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Research Article

Magnetic Nanoparticle Hyperthermia Using Pluronic-Coated Fe_3O_4 Nanoparticles: An *In Vitro* Study

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Magnetic nanoparticles are promising materials for hyperthermia treatment. The temperature rise under ac magnetic field, cytotoxicity, and *in vitro* hyperthermia effect of Fe_3O_4 nanoparticles coated with Pluronic f-127 were evaluated in this paper. The Pluronic-coated Fe_3O_4 nanoparticles exhibited no cytotoxic effect on HeLa cells. The optimal magnetic field of Pluronic-coated Fe_3O_4 nanoparticles was 16 kA/m (200 Oe) at the field strength of 210 kHz. Appropriate temperature rise significantly reduced the viability of HeLa cells and induced apoptosis.

1. Introduction

Hyperthermia is a cancer therapy; that is, increasing the temperature of the body or a particular region at temperature higher than 42°C. This hyperthermia treatment has great advantages of being less risky to the body, causing less side effects and providing the possibility of repeating treatment compared to traditional cancer treatment, such as surgical operation, chemotherapy, and radiation therapy. Dewey et al. reported the thermal sensitivity of Chinese hamster ovary (CHO) cells in 1977 [1]. The cells heated at more than 42.5°C greatly reduced their viability according to the temperature.

Magnetic nanoparticles have attracted attention as various biomedical applications including contrast agent for magnetic resonance imaging (MRI), carrier of drug delivery system, and the heat source for hyperthermia [2]. They possess unique properties such as magnetic transportation, magnetic isolation, and self-heating in an ac magnetic field. The magnetic nanoparticles can be injected intravenously and transferred to specific parts of the body with EPR (enhanced permeation and retention) effect and a magnetic field. The tumor is then treated with the heat, which is generated by the magnetic nanoparticles under an external ac magnetic field. Magnetic nanoparticle hyperthermia has great advantages of local treatment with specific targeting and combination therapy with drug delivery system. The temperature of magnetic nanoparticles is controlled by the strength and frequency of the external magnetic field.

In order to apply magnetic nanoparticles for bioapplications, it is significant to keep their biocompatibility and avoid the aggregation of each nanoparticle for the EPR effect and to reduce the chance of obstruction of blood capillaries. We have previously reported the magnetic properties and heat dissipation of Fe_3O_4 nanoparticles coated with polyethylenimine (PEI), oleic acid, and Pluronic F-127 [3]. The Pluronic-coated Fe_3O_4 nanoparticles, the heat dissipation of which was not related to surrounding viscosity and known as biocompatible material, will be suitable for a heat source of hyperthermia. Pluronic is a water-soluble triblock copolymer composed of a hydrophobic central segment of poly(propylene oxide) (PO) flanked by two hydrophilic segments of poly(ethylene oxide) (EO). Pluronic can be represented as EOa-POb-EOa, where a and b are the number of ethylene oxide and propylene oxide units, respectively. Pluronic F-127 contains 200.45 EO units (a = 100) and 65.17 PO units (b = 65) with a molecular weight

of 12,600 Da [4]. In this study, the efficacy of hyperthermia treatment using Pluronic-coated Fe_3O_4 nanoparticles was evaluated.

2. Experiments

2.1. Surface Coating. Fe_3O_4 nanoparticles (particle size of 20–30 nm) were used as samples (Nanostructured & Amorphous Materials, Inc.). The Fe_3O_4 nanoparticles were dispersed in a solution containing 100 mL of 1 mg/mL oleic acid (Nacalai Tesque) and 25 mL of ammonia solution by ultrasonication. This solution was then heated below the boiling point with vigorous stirring at 1,200 rpm for 90 min. The solution was then washed with ethanol four times by magnetic decantation to remove the excess oleic acid, and the sediment was then dried. The dried powders were redispersed in a solution containing 100 mL of Pluronic F-127 with vigorous stirring at 1,200 rpm for 4 h at room temperature. The solution was purified by centrifugation at 3,000 rpm for 15 min. The supernatant was then centrifuged at 10,000 g for 30 min. Finally, the precipitate was collected.

2.2. Heat Dissipation. The temperature rise of Pluronic-coated Fe_3O_4 nanoparticles was measured by applying an ac magnetic field of 4.0–20 kA/m (50–250 Oe) at a frequency of 210 kHz. The samples were dispersed in water. The weight concentrations of these samples were 3 mg/mL. The temperature rise of each sample was measured by optical fiber thermometer.

