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Biocompatibility of various ferrite nanoparticles evaluated by in vitro cytotoxicity assays using HeLa cells

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Abstract

Magnetic nanoparticles for thermotherapy must be biocompatible and possess high thermal efficiency as heating elements. The biocompatibility of Fe_3O_4 (20-30 nm), $ZnFe_2O_4$ (15-30 nm) and $NiFe_2O_4$ (20-30 nm) nanoparticles was studied using a cytotoxicity colony formation assay and a cell viability assay. The Fe_3O_4 sample was found to be biocompatible on HeLa cells. While $ZnFe_2O_4$ and $NiFe_2O_4$ were non-toxic at low concentrations, HeLa cells exhibited cytotoxic effects when exposed to concentrations of 100 μg/ml nanoparticles.

Keywords: magnetic nanoparticles, hyperthermia, biocompatibility, cell adhesion, viability

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Hyperthermia is a method of cancer treatments which is designed to raise the temperature of cancer cells. Cancer cells are sensitive to heat and tumor cells are more susceptible to heat than normal tissue. Hyperthermia treatment thus has the advantage of being less risky to the body, with less side effects and the possibility of repeating treatment, as compared to surgery, chemotherapy and radiation therapy. In recent years, magnetic nanoparticles have attracted attention as the heat source for hyperthermia due to their magnetic behavior such as magnetic transportation [1,2], magnetic isolation and self-heating in an ac magnetic field [1-4]. The magnetic nanoparticles can be injected intravenously and transferred to specific parts of the body with a magnetic field. The tumor is then treated by the heat generated by the magnetic nanoparticles from an external ac magnetic field. Using the magnetic nanoparticles as the heat source for hyperthermia is advantageous for cancer treatments. Tumors deeply located in the body can be treated and temperature of the heat source is controlled by the strength and frequency of the external magnetic field. Moreover, cancer cells are more radiosensitive when exposed to elevated temperatures [5]. The magnetic nanoparticles can be also utilized as the carrier of anticancer drug delivery. The availability of transferring the anticancer drug to specific part of the body reduces side-effect in chemotherapy. Therefore the magnetic nanoparticles are expected to enhance therapeutic effect and reduce side effect in the combined use with conventional cancer treatments.

Fe ferrite nanoparticles are biocompatible and in clinical use as contrast agents for magnetic resonance imaging (MRI). The temperature rise in an ac magnetic field and the ensuing the cytotoxicity of Fe_3O_4, dextran-coated magnetite [6,7], magnetite cationic liposome [8], and others [9,10] has been reported. The nanoparticles are coated with some materials in order to improve their biocompatibility, to provide the specific

functionalization to cancer cells and to avoid the aggregation among the particles by magnetic force. However, the biocompatibility of uncoated magnetic nanoparticles is also significant because of inadequate coating or detachment of entangling coated materials. In this paper, the cytotoxicity of uncoated Fe_3O_4, $ZnFe_2O_4$ and $NiFe_2O_4$ is studied in order to develop magnetic nanoparticles with higher thermal efficiency and biocompatibility for hyperthermia.

Ferrite nanoparticles of Fe_3O_4 (20-30 nm), $ZnFe_2O_4$ (15-30 nm) and $NiFe_2O_4$ (20-30 nm) were used as samples (commercially distributed by Nanostructured & Amorphous Materials, Inc.). The shapes of Fe_3O_4 and $ZnFe_2O_4$ samples were spherical while $NiFe_2O_4$ was nearly spherical. Figure 1 shows the magnetization curves for the samples measured at room temperature by a sample vibrating magnetometer (VSM). The self-heating property of the samples was also measured in an alternating magnetic field at a frequency of 110 kHz. Figure 2 shows the field intensity dependence of the temperature rise. $ZnFe_2O_4$ exhibited lower self-heating temperature, which was attributed to its lower magnetic susceptibility as indicated in Figure 1.

