Kaleidoscope

Volume 10 Article 28

August 2012

Biocomaptibility Analysis and Cancer Therapy Applications of Core-shell Composite Magnetic Nanoparticles

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1. Abstract

Composite magnetic nanoparticles provide the opportunity for direct tumor targeting, thermal therapy, and controlled drug release. However, the magnetic nanoparticles must be biocompatible in order to be used in cancer therapy applications. By coating the magnetic nanoparticles with hydrogels, we can decrease their toxicity while maintaining their property of heating in alternating magnetic fields. In this study, it was found that when the concentration of nanoparticles was increased, cell viability decreased. In the presented studies, the heat generated by these nanoparticle systems in an alternating magnetic field was not enough to induce cell death but it may be possible. For future studies, higher concentrations of particles and/or longer exposure times are recommended to induce cell death.

2. Introduction

2.1 Hyperthermia

Cancer is the second leading cause of death in the United States; therefore cancer therapy research is gaining importance among today's researchers. Even though vast advancements in the treatment of cancer have been made, there are still many types of cancer that have very low survival rates and poor treatment success rates. Thus, novel therapies based on nanotechnologies are being pursued due their unique properties and potential to improve treatments. For example, composite magnetic nanoparticles provide the opportunity for direct tumor targeting, thermal therapy, and controlled drug release as shown in Figure 1.

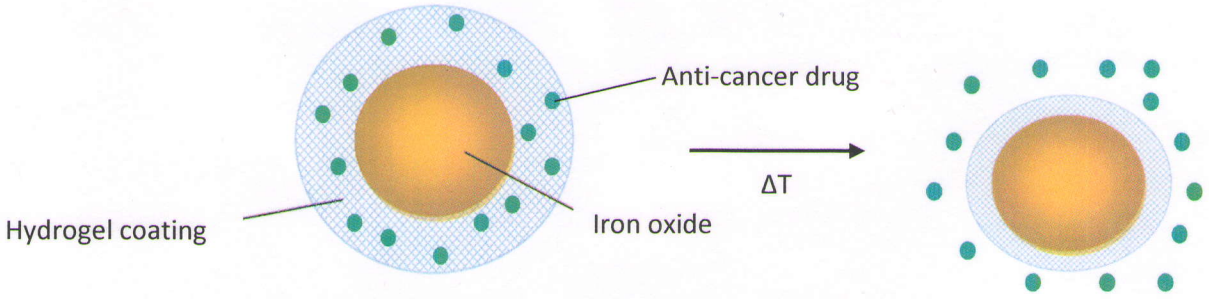


Figure 1. Iron oxide coated in a hydrogel provides the opportunity for controlled drug release.

Hyperthermia, the heating of cancer tissue to between 41 and 45°C, has previously been proven to induce cell death and when used in conjunction with other cancer treatments, such as chemotherapy and radiation therapy, has shown improved efficacy [1]. However, there are many setbacks to using hyperthermia as the main treatment of cancer. The most common forms of hyperthermia today are whole body hyperthermia and regional hyperthermia. Whole body hyperthermia utilizes heating

blankets or water baths in order to elevate the temperature of the body. Regional hyperthermia removes blood surrounding the tumor site, heats the blood, and then pumps it back into the body and through the tumor site. Many patients experience discomfort as well as burns near the tumor site, and it is difficult to have homogeneous distribution of heat across the tumor site [2]. Due to the lack of ability to localize hyperthermia to the cancer tissue, healthy tissue surrounding the cancer tissue is often damaged. In the presence of an alternating magnetic field, composite magnetic nanoparticles present the opportunity for localized hyperthermia within cancer tissue due to their superparamagnetic properties [3]. As the magnetic field alternates, heat can be generated through Neel and Brownian relaxation. In high enough concentrations of nanoparticles, the heat generated could induce cell death.

2.2 Tumor targeting magnetic nanoparticles

Composite magnetic nanoparticles also provide the opportunity for direct tumor targeting. These composite magnetic nanoparticles, which consist of a magnetic core and a polymer coating loaded with anti-cancer drug, can be injected into the tumor or directed to the tumor site through the use of an external magnet. The vasculature of cancerous tissue is deformed and irregular which allows the nanoparticles systems to permeate through the tissue and remain there due to the cancer tissue's inability to remove waste, otherwise known as the enhanced permeability and retention effect (EPR) [4, 5]. Uptake of the nanoparticles into the cells has also been observed. Therefore, daughter cells of the particle containing parent cells should contain up to 50% of the original particle amount [3]. This provides the opportunity for multiple heating sessions and an increased probability of inducing cell death on descendants of particle containing cells. However, EPR targeting methods do not require particle uptake. Localized heating of the tumor tissue is can be enough to induce cell death.

2.3 Stealth and temperature responsive polymers

One of the main difficulties of the composite magnetic nanoparticles is their biocompatibility. The healthy tissue surrounding the tumor will be exposed to the composite magnetic nanoparticles so the particles should not induce cell death unless in the presence of an alternating magnetic field. By coating the iron oxide nanoparticles with a biocompatible polymer, their cytotoxicity is decreased [6], agglomeration of particles is prevented and circulation time increases [7]. PEG400DMA is a stealth polymer used to decrease the toxicity of the iron oxide nanoparticle, but it does not provide the opportunity for drug loading and controlled drug release.

Poly(N-isopropylacrylamide) (PNIPAAm) is a temperature responsive polymer with a lower critical solution temperature (LCST) of about 32 °C in water. Below this LCST, the polymer is hydrated and hydrophilic. Above the LCST, PNIPAAm is hydrophobic and collapses on itself. By crosslinking PNIPAAM with other polymers, the LCST can be increased to above body temperature. The behavior of PNIPAAm and other temperature responsive polymers allows for anti-cancer drug to be loaded into the polymer when it is below the LCST, and then released during the sudden collapse above the LCST [5]. Coating magnetic nanoparticles with temperature responsive polymers would allow for anti-cancer drug to be delivered directly to the tumor site [8]. Heating the iron oxide core by an external alternating magnetic field would also heat the temperature responsive polymer coating. If the polymer coating was heated to a temperature above its LCST, the polymer would collapse and release the drug into the cancerous tissue [9]. Therefore, coating the iron oxide nanoparticles with polymer does not only decrease the toxicity of the particles, but it also provides a method for direct drug delivery. In this study, the stealth polymer poly (ethylene glycol) 400 dimethylacrylate (PEG400DMA) was used to a decrease in the toxicity of the nanoparticles, but plans for future experiments include testing the toxicity of nanoparticles coated with temperature responsive polymer systems.