2.3. Cytotoxicity. A cytotoxicity study of Pluronic-coated Fe_3O_4 nanoparticles was conducted on human cervical carcinoma cells (HeLa cells). HeLa cells were cultured in Dulbecco's modified eagle medium (DMEM; GIBCO) with 10% fetal bovine serum (Equitec-bio, Inc.) and 1% penicillin streptomycin (GIBCO); they were incubated at 37°C in 5% CO_2 atmosphere. HeLa cells were seeded at a density of 2×10^4 cells/well in 24-well plates and incubated at 37°C in a 5% CO_2 atmosphere. After 24 h of incubation, HeLa cells were exposed to 10–500 μg/mL of each nanoparticle dispersed in the medium. The HeLa cells were observed for 3 days after exposure to the nanoparticles. The medium was removed and the nanoparticles were washed with phosphate-buffered saline (PBS). Then the cells were trypsinized and the number of the living cells was counted using Burker-Turk hemocytometer.

2.4. In Vitro Hyperthermia. HeLa cells were subjected to hyperthermia treatment using Pluronic-coated Fe_3O_4 nanoparticles (20–30 nm). HeLa cells were seeded at a density of 5×10^5 cells/well in 30 mmφ dishes and incubated at 37°C in 5% CO_2 atmosphere. After 24 h of incubation, the HeLa cells were exposed to 500 μg/mL of Pluronic-coated Fe_3O_4 nanoparticles dispersed in a medium. Next, the HeLa cells were exposed to an ac magnetic field of 16 kA/m (200 Oe) and 20 kA/m (250 Oe) at 210 kHz for a period of 15–60 min. After the hyperthermia treatment, the medium containing the magnetic nanoparticles was washed with phosphate-buffered saline (PBS) and fresh medium was added. After 24 h of incubation, the cells were trypsinized and the number of the living and dead cells was counted using the Burker-Turk hemocytometer. The viability of the cells was evaluated by the trypan blue method. The viability was calculated by the following equation:

$$\text{Viability } [\%] = \frac{\text{number of living cells [cells]}}{\text{total number of cells [cells]}}. \quad (1)$$

2.5. Apoptosis. After the hyperthermia treatment, the medium containing magnetic nanoparticles was washed with phosphate-buffered saline (PBS) and a fresh medium was added. After 24 h of incubation, the cells were trypsinized and the number of the cells treated under each condition was fixed at the same density. Then, mitochondrial membrane potential and activation of caspase 3 were measured using the Dual Sensor: MitoCasp (Cell Technology Inc.,) and Caspase-Glo 3/7 Assay (Promega), respectively, according to the manufacturers' protocol. The mitochondrial membrane potential dye contains a cationic mitochondrial dye that accumulates in intact mitochondria to emit red fluorescence. Caspase-Glo 3/7 Assay uses a proluminescent substrate containing a DEVD sequence, which is recognized and activated by caspase 3 and caspase 7, and the luminescence signal is proportional to the net activation of caspase 3 and caspase 7.

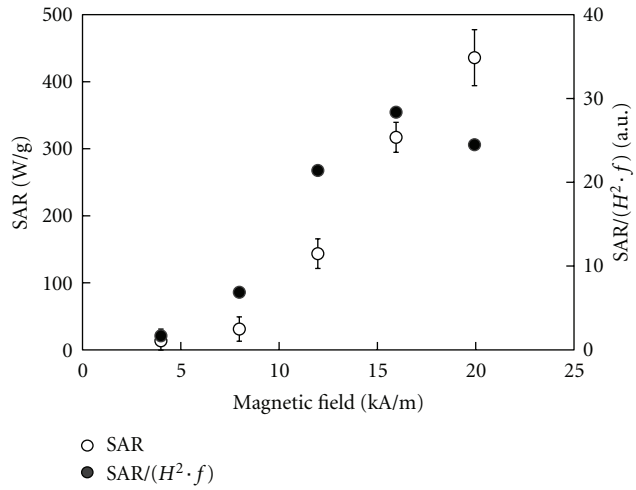

Figure 1: Dependence of magnetic field strength on specific absorption rate (SAR) of Pluronic-coated magnetic nanoparticles (open circles). Energy efficiency of applied magnetic field to generate self-heating (closed circles). The temperature rise was divided by $H^2 \times f$, where H and f are the amplitude and frequency of applied ac magnetic field, respectively. The ac field frequency was 210 kHz, and the amplitude was varied from 4 to 20 kA/m (50 to 250 Oe).

