributable to the difference between theoretical values of 2.531 Å for CoFe₂O₄ (JCPDS# 22-1086) and 2.532 Å for Fe₃O₄ (JCPDS# 19-0629). The crystallite size calculated from XRD by applying Scherrer's formula to the (311) diffraction peak was ~8 nm (in diameter) for the both patterns (a) and (b), which is consistent with the TEM results. Thus, products (a) and (b) were identified to be CoFe₂O₄ and Fe₃O₄, respectively, with a spinel structure. The results of TEM, EDX analysis, elemental mapping, and XRD measurements suggest that CoFe₂O₄ nanoparticles with a diameter of ~10 nm were synthesized without composites of Co₃O₄, Fe₃O₄, and γ -Fe₂O₃. The formation of CoFe₂O₄ likely progressed by a generally known co-precipitation reaction represented by the following equation [28]:

Fig. 2 shows the magnetization curves of CoFe₂O₄ and Fe₃O₄ nanoparticles at 300 K. As depicted in Fig. 2, the magnetization value at the 10000 Oe field of both CoFe₂O₄ and Fe₃O₄ nanoparticles was found to be ~60 emu·g⁻¹. The magnetization values observed for the synthesized nanoparticles with a diameter of ~10 nm were slightly smaller than those of bulk materials, which could be due to the effect of increased

thermal fluctuation near the surface of nanoparticles or that of the magnetically disordered surface formed because of the large surface-to-volume ratio [20]. As also shown in Fig. 2, the coercivity of CoFe₂O₄ was ~200 Oe and that of Fe₃O₄ was less than 10 Oe, suggesting that CoFe₂O₄ nanoparticles show a ferromagnetic type of behavior, whereas Fe₃O₄ demonstrate superparamagnetic behavior. Hence, the heat derived from CoFe₂O₄ is generated by hysteresis loss, and the heat generated by Fe₃O₄ is largely controlled by Néel and Brown relaxation under the AC magnetic field [17-19,26]. Thus, the substitution of Fe²⁺ with Co²⁺ is likely to result in an increase in coercivity.

Fig. 3 shows the temperature curve measured for 500 µL of water containing 1 mg of nanoparticles without cells under an AC magnetic field (569.1 A, 325 kHz) for 20 min using a 3-turn coil with an outer diameter of 40 mm (the strength of magnetic field was theoretically calculated to be 536 Oe). The temperature of CoFe₂O₄ nanoparticles was higher than that of Fe₃O₄, whose average temperature measured between 400 and 1200 s was ~56°C for CoFe₂O₄ and ~45°C for Fe₃O₄. From the initial slope of these time-dependent temperature curves in the first 100 s, specific absorption rates (SARs) of 404 and 319 W·g⁻¹ was obtained for CoFe₂O₄ and Fe₃O₄ nanoparticles, respectively, under this condition by the following equation:

$$SAR = \frac{\sum_i C_{pi} m_i}{m_{NPs}} \cdot \frac{\Delta T}{\Delta t} \quad (2)$$

where C_{pi} and m_i are specific heat capacity and mass for each substance, and m_{NPs} is the mass of nanoparticles.

We next investigated the influence of nanoparticles on human breast cancer MCF-7 cells. Fig. 4 shows the percentage of cells containing nanoparticles (A) and the percentage of non-viable cells (B). As depicted in Fig. 4A, the percentage of cells containing nanoparticles tended to increase at the higher doses of both types of particles: at 200 μ g, the percentage of cells containing nanoparticles was 50–60% and at 800 μ g, the percentage of cells containing nanoparticles was $\sim$ 80%. No more than 10% cell mortality was observed following the addition of nanoparticles at concentrations from 0 to 800 μ g. Neither Fe_3O_4 nor $CoFe_2O_4$ nanoparticles killed MCF-7 cells despite cellular uptake. Although the percentage of MCF-7 cell death observed with $CoFe_2O_4$ nanoparticles seems slightly higher than that for Fe_3O_4 at all doses, it should be noted that this tendency was observed even in the control experiment (without nanoparticles); the cell mortality was constant at the dose range from 0 to 800 μ g for both Fe_3O_4 and $CoFe_2O_4$ nanoparticles. However, $CoFe_2O_4$ nanoparticles might derive cytotoxicity from Co^{2+} [29,30] eluted from $CoFe_2O_4$. For example, Catelas et al. [29] reported that Co^{2+} induces apoptosis via a caspase-3 pathway. To consider the influence of dissolved Co^{2+} , we examined Co^{2+} elution by ICP-MS under almost identical conditions as those