The overall objective of this study was to synthesize and characterize composite magnetic nanoparticles for use as hyperthermia and chemotherapeutic-based treatments of cancer. More specifically, the goals were to prepare iron oxide nanoparticles coated with citric acid and PEG400DMA, compare the viability of NIH 3T3 fibroblasts and A549 lung cancer cells exposed to PEG400DMA coated iron oxide nanoparticles, and to show increased nanoparticle toxicity in the presence of an alternating magnetic field.

3. Materials and Methods

3.1 Materials

The iron (II) chloride tetrahydrate and iron (III) chloride hexahydrate were purchased from Sigma-Aldrich (St. Louis, MO). Citric acid and ammonium hydride were obtained from Sigma-Aldrich. The 2,2-Dipyridyl (Bpy)and copper (I) bromide (CuBr)that were used in polymerization were also purchased from Sigma-Aldrich. The initiator, 3-bromopropyl trimethoxysilane (BPTS), was purchased from Gelest (Morrisville, PA). Poly (elthylene glycol) 400 dimethylacrylate (PEG400DMA) and poly(N-isopropylacrylamide) (PNIPAAm), which were monomers used to coat the iron oxide nanoparticles, were purchased from Polysciences, Inc. (Warrington, PA).

3.2 Iron oxide nanoparticles preparation

Nanoparticle systems were created using a one-pot co-precipitation method. Iron (III) chloride and iron (II) chloride were combined in a 2:1 ratio respectively with NH_4OH in a three neck flask and vented with nitrogen gas. The temperature of the system was set to 85°C, and the speed of the impeller was set to 300 rpm. After the system reached the specified temperature, 1.68 grams of 2M citric acid was added. The reaction was carried out for one hour, and the nanoparticles were separated by magnetic decanting, washed with ethanol, and vacuum dried.

In order to coat the iron oxide nanoparticles with polymer, the nanoparticles were first coated with an initiator, 3-bromopropyl trimethoxysilane (BPTS), via a ligand exchange with the citric acid. BPTS puts bromine on the outside of the nanoparticles which becomes a radical initiator when heated. This creates "seeds" for the polymer coating. BPTS and the iron oxide ratio are added to a three-neck flask in a 2.5:1 ratio, respectively. The system was purged with nitrogen gas and then sealed. The reaction was run for 24 hours at room temperature with the impeller rotating at 300 rpm.

3.3 Polymerization techniques

In order to coat the nanoparticles in polymer, the steps to coat the nanoparticles with BPTS initiator were first completed. The nanoparticles were then added to a three neck flask with Bpy and CuBr, which were each diluted with ethanol. The system was set to run at 60°C, 300 rpm and for 6 hours. When the system reached 60°C, 5 grams of PEG400DMA was added. After 6 hours, then PEG400DMA coated nanoparticles were washed in ethanol and vacuum dried. This same polymerization technique was used to synthesize the pNIPAAmPNIPAAm-PEG400DMA 15 weight% coated iron oxide nanoparticles used in the NIH 3T3 cytotoxicity study as well. The structures of the polymers and initiator are shown in Figure 2.

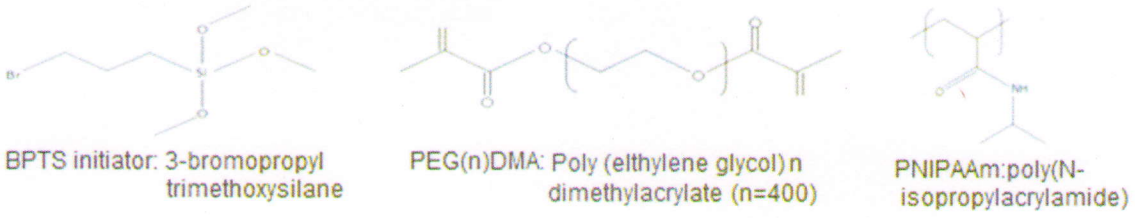


Figure 2. Structures of BPTS initiator, PEG400DMA and PNIPAAm.

3.4 Characterization

Fourier Transform Infrared (FTIR) Spectra. Attenuated total reflectance FTIR (ATR-FTIR) was used to confirm the existence of specific functional groups on the surface of the iron oxide nanoparticles using a Varian 7000e FTIR spectrophotometer. The spectrum was obtained from 800 to 4000cm^{-1} at a resolution of 8 cm^{-1} for 32 scans. Dried composite magnetic nanoparticles samples were placed on the diamond ATR crystal in order to collect the spectrum.

*Thermogravimetric Analysis (TGA).*Weight percent measurements were obtained from a Q500 TA instrument. About 10mg of a sample was used at a heating rate of 20 °C/min. The sample was heated to 120°C and remained there for 10 minutes in order to evaporate any remaining water. The process was completed under nitrogen flow, and the TGA curves were normalized to 120°C.

3.5 Controlled heating

Iron oxide nanoparticles coated with PEG400DMA and NIAAm-PEG400DMA 15wt% were suspended in A549 lung cancer media and exposed to an alternating magnetic field. Each of the nanoparticle systems was added to the media at concentrations of 100µg/ml and 500µg/ml, which are the same concentrations the A549 lung cancer cells were exposed to in the cytotoxicity study. A one milliliter sample of the nanoparticles solution was placed into a 2ml centrifuge tube, which was then placed inside Teflon tube inside the coil, and a fiber optic temperature probe (Luxtron FOT Lab Kit) was used to record the temperature of the nanoparticle solutions. The alternating magnetic field was turned on 30 seconds after the start time, and left on for 10 minutes. This procedure was completed three times for each concentration of the two nanoparticles systems.