3. Results and Discussion

3.1. Surface Coating. Morphology and hydrodynamic particle size of the Pluronic-coated Fe_3O_4 nanoparticles have been

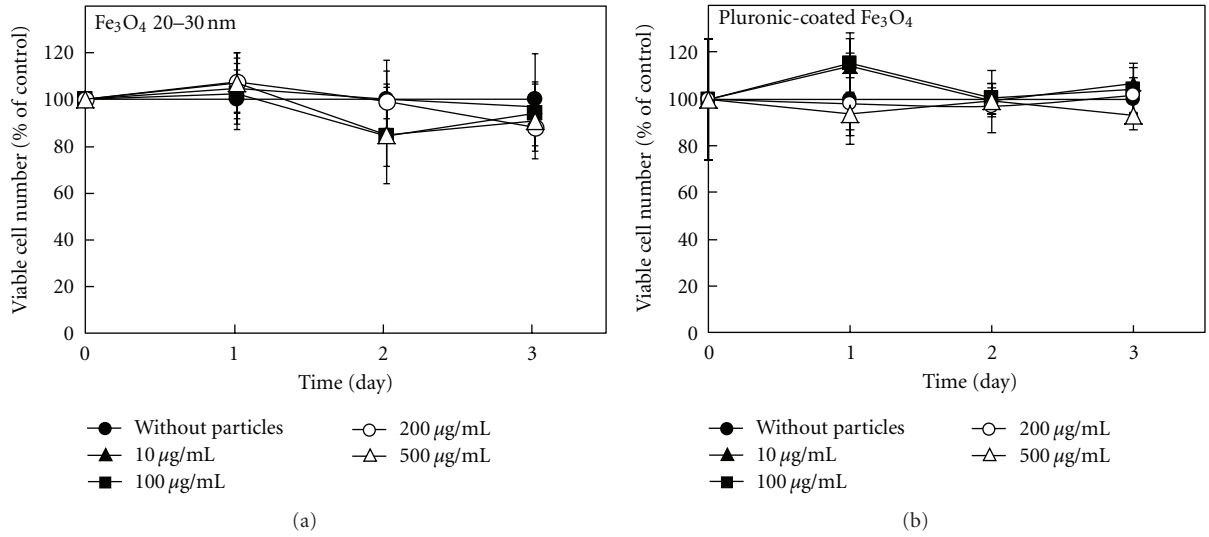

Figure 2: Viable cell number of the HeLa cells treated with Fe_3O_4 nanoparticles and without nanoparticles at concentration of 10–500 μg/mL. (a) uncoated, (b) Pluronic-coated Fe_3O_4 nanoparticles.

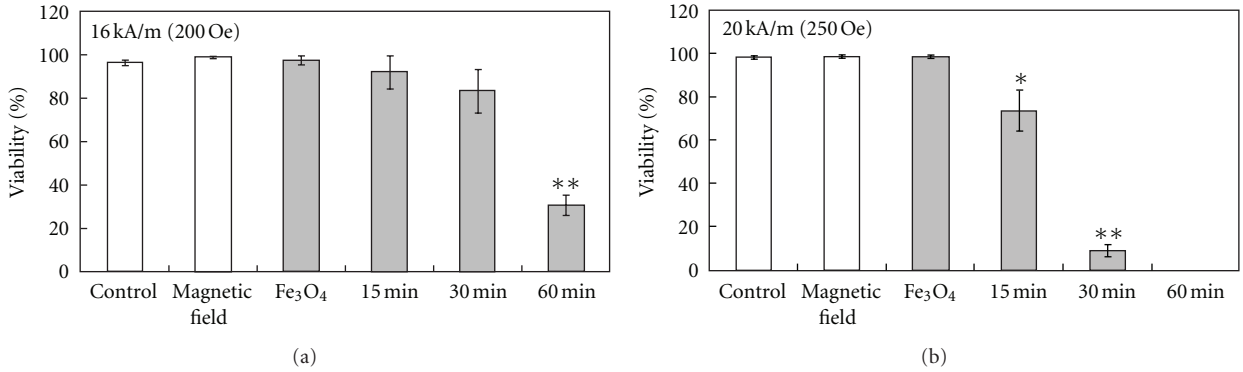

Figure 3: Viability of HeLa cells treated with hyperthermia treatment using the Pluronic-coated Fe_3O_4 nanoparticles at the field strength of (a) 16 kA/m (200 Oe) and (b) 20 kA/m (250 Oe) for 15, 30, and 60 min. $^*P < 0.05$, $^{**}P < 0.01$, $n = 3$.

reported previously [3]. The hydrodynamic particle size of the Pluronic-coated Fe_3O_4 nanoparticles was 181 nm. The uncoated magnetic nanoparticles formed large clusters with hydrodynamic sizes in the order of tens of micrometers.