used for the cytotoxicity test: the concentration was 1 mg of CoFe_2O_4 nanoparticles per 3 mL of H_2O at 37°C . As shown in Table 1, few Co^{2+} were eluted into the water from CoFe_2O_4 nanoparticles: the concentration of Co^{2+} in the sample was $0.785 \mu\text{g}\cdot\text{mL}^{-1}$ (0.9% of Co was eluted) after a 24-h incubation, and $0.465 \mu\text{g}\cdot\text{mL}^{-1}$ (0.6% of Co was eluted) after a 72-h incubation. The decrease of Co^{2+} concentration from 24 h to 72 h seems to be related to the adsorption of Co^{2+} to the surface of the polypropylene tube. Indeed, Kwon et al. [30] investigated the cytotoxicity of Co ions released from Co nanoparticles on RAW263.7 macrophage cells *in vitro*, and reported that Co ions at a concentration of $0.630 \mu\text{g}\cdot\text{mL}^{-1}$ show no cytotoxic effect, but $4.777 \mu\text{g}\cdot\text{mL}^{-1}$ of Co ions was cytotoxic. Based on this information, we conclude that an extensive elution of Co^{2+} from CoFe_2O_4 nanoparticles was not observed in our case, but further surface modification of CoFe_2O_4 should be the key when their practical application to magnetic hyperthermia is considered.

Fig. 5 shows the dose dependence of the uptake of CoFe_2O_4 and Fe_3O_4 nanoparticles by MCF-7 cells. The CoFe_2O_4 nanoparticles were incorporated at a lower concentration than Fe_3O_4 nanoparticles. Approximately 75 pg of both types of nanoparticles was incorporated into each cell at a dose of 200 μg per 5×10^5 cells, and ~200 pg (for CoFe_2O_4) or ~230 pg (for Fe_3O_4) of nanoparticles was incorporated per cell at the dose

of 800 μg . Based on our previous finding [22], this slight difference in uptake could be induced less by the composition of the nanoparticles than by their zeta potential. The zeta potential in water at pH ~ 7 was +2.6 mV for CoFe_2O_4 nanoparticles and +9.3 mV for Fe_3O_4 nanoparticles. The positive value of the zeta potential at pH ~ 7 is attributable to the cationic form of amine groups in spermine adsorbed on the surface of nanoparticles [22].

The uptake of nanoparticles into MCF-7 cells was observed with an optical microscope at the dose of 200 μg . In Fig. 6, MCF-7 cells were stained red and nanoparticles were stained blue by iron stain. Compared with the image of MCF-7 cells without the dose of nanoparticles (Fig. 6A), the incorporation of nanoparticles did not alter the apparent morphology of cells for both CoFe_2O_4 (Fig. 6B) and Fe_3O_4 (Fig. 6C). The nanoparticles were observed within the cytoplasm but not in the nucleus. The ratio of cells containing nanoparticles observed in those optical microscopic images was comparable to that evaluated by flow cytometry (Fig. 4). In addition, the amount of incorporated nanoparticles appeared to be similar for both CoFe_2O_4 and Fe_3O_4 nanoparticles, which is consistent with the result shown in Fig. 5. Therefore, cellular uptake of nanoparticles was evidenced by the results shown in Figs. 4, 5, and 6.

To determine the potential of $\sim 10\text{-nm}$ CoFe_2O_4 nanoparticles for efficiently inducing

cancer cell death, MCF-7 cells with incorporated nanoparticles were subjected to an AC magnetic field (using a 3-turn coil with an outer diameter of 40 mm at 325 kHz and 569.1 A) for 20 min, of which strength was calculated to be 536 Oe. Based on the temperature curves measured for nanoparticles under the AC magnetic field (Fig. 7), the temperature of CoFe_2O_4 nanoparticles was greater than that of the Fe_3O_4 nanoparticles incubated at the same dose, suggesting that CoFe_2O_4 nanoparticles convert the magnetic field into heat more efficiently than did Fe_3O_4 nanoparticles under this condition. Considering the magnetic properties of the nanoparticles (Fig. 2) and the amount taken up by the cells (Fig. 5), the greater temperature reached by CoFe_2O_4 nanoparticles is strongly expected to be attributable to their coercivity, even though the amount of CoFe_2O_4 nanoparticles incorporated into a cell was smaller than that of the Fe_3O_4 nanoparticles. Following exposure to the AC magnetic field, the percentage of non-viable MCF-7 cells incubated with every dose of nanoparticles was evaluated by flow cytometry. As shown in Fig. 8, ~53% cell death could be induced using CoFe_2O_4 nanoparticles and the AC magnetic field. On the other hand, Fe_3O_4 nanoparticles did not significantly induce cell death as only ~17% of non-viable cells was observed even at the 800 μg dose of nanoparticles. Based on the temperature shown in Fig. 8 which is the average temperature measured between 400 and 1200 s in Fig. 7, the higher temperature