3.6 Cytotoxicity

NIH 3T3 murine fibroblasts at passages 6-8 were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10 % v/v calf bovine serum, 10 µg/ml Fungizone (Invitrogen), and 2 µg/ml Penicillin-Streptomycin-Glutamine (ATCC) in an incubator at 37°C and 5% CO_2. The fibroblasts were then seeded into 12-well plates at 5000cells/cm^2 and incubated for 24 hours. Cytotoxicity studies of A549 lung cancer cells were also completed. For these studies, A549 lung cancer cells at passages 3 and 4 were cultured in F-12K medium supplemented with 10% fetal bovine serum, 10 µg/ml Fungizone, and 2 µg/ml Penicillin-Streptomycin-Glutamine and seeded into 12-well plates at 6000cells/cm^2 and incubated for 24 hours. After 24 hours, the cells were exposed to the various concentrations of nanoparticles. Nanoparticles were added to cell media at concentrations of 100µ/ml and 500µ/ml. The nanoparticle

solutions were vortexed and sonicated to dissolve the nanoparticles into the media. After removal of the spent media, one milliliter of the nanoparticles solutions was added to each well containing the cells. The control, which was exposed to no nanoparticles, also had a change of media. The well plates were then put back in the incubator for 24 and 48 hours.

There were two methods of analyzing cytotoxicity. The first technique was completed using the cellometer (Nexcelom Bioscience, Lawrence, MA). After 24 and 48 hours, the cells were removed from the incubator, and the media was removed and placed into respective centrifuge tubes. The wells were then washed with 125µl of trypsin, and then incubated with 250µl of trypsin to detach the cells from the well plate. After incubating for seven minutes, the cells and trypsin were removed from the well plates and placed into the centrifuge tubes. A 2ml DPBS wash was then completed to insure all the cells were being collected. The cell solutions were centrifuged on a setting of three for three minutes. The supernatant was then removed from the tubes, and the cells were re-suspended in 0.5 ml of live/dead assay solution. The centrifuge tubes were then placed back into the incubator for 20 minutes to allow the cells to stain. After incubation, three 20µl samples were taken from each centrifuge tube and analyzed using the cellometer. The cellometer provides the cell viability of three 20µl samples, which are then averaged to get the cell viability for that trial. In addition to cell viability, the cellometer also gives the cell count, cell concentration, and mean size of the cells.

The second analyzing method used in the cytotoxicity studies involved a fluorescent microscope (Nikon Eclipse LV 100, Melville, NY) and NIS-Elements BR 3.0 imaging software. After 24 and 48 hours, the cells were removed from the incubator, and the media was removed and discarded. The 2ml DPBS wash was then completed to remove all remaining media. After discarding the DPBS wash, the live/dead assay solution was added at 0.5ml per well. The cells were then returned to the incubator for 20 minutes before imaging. Five images (live and dead) were taken of each well. The number of live and dead cells in each image were then counted using NIS-Elements BR 3.0 imaging software and averaged to find the average cell viability of each well.

3.7 Hyperthermia Demonstration

A hyperthermia demonstration was completed on A549 lung cancer cells. The cells were cultured in F-12K media and seeded into 35mm dishes at 6000 cells/cm^2. After seeding for 24 hours, the cells were exposed to the nanoparticles solutions. For this study, cells were exposed to citric acid coated iron oxide nanoparticles, and PEG400DMA coated iron oxide nanoparticles. Two concentrations (100µg/ml and 500µg/ml) of each system were studied. The first control was cells not exposed to particles, but

exposed to the alternating magnetic field, and the second control was cells which were neither exposed to nanoparticles or the alternating magnetic field. After exposure to the nanoparticles solutions, the cells were placed in the incubator for three hours. The cells were then exposed to the alternating magnetic field. The 35mm dishes were placed on the stage, centered on the coil, and exposed to the alternating magnetic field for 10 minutes. After termination of the alternating magnetic field, the dish was removed from the stage and placed back into the incubator for two hours. Cells were then stained with Calcien AM and ethidium homodimer-1 so the live cells are stained green and the dead cells are stained red. Each dish was then imaged using the Nikon Eclipse LV100 fluorescent microscope and NIS-Elements BR 3.0 imaging software.

4. Results and Discussion

4.1 Characterization

The FTIR results for iron oxide nanoparticles coated in PEG400DMA and NIPAAm-PEG400DMA are shown in Figures 3 and 4 respectively. In Figure 3, the line drawn at 1715 cm^{-1} indicates the presence of a C=O and the line drawn at 1105 cm^{-1} locates the valley corresponding to the C-O-C bonds. In Figure 4, the lines drawn at 1656 cm^{-1} and 1620cm^{-1} correspond to the peaks which represent the C=O stretch and 1540 cm^{-1} locates the valley which corresponds to N-H bending. Therefore, the presence of PEG400DMA and NIPAAm-PEG400DMA 15wt% is confirmed due to the presence of these peaks and valleys.

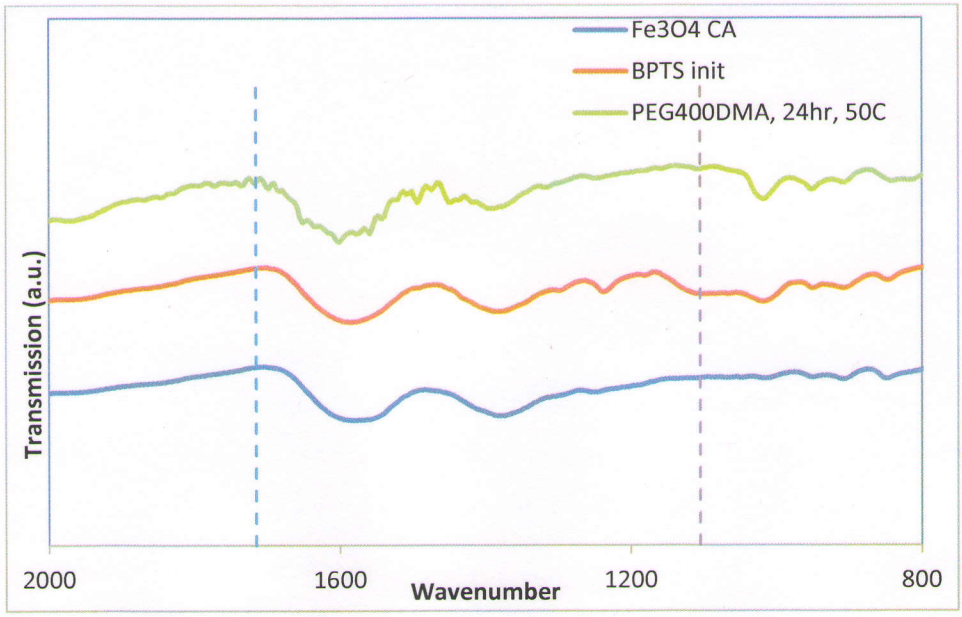


Figure 3. FTIR spectra of citric acid coated iron oxide, BPTS initiator, and functionalization with PEG400DMA.