In vitro cytotoxicity of samples was then investigated by two methods, colony formation assay and cell viability assay (trypan blue dye exclusion). The colony formation assay is a commonly used assay to evaluate the cellular proliferative potential and is widely used in the field of radiation biochemistry [11], chemotherapy and toxicology [12]. In these experiments, cultured cells were exposed to magnetic nanoparticles, and the number of colonies which adhered normally to culture vessels evaluated. The ratio of colonies was defined as the number of colonies counted 7 days after exposing the cells to magnetic nanoparticles compared with the number of colonies counted with no added nanoparticles. The trypan blue assay is the most simple

assessment to investigate probability of cell survival [13]. This assay enables to discriminate living cells from blue stained dead cells and to evaluate cellular viability. The viability was calculated as the ratio of living cell number exposed to magnetic nanoparticles against the cell number of control (without nanoparticles) cells. In this paper, toxicity is determined when the cells exposed to magnetic nanoparticles have both low proliferation ability in colony formation assay and simultaneously low viability in cell viability assay.

Human cervical carcinoma (HeLa) cells were chosen for the cell culture experiments. The cells were grown in modified eagle medium (MEM) containing 10% calf serum (CS) and incubated at 37 °C under 5% CO_2 atmosphere. Nearly confluent cells in 50 ml tissue culture flask were washed twice with Hanks' Balanced Salt Solutions (HBSS) to remove unattached cells and medium. Then the cells were trypsinized by 0.1% trypsin solution and centrifuged at 1000 g for 3 minutes. The cell pellet was resuspended in fresh media.

In the colony formation assay, HeLa cells were seeded at a density of 100 cells in 60 mm Petri dishes with 5 ml MEM. After 24 hours culture at 37 °C in 5% CO_2 atmosphere, the cells were exposed to each sample of Fe_3O_4, $ZnFe_2O_4$ and $NiFe_2O_4$ at a concentration of 10 μg/ml and 100 μg/ml for 7 days. After 7 days, the medium was removed and the cells were rinsed in HBSS twice. The colonies were fixed with methanol and stained for 30 minutes with Giemza staining solution. Then, the number of adhered colonies in each dishes was counted. The mean value of three trials under the same condition was calculated for each experiment throughout this study.

The ratio of the number of adhered colonies of HeLa cells exposed to Fe_3O_4, $ZnFe_2O_4$ and $NiFe_2O_4$ compared to that without nanoparticles was calculated. As shown in Figure

3, the number of colonies exposed to Fe_3O_4, $ZnFe_2O_4$ and $NiFe_2O_4$ at the nanoparticle concentration of 10 μg/ml was almost equivalent to that of the control group. At the concentration of 100 μg/ml, HeLa cells exposed to Fe_3O_4 and $ZnFe_2O_4$ were highly adhered to the cultured vessels, but the adhered cells ratio for $NiFe_2O_4$ was only 55%.

In the cell viability assay, HeLa cells were seeded at a density of 1×10^4 cells/ml to each well of a 24 well plates. After 24 hours, the cells were exposed to the nanoparticle samples (Fe_3O_4, $ZnFe_2O_4$, $NiFe_2O_4$) at a concentration of 10 μg/ml and 100 μg/ml for 24 and 48 hours. The medium was then removed and the cells rinsed in HBSS twice. The rinsed cells were harvested by adding 200 μl of 0.1% trypsin for 3 minutes, then adding 800 μl of MEM with 0.1% trypan blue and counting the number of living cells.

The survival rate of HeLa cells exposed to Fe_3O_4, $ZnFe_2O_4$ and $NiFe_2O_4$ (Figure 4) at the concentration of 10 μg/ml for 24 or 48 hours was above 80%. At the concentration of 100 μg/ml, however, the cells exposed to $ZnFe_2O_4$ and $NiFe_2O_4$ showed low viability of 77.6% and 70.5% for 24 hours, and 59.4% and 52.4% for 48 hours, respectively. Figure 5 shows photographs of HeLa cells exposed to each samples at the concentration of 100 μg/ml for 48 hours. There was almost no difference between colonies formed by the cells exposed to Fe_3O_4 and that of the cells without particles. But the colonies exposed to $ZnFe_2O_4$ and $NiFe_2O_4$ were smaller. In the experiment, it was also found that adhesion between cells in their colonies was weak.