3.2. Heat Dissipation. Temperature rise of the Pluronic-coated Fe_3O_4 nanoparticles under an ac magnetic field was measured. The magnetic field strength was fixed at 16 kA/m (200 Oe) and 20 kA/m (250 Oe), and frequency was 210 kHz. The temperature reached at 45°C and 51°C within 30 min under ac magnetic fields of 16 kA/m (200 Oe) and 20 kA/m (250 Oe), respectively. Figure 1 shows the specific absorption rate (SAR) of Pluronic-coated Fe_3O_4 nanoparticles and the efficiency of applied energy to generate self-heating. The ability of heat dissipation of magnetic nanoparticles is usually described as the specific absorption rate (SAR). The SAR values (W/g) were calculated by the following equation:

$$\mathrm{SAR} = C\frac{\Delta T}{\Delta t}\frac{1}{m}, \tag{2}$$

where C is the specific heat capacity, m the weight of the sample, and $\Delta T/\Delta t$ the initial slope of the time-dependent temperature rise. The specific heat capacity of the sample is almost that of water $C \approx C_{\mathrm{water}} = 4.18\,\mathrm{Jg}^{-1}\,\mathrm{K}^{-1}$.

The efficiency of applied energy was determined by the temperature rise divided by $H^2 \times f$, where H and f are the amplitude and frequency of applied ac magnetic field. The energy applied to generate a magnetic field is proportional to the product of H^2 and f. Figure 1 shows that the optimum field strength to generate heat was 16 kA/m (200 Oe) at a frequency of 210 kHz for the Pluronic-coated Fe_3O_4 nanoparticles. Therefore, the magnetic field strength of 16 kA/m (200 Oe) will be suitable for hyperthermia treatment using the Pluronic-coated Fe_3O_4 nanoparticles.

3.3. Cytotoxicity. The viable cell number of the HeLa cells exposed to the uncoated and Pluronic-coated Fe_3O_4 nanoparticles is shown in Figure 2. The HeLa cells exposed to the uncoated Fe_3O_4 nanoparticles exhibited a viable cell number of higher than 84%. Fe_3O_4 nanoparticles are widely

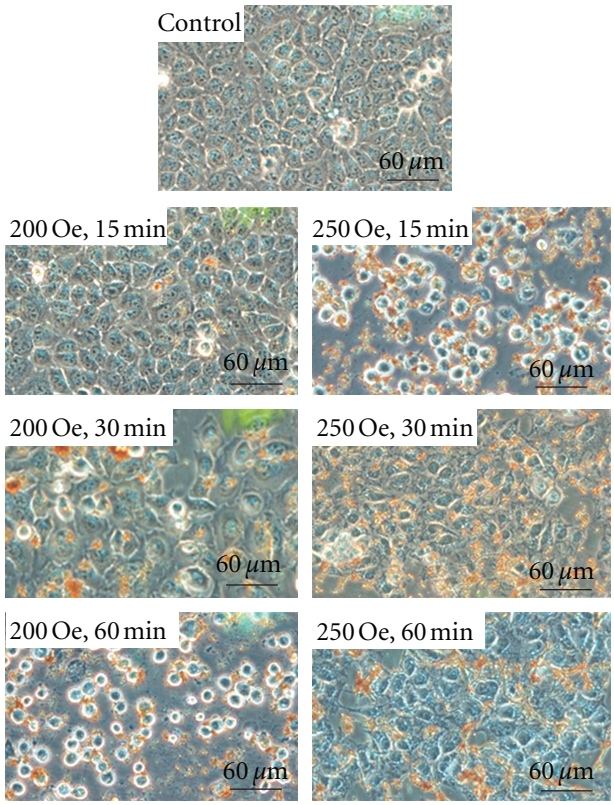

Figure 4: Morphology of HeLa cells treated with hyperthermia treatment using Pluronic-coated Fe_3O_4 nanoparticles at the field strength of 16 kA/m (200 Oe) and 20 kA/m (250 Oe) for 15, 30, and 60 min.