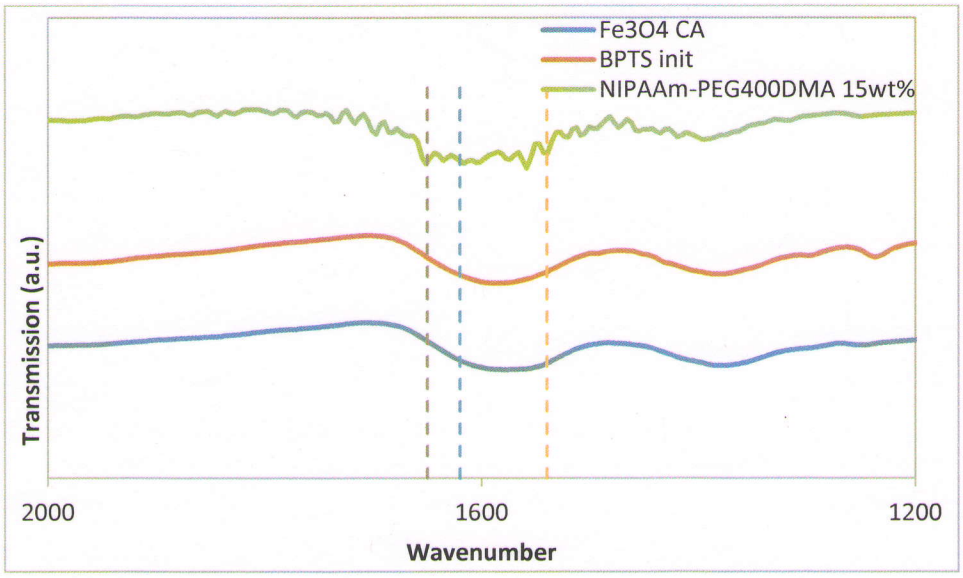


Figure 4. FTIR spectra of citric acid coated iron oxide, BPTS initiator, and functionalization with NIPAAm-PEG400DMA 15wt%

Thermogravimetric analysis can also be used to confirm the presence of a polymer coating on the iron oxide nanoparticles and quantify the weight percent of polymer, but it also confirms the theory that coating the iron oxide nanoparticles with polymer increases their stability. The citric acid coating begins to degrade at a lower temperature than the PEG400DMA coating as displayed in Figure 5. The rate of degradation is also much higher for citric acid than PEG400DMA, which further confirms an increase in stability by coating the nanoparticles with polymer. The final weight of the nanoparticles coated with citric acid is also greater than the final weight of the iron oxide nanoparticles coated in PEG400DMA, indicating a greater coating of polymer than citric acid. In the PEG400DMA coated iron oxide nanoparticles, approximately 12% of the nanoparticle is polymer, and the other 88% is the iron oxide core.

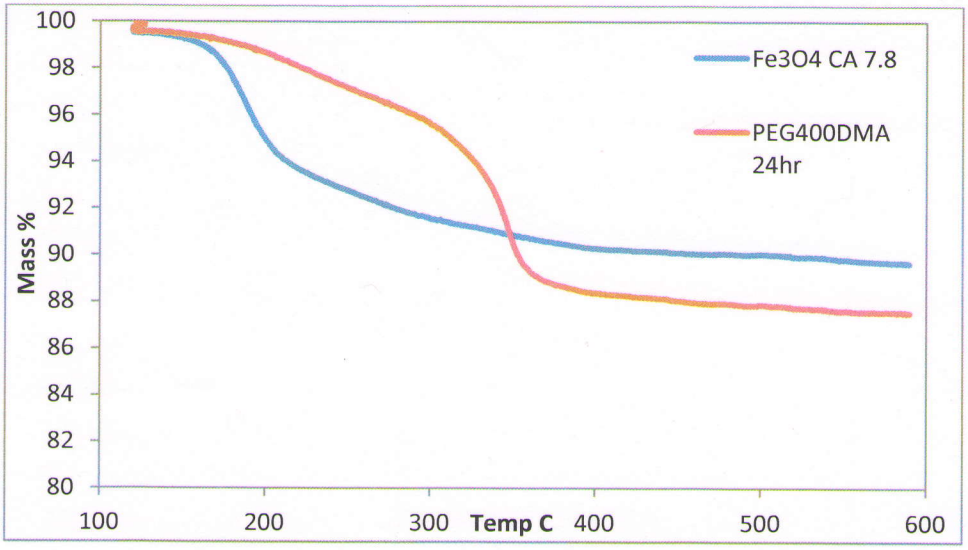


Figure 5. TGA of citric acid coated iron oxide nanoparticles and PEG400DMA, BPTS initiator, coated iron oxide nanoparticles.

The AMF heating capabilities of the composite magnetic nanoparticles were determined using alternating magnetic field heating. The particles were suspended in F-12K A549 lung cancer cell medium, at various concentrations. As the concentration of nanoparticles in the cell medium increased, the amount of heat generated during exposure to an alternating magnetic field also increased. It was also observed that the nanoparticles coated in NIPAAm-PEG400DMA 15wt% were heated to greater temperatures than those coated in PEG400DMA at both concentrations. Since the nanoparticles do generate heat in the presence of an alternating magnetic field, as shown in Figure 6, this presents the question of whether the heat generated is enough to be used as hyperthermia to induce cell death.