The viability of HeLa cells exposed to 100 μg/ml of $ZnFe_2O_4$ and $NiFe_2O_4$ is lower than that to 10 μg/ml, which indicates that higher concentration of magnetic nanoparticles show more cytotoxicity. The cytotoxicity of magnetic fluids depending on the concentration of ferrites and reduced cell viability at the high concentration above 100 μg/ml have been also reported [14,15]. When cells are exposed to magnetic

nanoparticles, most nanoparticles are first adhered to the surface, internalized to the cells as a result of endocytosis, and accumulated in digestive vacuoles. The cytotoxicity is thus very likely caused by particle overload to cells [16].

In the case of exposure to $ZnFe_2O_4$, HeLa cells in colony formation assay showed high ratio of colonies while the survival rate in cell viability assay in case of 100 μg/ml was low. The colony formation assay is to evaluate cellular proliferative potential and the trypan blue assay is to investigate survival capacity of cells. The difference between these two assays reveals that HeLa cells are affected greatly by $ZnFe_2O_4$ in 48 hours, however, they were able to recover their proliferative ability. As for $NiFe_2O_4$ cases in two assays, both of the colony adhered rate and cell viability at 100 μg/ml was lower than that of Fe_3O_4 as indicated in Figures 3 and 4. It means that HeLa cells exposed to $NiFe_2O_4$ are less likely to survive and maintain their proliferative capacity. This indicates that $NiFe_2O_4$ is much more cytotoxic than Fe_3O_4 and $ZnFe_2O_4$.

The difference in cytotoxicity for magnetic nanoparticles made of different materials has been reported. For example, $CoFe_2O_4$ is less biocompatible than Fe_3O_4 and $MnFe_2O_4$ [14]. The coating materials, such as dextran or other polymers, on magnetic nanoparticles also influence their biocompatibility [6-10]. On the other hand it has been reported that $CoFe_2O_4$ coated with lauric acid showed higher loss of cell viability compared to Fe_3O_4 and $MnFe_2O_4$ coated with same material [15]. These results indicate that the biocompatibility of nanoparticles depends on the coating materials, but also the core material of nanoparticle. Moreover, it has been reported that enzymatic degradation of the dextran shell within cells causes the cytotoxicity of dextran-coated magnetite nanoparticles [17]. It is obvious that the biocompatibility of uncoated various ferrite nanoparticles is significant.

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In summary, the toxicity of Fe_3O_4 (20-30 nm), $ZnFe_2O_4$ (15-30 nm) and $NiFe_2O_4$ (20-30 nm) to HeLa cells at the concentration of 10 μg/ml and 100 μg/ml were investigated. Fe_3O_4 is highly biocompatible in both colony formation assay and cell viability assay. HeLa cells exposed to 10 μg/ml $ZnFe_2O_4$ had a high proliferation ability and high viability, however, HeLa cells exposed to 100 μg/ml $ZnFe_2O_4$ showed only slight changes on proliferation, in spite of low viability. $NiFe_2O_4$ also showed only minimal changes on HeLa cell proliferation at concentrations of 10 μg/ml, but low viability at concentrations of 100 μg/ml. The obtained result reveals that non-toxicity of three ferrite nanoparticles (Fe_3O_4, $ZnFe_2O_4$ and $NiFe_2O_4$) at the concentration lower than 10 μg/ml and the cytotoxicity of $NiFe_2O_4$ at concentrations of 100 μg/ml. To use these nanoparticles as the heat source for hyperthermia, this threshold is essential for non-target tissues.

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Fig. 1. Magnetization curves of Fe_3O_4 (20-30 nm), $ZnFe_2O_4$ (15-30 nm) and $NiFe_2O_4$ (20-30 nm) measured by VSM at room temperature.