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3 induced greater cell mortality, which was especially significant when the temperature
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6 reached $\sim 43^{\circ}\text{C}$ (critical temperature for cell death). Thus, under the tested conditions,
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9 the heat generated by $\sim 10\text{-nm}$ CoFe_2O_4 nanoparticles under an AC magnetic field was
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12 found to be sufficient for human breast cancer cell death.
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19 **4. Conclusions**

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22 In this report, we applied a synthetic method that we previously developed for Fe_3O_4
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25 nanoparticles to the synthesis of CoFe_2O_4 nanoparticles and compared certain
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28 characteristics of similarly synthesized CoFe_2O_4 and Fe_3O_4 nanoparticles having a mean
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31 diameter of $\sim 10\text{ nm}$. We demonstrated that the CoFe_2O_4 nanoparticles synthesized in
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34 this study exhibit greater coercivity than Fe_3O_4 nanoparticles with almost the same
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37 diameter. An increase in coercivity derived from the substitution of Fe^{2+} with Co^{2+} was
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40 confirmed to cause a higher heating efficiency of nanoparticles under an AC magnetic
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43 field. We also demonstrated that cell death attributable to heat was observed rapidly
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46 following exposure to an AC magnetic field in human breast cancer MCF-7 cells
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49 containing CoFe_2O_4 nanoparticles with a diameter of $\sim 10\text{ nm}$, whereas MCF-7 cell
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52 death was not induced using $\sim 10\text{-nm}$ Fe_3O_4 nanoparticles under the same conditions.
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57 These results will assist in the design of magnetic nanoparticles as heat elements for
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3 magnetic hyperthermia, although further investigation into surface modifications to
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6 prevent Co^{2+} elution from CoFe_2O_4 will be necessary.
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Figure Captions

Fig. 1. Characterization of CoFe_2O_4 and Fe_3O_4 nanoparticles employed in this study. TEM images of CoFe_2O_4 nanoparticles (A) and Fe_3O_4 nanoparticles (B), and XRD patterns with Co $K\alpha$ radiation (C): CoFe_2O_4 (a) and Fe_3O_4 (b).

Fig. 2. Magnetization curves of CoFe_2O_4 and Fe_3O_4 nanoparticles. The curves were measured by SQUID at 300 K between -10000 and 10000 Oe of magnetic field strength.

Fig. 3. Time dependence of the temperature curve of 1 mg of CoFe_2O_4 or Fe_3O_4 nanoparticles in 500 μL of water under an AC magnetic field.

Fig. 4. Dependence of the percentage of cells containing nanoparticles (A) and the percentage of non-viable cells (B) on the amount of CoFe_2O_4 (circles) or Fe_3O_4 (triangles) nanoparticles added to 5×10^5 MCF-7 cells.

Fig. 5. Dependence of the amount of incorporated nanoparticles per cell on the amount

of CoFe_2O_4 (circles) or Fe_3O_4 (triangles) nanoparticles added to 5×10^5 MCF-7 cells.

Fig. 6. Optical microscopic images of MCF-7 cells without nanoparticles (A), those containing CoFe_2O_4 nanoparticles (B), and those containing Fe_3O_4 nanoparticles (C). In these images, cells were stained red whereas nanoparticles were stained blue by iron stain.

Fig. 7. Time dependence of the temperature change in MCF-7 cells containing CoFe_2O_4 (a) or Fe_3O_4 (b) nanoparticles under an AC magnetic field. Control cells were not treated with nanoparticles. The inserted 200, 400, 600, and 800 μg indicate the amount of nanoparticles added to 5×10^5 MCF-7 cells, not the amount of incorporated nanoparticles.

Fig. 8. Dependence of the percentage of non-viable MCF-7 cells containing CoFe_2O_4 (a) or Fe_3O_4 (b) nanoparticles soon after exposure to an AC magnetic field (ACMF; 325 kHz, the strength was calculated to be ~ 500 Oe) for 20 min on the amount of nanoparticles added to 5×10^5 cells. The temperatures indicated are the average temperatures between 400 and 1200 s in Fig. 7.

Table 1.

Co²⁺ elution at the concentration of 1 mg of CoFe₂O₄ nanoparticles per 3 mL of H₂O.

	Concentration / μg·mL ⁻¹	Elution percentage (%)
Water	0.00004	0.0
24 h	0.785	0.9
72 h	0.465	0.6

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

A

B

C

Fig. 7

Fig. 8