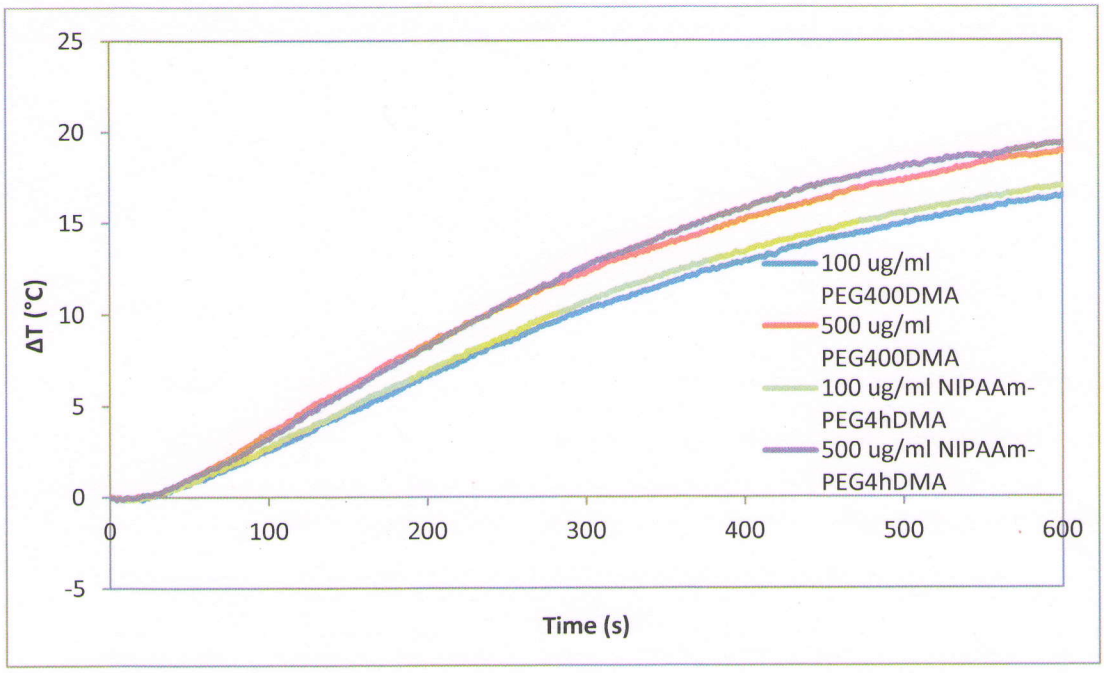


Figure 6. Heating profiles of composite magnetic nanoparticles in F-12K medium in an alternating magnetic field strength of 59.5kA/m.

4.2 Cytotoxicity of composite magnetic nanoparticles

NIH 3T3 fibroblasts model a common cell type found in the body. Cytotoxicity studies were completed on this cell line to model how composite magnetic nanoparticles interact with and effect non-cancerous cells. NIH 3T3 were exposed to four different nanoparticles systems: Fe_3O_4, Fe_3O_4 coated with citric acid, PEG400DMA coated iron oxide, and NIPAAm-PEG400DMA 15wt% coated iron oxide. Three concentrations (100µg/ml, 500µg/ml and 1mg/ml) of each nanoparticles system were used. Cell viabilities were calculated using microscope imaging. Five images were taken per well. The live and dead cells in each image were counted and averaged with the other images of that well. Then, the three wells of the same nanoparticle system and concentration were averaged to get an overall cell viability.

Figure 7 shows that the cell viabilities of the NIH 3T3 fibroblasts exposed to the 100µg/ml nanoparticles solutions were not significantly different from the control (not exposed to nanoparticles) cell viability. The 500µg/ml nanoparticles solutions proved to be more toxic to the cells than the 100µg/ml concentration. Iron oxide without coating and PEG400DMA coated iron oxide were the only two nanoparticles systems with not significantly different cell viabilities from the control for either 48 or 24 hours. The 1mg/ml concentration appears to be very toxic due to all nanoparticle systems, except for

PEG400DMA coated, to have a significant difference in cell viability from the control. This trend of increasing nanoparticles concentration, decreasing cell viability is to be expected.

Within each concentration of nanoparticles, it was expected that the polymer coated nanoparticles would increase cell viability when compared to the non-coated iron oxide and citric acid coated iron oxide. Although in many cases the viability of the cells exposed to polymer coated nanoparticles was greater than the others, it was not significant, as shown in Table 1. The p-values comparing the non-coated and citric acid coated iron oxide core nanoparticles to the polymer coated nanoparticles were greater than 0.05, not indicating a significant difference. However, there was a significant difference between increasing concentrations of the same nanoparticles systems. Therefore, we can say with confidence that increasing the nanoparticles concentration does decrease cell viability.