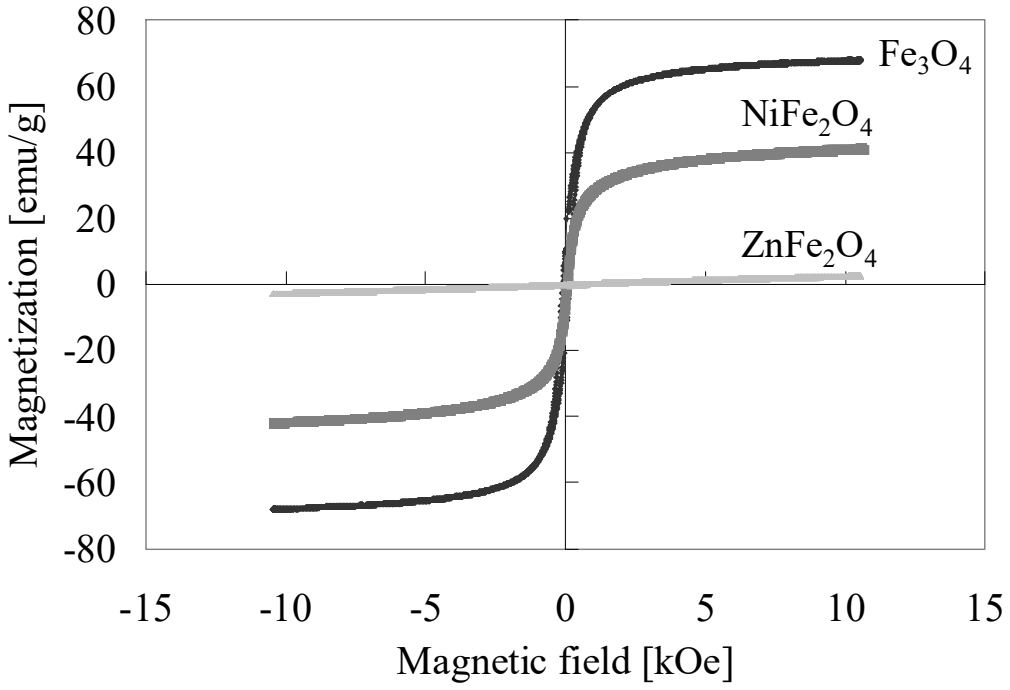

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Fig. 2. Temperature rise of Fe_3O_4 (20-30 nm), $ZnFe_2O_4$ (15-30 nm) and $NiFe_2O_4$ (20-30 nm) measured by applying an ac magnetic field at 110 kHz.