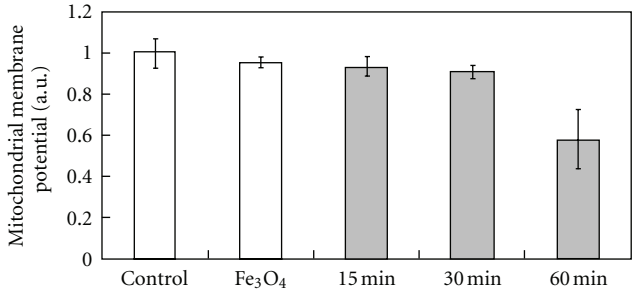

Figure 5: Mitochondrial membrane of the HeLa cells treated with hyperthermia using the Pluronic-coated Fe_3O_4 nanoparticles at the field strength of 16 kA/m (200 Oe) for 15, 30, and 60 min. They are normalized to untreated control cells.

accepted as biocompatible materials, but the cytotoxicity of nanoparticles is not fully understood. The viability reduction of cells exposed to uncoated Fe_3O_4 nanoparticles has been reported, while no cytotoxicity was found with the exposure of Fe_3O_4 nanoparticles coated with biocompatible substances [5, 6]. On the other hand, no significant difference in the cytotoxicity of uncoated nanoparticles between coated Fe_3O_4 nanoparticles has been reported [7]. These differences in cytotoxicity may be due to the use of different cell lines and particle size.

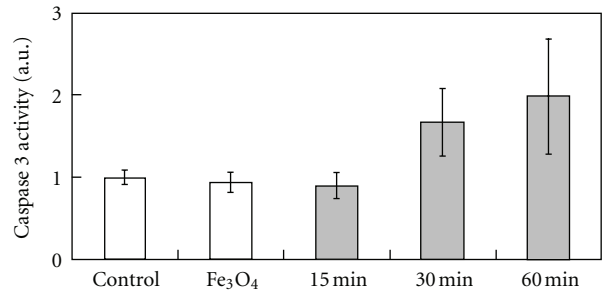

Figure 6: Caspase 3 activity of the HeLa cells treated with hyperthermia using the Pluronic-coated Fe_3O_4 nanoparticles at the field strength of 16 kA/m (200 Oe) for 15, 30, and 60 min. They are normalized to untreated control cells.

No cytotoxic effect was observed for the HeLa cells exposed to the Pluronic-coated Fe_3O_4 nanoparticles even at the concentration of 500 μg/mL. Pluronics have attracted attention for use in drug delivery systems because of their biocompatibility [8, 9] and long blood-circulation time [10, 11]. Our results corresponded to these reports.

3.4. In Vitro Hyperthermia. Viability and morphology of HeLa cells treated with magnetic nanoparticle hyperthermia are shown in Figures 3 and 4, respectively. The viability of HeLa cells treated with hyperthermia at the field strength of 16 kA/m (200 Oe) for 15 min, 30 min, and 60 min was 90%, 83%, and 46%, respectively. Hyperthermia treatment significantly reduced the viability of the HeLa cells. The HeLa cells were observed to shrink with hyperthermia treatment for 30 min and 60 min. Lower viability was observed for the HeLa cells treated at the field strength of 20 kA/m (250 Oe). The viability of HeLa cells treated for 15 min, 30 min, and 60 min was 74%, 9%, and 0%, respectively. HeLa cells exposed to the magnetic field for more than 30 min did not shrink.

3.5. Apoptosis. Mitochondrial membrane potential and caspase 3 activity normalized in untreated control cells are shown in Figures 5 and 6, respectively. In Figure 5, a clear collapse of the mitochondrial membrane potential is observed in the case of hyperthermia treatment for 60 min. Figure 6 shows that caspase 3 activity increased in HeLa cells treated for 30 min and 60 min.

Apoptosis can be triggered by various stimuli such as UV radiation, chemotherapy, and heat. An apoptotic cell changes its morphology. The cell shrinks, its chromatin condenses, and fragmentation of nucleus occurs [12]. There are two main apoptotic pathways: the extrinsic (death receptor) pathway and the intrinsic (mitochondrial) pathway. Mitochondrial permeability transition is an important step in the induction of the intrinsic apoptosis pathway. During this process, the mitochondrial membrane potential collapses. Activation of caspase 3 is a downstream effector of the apoptotic pathway. These results indicate that hyperthermia treatment using the Pluronic-coated Fe_3O_4 nanoparticles mediates apoptosis through the mitochondrial pathway.