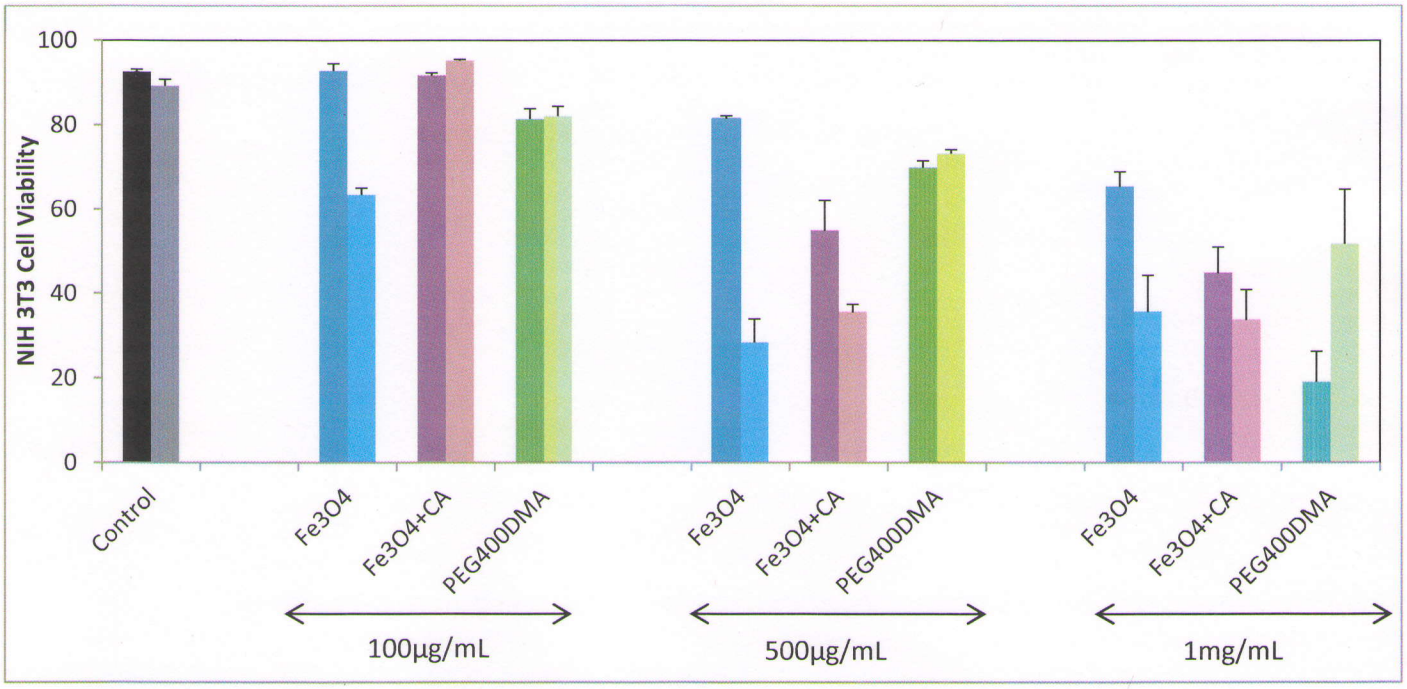


Figure 7. NIH 3T3 fibroblast cell viability after exposure to Fe3O4, Fe3O4+CA and PEG400DMA nanoparticles. Dark bars represent 24 hours and light bars represent 48 hours. The error is reported as standard error.

Statistical Analysis of NIH 3T3 Viabilities			
	100 ug/ml	500 ug/ml	1 mg/ml
Fe3O4 (24 hours)	p>0.05	p>0.05	p<0.01
Fe3O4 (48 hours)	p>0.05	p<0.01	p<0.01
Fe3O4 + Citric Acid (24 hours)	p>0.05	p<0.001	p<0.001
Fe3O4 + Citric Acid (48 hours)	p>0.05	p<0.01	p<0.01
PEG400DMA (24 hours)	p>0.05	p<0.05	p<0.001
PEG400DMA (48 hours)	p>0.05	p>0.05	p>0.05

Table 1. Statistical analysis of NIH 3T3 fibroblast viabilities. This table compares the viabilities of the control cells to the cells which have been exposed to nanoparticle systems. A p-value greater than 0.5 (shaded in green) indicates no significant difference between the control viability and the nanoparticle system viability. A p-value less than 0.5 (shaded in red) indicates a significant difference in cell viability between the control and the nanoparticle system, therefore, they could be considered toxic.

Cytotoxicity studies with A549 lung cancer cells were also completed to determine how the composite magnetic nanoparticles interact with cancer cells. The cells were cultured as stated above, and exposed to two nanoparticle systems and two concentrations of each system. Cell viabilities were calculated using the cellometer method outlined in the materials and methods section.

As with the NIH 3T3 fibroblasts, the 100µg/ml nanoparticle solutions do not significantly decrease cell viability as shown in Figure 8. Only the 100µ/ml PEG400DMA became toxic after 48 hours. Not experiencing a significant decrease in the viability of cells exposed to the 100µg/ml concentrations of the nanoparticle systems, could indicate biocompatibility at this concentration. However, when the concentration is increased to 500µg/ml all the nanoparticle systems caused a decrease in cell viability, except when exposed to PEG400DMA for only 24 hours.

A549 lung cancer cells did not experience a significant drop in viability when the concentration of the same nanoparticle system was increased. For example, Table 2 does not show a statistical difference between the viabilities of cells exposed to 100µg/ml Fe_3O_4+CA and does show a statistical significance to those exposed to 500µg/ml Fe_3O_4+CA after 24 hours when compared to the control.

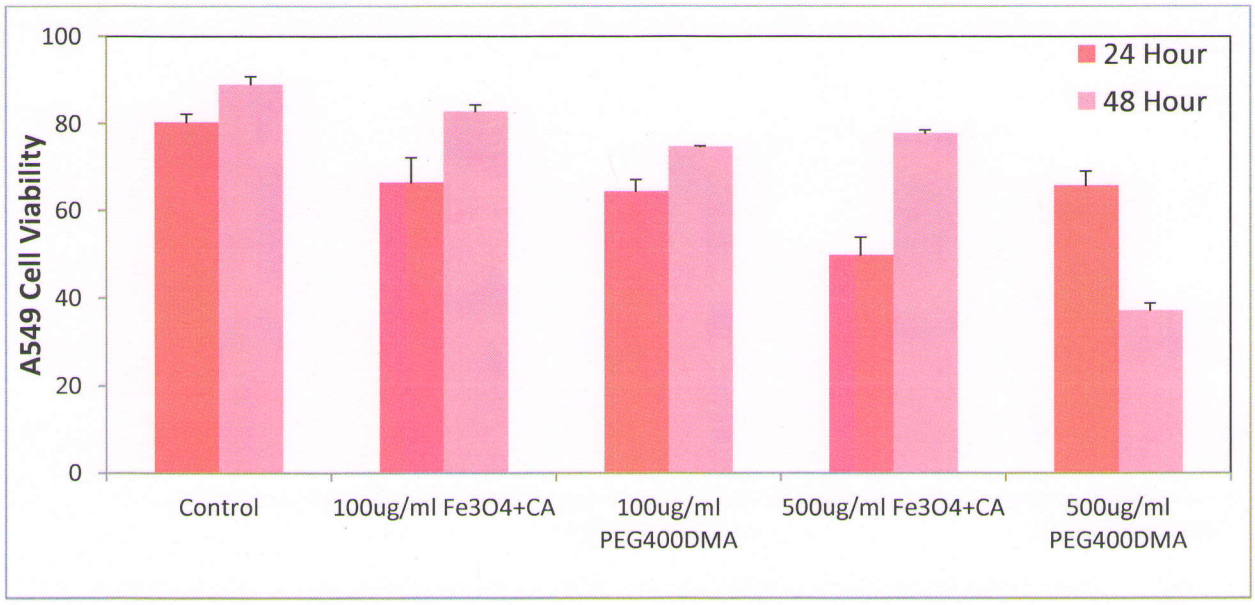
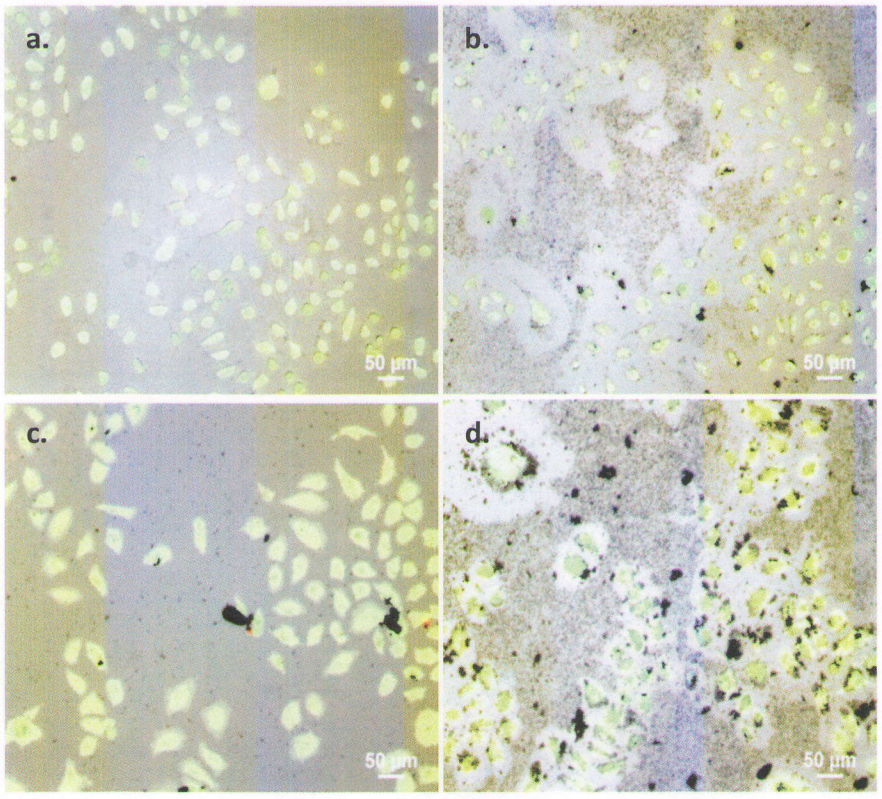