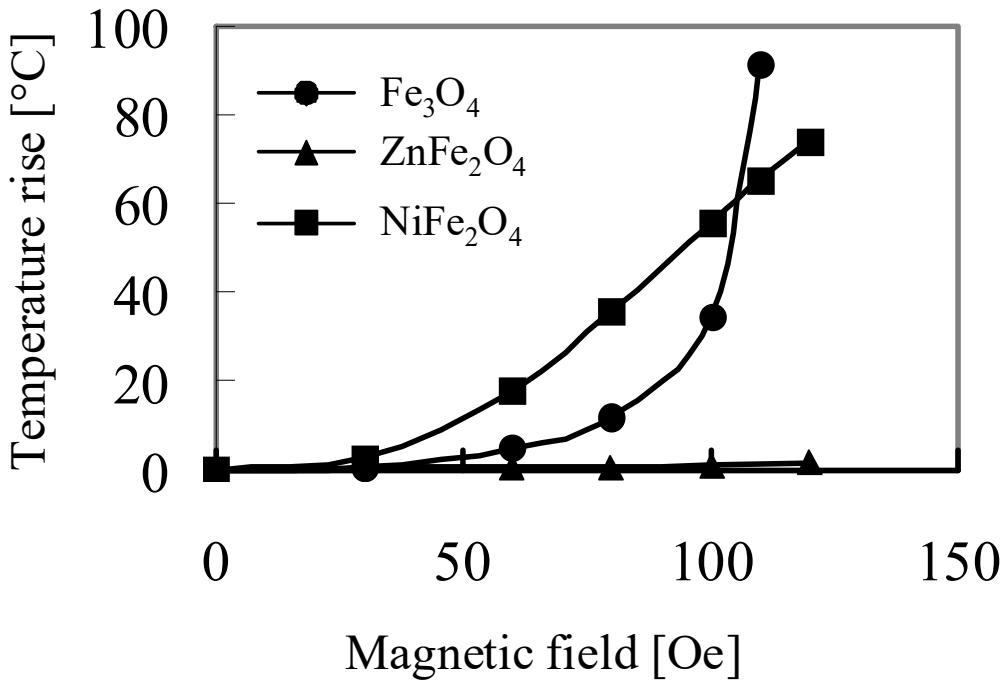

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Fig. 3. The ratio of the number of adhered colonies of HeLa cells exposed to magnetic nanoparticles of Fe_3O_4 (20-30 nm), $ZnFe_2O_4$ (15-30 nm) and $NiFe_2O_4$ (20-30 nm) compared to the number of colonies without nanoparticles in cell adhesion study.

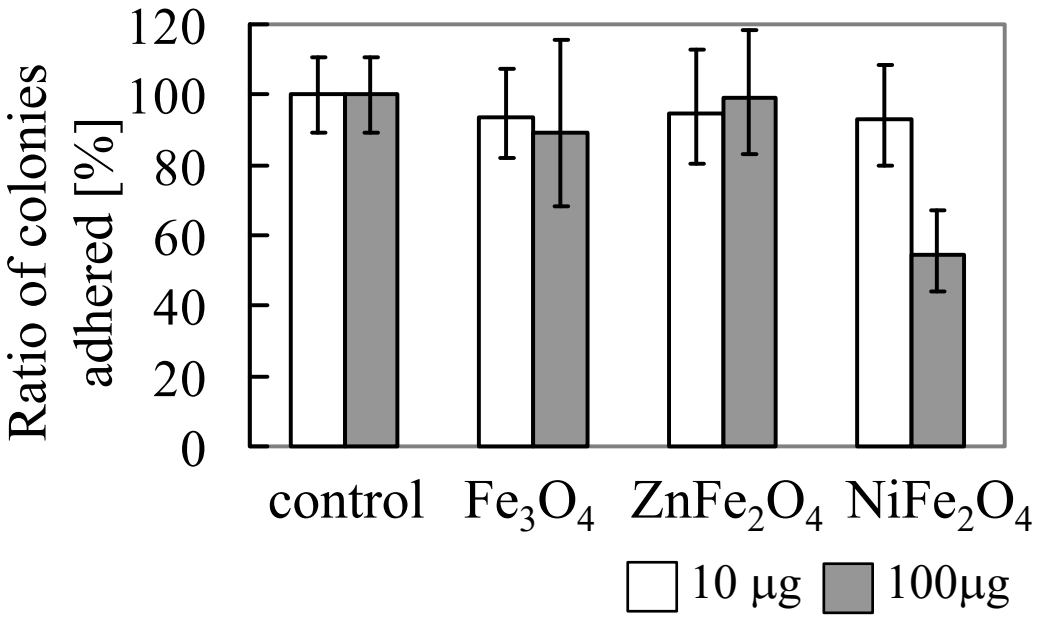

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Fig. 4. Survival rate of HeLa cells exposed to magnetic nanoparticles of Fe_3O_4 (20-30 nm), $ZnFe_2O_4$ (15-30 nm) and $NiFe_2O_4$ (20-30 nm) compared to cells without nanoparticles in cellular viability study.