Hyperthermia treatment is suitable for inducing both necrosis and apoptosis depending on the temperature. Cells heated at temperatures in the range of 41°C to 47°C begin to show signs of apoptosis, whereas increasing temperatures (above 50°C) are associated with decreased apoptosis and increased necrosis [13]. Our results confirm that apoptosis is induced by thermal treatment at 45°C. HeLa cells heated at 45°C showed shrinkage, although those heated at 51°C did not show shrinkage (Figure 4). Hyperthermia treatment at a higher temperature might induce necrosis instead of apoptosis.

4. Conclusion

The temperature rise under ac magnetic field, cytotoxicity, and *in vitro* hyperthermia effect of Pluronic-coated Fe_3O_4 nanoparticles was evaluated in this study. Appropriate temperature rise was achieved under an ac magnetic field of 16 kA/m (200 Oe) and 20 kA/m (250 Oe) at 210 kHz. No cytotoxic effect was observed on the HeLa cells. *In vitro* hyperthermia treatment using Pluronic-coated Fe_3O_4 nanoparticles significantly reduced the viability of HeLa cancer cells. The collapse of mitochondria membrane potential and caspase 3 activity were observed. Hyperthermia using the Pluronic-coated Fe_3O_4 nanoparticles induced cell death related to apoptosis through mitochondrial pathway.

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References

[1] W. C. Dewey, L. E. Hopwood, S. A. Sapareto, and L. E. Gerweck, "Cellular responses to combinations of hyperthermia and radiation," *Radiology*, vol. 123, no. 2, pp. 463–474, 1977.

[2] M. Arruebo, R. Fernandez-Pacheco, M. R. Ibarra, and J. Santamaria, "Magnetic nanoparticles for drug delivery," *Nanotoday*, vol. 2, pp. 22–32, 2007.

[3] A. Tomitaka, K. Ueda, T. Yamada, and Y. Takemura, "Heat dissipation and magnetic properties of surface-coated Fe_3O_4 nanoparticlesfor biomedical applications," *Journal of Magnetism and Magnetic Materials*. In press.

[4] T. K. Jain, S. P. Foy, B. Erokwu, S. Dimitrijevic, C. A. Flask, and V. Labhasetwar, "Magnetic resonance imaging of multifunctional pluronic stabilized iron-oxide nanoparticles in tumor-bearing mice," *Biomaterials*, vol. 30, no. 35, pp. 6748–6756, 2009.

[5] A. K. Gupta and M. Gupta, "Cytotoxicity suppression and cellular uptake enhancement of surface modified magnetic nanoparticles," *Biomaterials*, vol. 26, no. 13, pp. 1565–1573, 2005.

[6] M. Kim, J. Jung, J. Lee, K. Na, S. Park, and J. Hyun, "Amphiphilic comblike polymers enhance the colloidal stability of Fe_3O_4 nanoparticles," *Colloids and Surfaces B*, vol. 76, no. 1, pp. 236–240, 2010.

[7] D. H. Kim, K. N. Kim, K. M. Kim, and Y. K. Lee, "Targeting to carcinoma cells with chitosan- and starch-coated magnetic nanoparticles for magnetic hyperthermia," *Journal of Biomedical Materials Research. Part A*, vol. 88, no. 1, pp. 1–11, 2009.

[8] J. Qin, S. Laurent, Y. S. Jo et al., "A high-performance magnetic resonance imaging T_2 contrast agent," *Advanced Materials*, vol. 19, no. 14, pp. 1874–1878, 2007.

[9] J. Y. Kim, W. I. Choi, Y. H. Kim et al., "In-vivo tumor targeting of pluronic-based nano-carriers," *Journal of Controlled Release*, vol. 147, no. 1, pp. 109–117, 2010.

[10] M. A. Morales, T. K. Jain, V. Labhasetwar, and D. L. Leslie-Pelecky, "Magnetic studies of iron oxide nanoparticles coated with oleic acid and Pluronic block copolymer," *Journal of Applied Physics*, vol. 97, no. 10, Article ID 10Q905, pp. 1–3, 2005.

[11] T. K. Jain, J. Richey, M. Strand, D. L. Leslie-Pelecky, C. A. Flask, and V. Labhasetwar, "Magnetic nanoparticles with dual functional properties: drug delivery and magnetic resonance imaging," *Biomaterials*, vol. 29, no. 29, pp. 4012–4021, 2008.

[12] A. Gewies, "Introduction to Apoptosis," *ApoReview*, pp. 1–26, 2003.

[13] P. Cherukuri, E. S. Glazer, and S. A. Curley, "Targeted hyperthermia using metal nanoparticles," *Advanced Drug Delivery Reviews*, vol. 62, no. 3, pp. 339–345, 2010.