Figure 8. A549 lung cancer cell viabilities 24 and 48 hours after exposure to iron oxide coated in citric acid and PEG400DMA coated iron oxide nanoparticles. Error is reported as standard error.



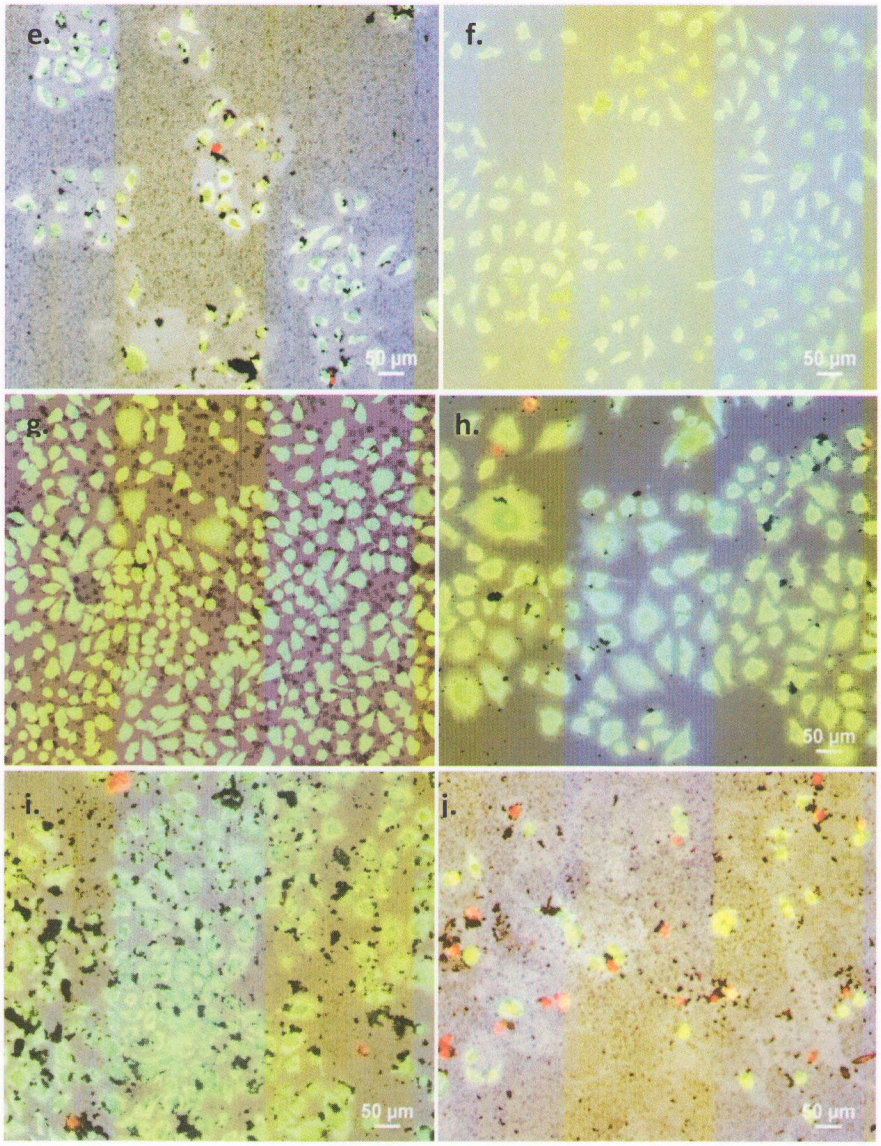


Figure 9. Images a-e are images taken 24 hours after A549lung cancer cells were exposed to the nanoparticles and images f-j are images taken 48 hours after nanoparticle exposure. Cells were exposed to a/f.) no particles b/g.) 100µg/ml Fe3O4 coated with citric acid c/h.) 100µg/ml PEG400DMA coated iron oxide d/i.) 500µg/ml Fe3O4 coated with citric acid e/j.) 500µg/ml PEG400DMA coated iron oxide.