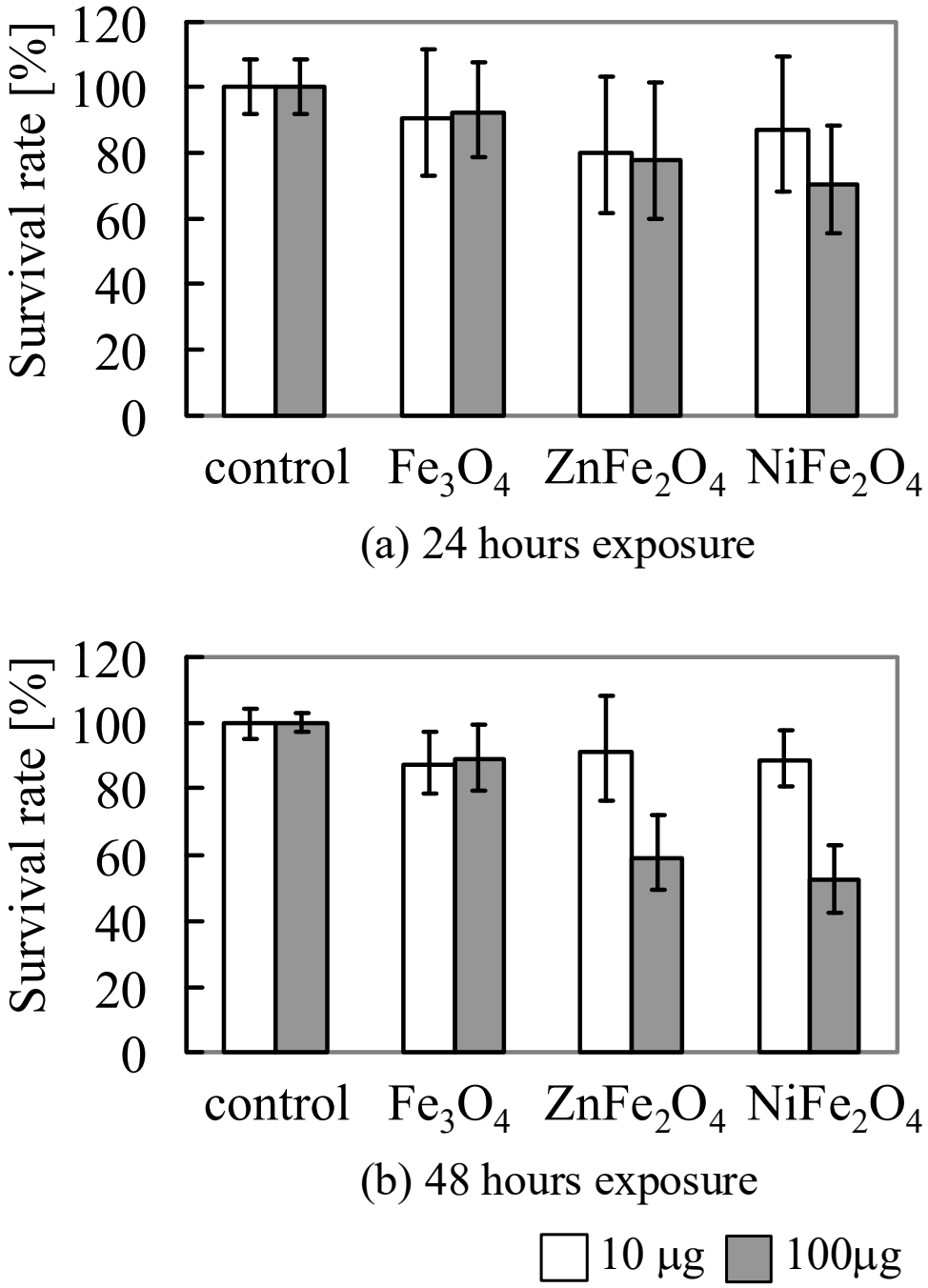

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Fig. 5. Photographs of HeLa cells (a) without particles, and exposed to 100 μg/ml (b) Fe_3O_4 (20-30 nm), (c) $ZnFe_2O_4$ (15-30 nm), and (d) $NiFe_2O_4$ (20-30 nm).