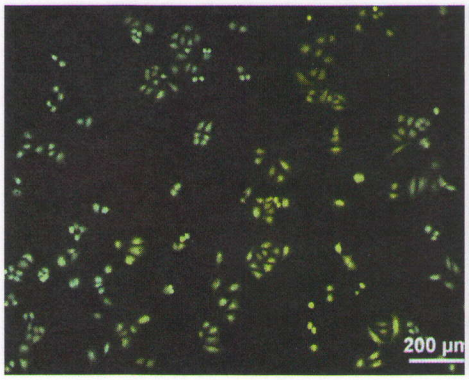
Statistical Analysis of A549 lung cancer viabilities		
	100 ug/ml	500 ug/ml
Fe3O4 + Citric Acid (24 hours)	p>0.05	p<0.01
Fe3O4 + Citric Acid (48 hours)	p>0.05	p<0.01
PEG400DMA (24 hours)	p>0.05	p>0.05
PEG400DMA (48 hours)	p<0.001	p<0.001

Table 2. Statstical analysis of A549 lung cancer cells. A p-value greater than 0.5 (shaded in green) indicates no statistical difference between the control viability and the nanoparticle system. A p-value

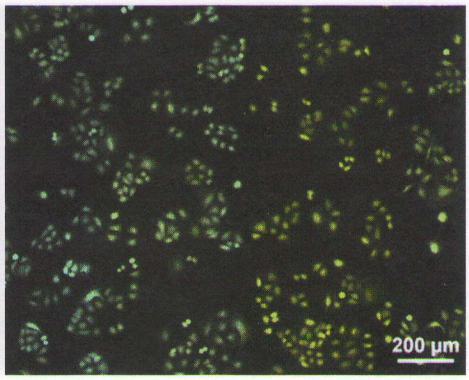
less than 0.5 (shaded in red) indicates a significant difference between the control viability and the nanoparticle system, therefore, they could be considered toxic.

4.3 Hyperthermia Demonstration

A549 lung cancer cells which had been exposed to composite magnetic nanoparticles were also exposed to an alternating magnetic field, in hopes of proving that the heat generated by the nanoparticles is enough to induce cell death. Figure 5 showed that in the presence of an alternating magnetic field, the magnetic nanoparticles do generate heat. However, when the cells and nanoparticles were exposed to the same alternating magnetic field, the heat generated was not enough to induce cell death. The images in Figure 10 were taken at the center of the 35 mm dish. The cells in these images are alive with minimal dead cells. Had the heat generated by the particles been enough to induce cell death, it would have been expected to see a majority of dead cell at the center of the dish. It is possible to increase the heat generated by increasing the nanoparticle concentrations, however, this could also decrease cell viability in cells not exposed to an alternating magnetic field. Future experiments will be completed to determine the optimum concentration of nanoparticles that will both generate enough heat to induce cell death and allow for high cell viability when not exposed to an alternating magnetic field.



Control-No AMF/No particles



Control-AMF/No particles

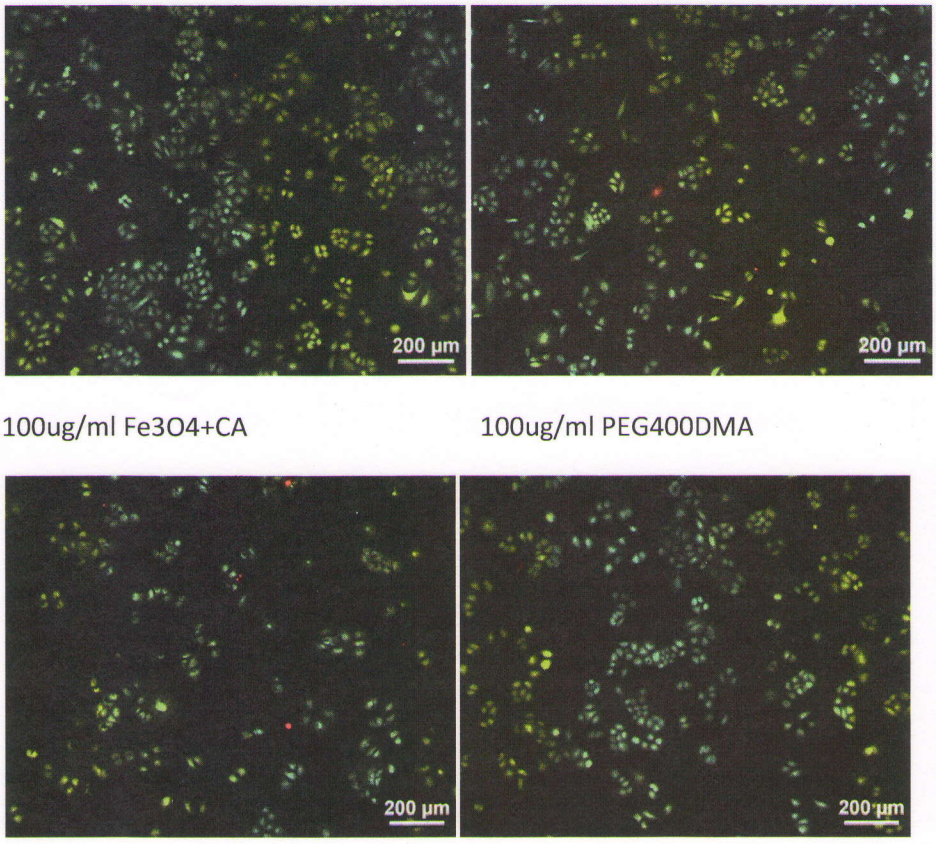


Figure 10. Live/dead images of A549 cells exposed to nanoparticle systems and then exposed to an alternating magnetic field for 10 minutes.

5. Conclusions

It was found that increasing the nanoparticle concentration to which cells are exposed, decreases cell viability. This trend was observed when using both NIH 3T3 fibroblasts and A549 lung cancer cells. The 100µg/ml concentration of the nanoparticle solutions did not produce significantly different cell viabilities from the control, and therefore could be considered biocompatible. However, as the concentration was increased, the nanoparticles became toxic to the cells, as the cell viabilities became significantly less than the control. In the future, we hope to prove that by coating iron oxide nanoparticles with polymer it is possible to make them more biocompatible. While some of the results support this trend, the results are not significant.

When magnetic nanoparticle suspended in A549 cell media were exposed to an alternating magnetic field, the nanoparticles generated heat. However, this heat was not enough to induce cell

death when both A549 lung cancer cells and magnetic nanoparticles were exposed to an alternating magnetic field. This could be due to the concentration of nanoparticles not being high enough, but the concentrations must remain low enough so that cell viability when not exposed to the alternating magnetic field remains high. Therefore, the next step of this research will be to develop nanoparticles that generate enough heat in an alternating magnetic field to induce cell death, but do not induce cell death otherwise.

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