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Photothermal effects and toxicity of Fe_3O_4 nanoparticles *via* near infrared laser irradiation for cancer therapy

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ABSTRACT

The photothermal effect of magnetite (Fe_3O_4) nanoparticles was characterized by photonic absorption in the near-infrared (NIR) region. Upon laser irradiation at 785 nm, the Fe_3O_4 nanoparticles generate localized hyperthermia in tumorous lesions, which is an effective strategy for cancer therapy; however, uncoated magnetite possesses an innate toxicity which can lead to drawbacks in the clinical setting. To reduce innate toxicity, a poly(acrylic acid) (PAA) coating on the nanoparticles was investigated in order to determine the alterations to stability and the degree of toxicity in an attempt to create a higher utility vector. It was found that the PAA coating significantly reduced the innate toxicity of the uncoated magnetite. Furthermore, the efficacy of PAA-coated magnetite nanoparticles (PAA-Fe_3O_4) was investigated for treating MDA-MB-231 (human mammary gland adenocarcinoma) cultures in viable concentration ranges (0.1–0.5 mg/ml). An appropriate PAA-Fe_3O_4 concentration range was then established for inducing significant cell death by hyperthermic ablation, but not through innate toxicity.

1. Introduction

The development of minimally invasive cancer treatments by the effective delivery of drug "payloads" to targeted lesions is of great interest to oncologists. The photothermal effect of Fe_3O_4 nanoparticles has been recently discovered and investigated for applicability in tumor treatments [1–8]. Upon near-infrared laser irradiation, the Fe_3O_4 magnetic nanoparticles generate sufficient energy to thermally ablate cancer cells. The main advantages of photothermal treatment are easy modifications to application areas and manipulation of thermal energy in targeted regions. This ease of application and manipulation of thermal energy generation requires that localized uptake of the nanoparticles reaches a sufficient concentration applicable for optical hyperthermia. A concern in clinical studies is that the innate toxicity of nanoparticle complexes at concentrations required for sufficient thermal energy generation proves to be significant. Investigations on the concentration dependent innate toxicity of uncoated magnetite nanoparticles have shown a significant impact on cellular viability for several types of cell lines. For the murine macrophage cell line J774 and glial cell line SVGp12, pronounced toxicity is observed beginning at 100 μg/ml [9,10]. However as iron oxide nanoparticle species have been previously shown to produce reactive oxygen species (ROS) through the release of free iron ions *in-vitro*, light-activated release of ROS species by photosensitizers coupled to the nanoparticle surface or protective surface coatings may improve photodynamic efficacy by reducing the required concentration required for elicitation of significant toxicity upon electromagnetic irradiation or by reducing innate toxicity [11–15].

The elevated vasculature endothelial growth and permeability factors mixed with disrupted endothelial, media, and adventitia layers, lead to leaky vasculature that is not usually seen in normal, healthy tissue [16–18]. As such, preferential accumulation of nanoparticle systems will occur in tumor tissue that provides a 10–50 fold increase in local concentration within 1–2 days [16]. Conveniently the nanoparticles in the endocytotic size range (<100 nm) exist within the size range for utilization of enhanced permeability and retention (EPR) effect [16,18–20]. This suggests that nanoparticles designed to utilize the EPR effect are likely to become internalized by cell lines once the nanoparticles leave the vasculature and, based on their size, become sequestered in lysosomal and other endosomal compartments. However, the EPR effect is not solely restricted to solid tumor masses and is observed in other inflammatory regions [17]. It is therefore critical

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that the concentration of nanoparticles be optimized in regions that experience the EPR effect, either cancerous or non-cancerous, such that the innate toxicity is minimized. Moreover upon the controlled application of mediating factors, significant toxicity during treatment may be elicited thereby allowing a highly efficacious and selective treatment regimen [1,7,21].

Quantification of innate toxicity reduction of uncoated magnetite nanoparticles (UC-Fe_3O_4) utilizing a one-pot, hydrothermal synthesis method for the surface deposition of poly(acrylic acid) (PAA) was investigated on MDA-MB-231 (human mammary gland adenocarcinoma) cultures. Furthermore, the size, colloidal stability, and photothermal effect under near-infrared irradiation of poly(acrylic acid)-coated magnetite nanoparticles (PAA-Fe_3O_4) and the UC-Fe_3O_4 variant were investigated for determination of the applicability of the chosen synthesis method and capping agent of the magnetite species for future *in-vivo* optical hyperthermia.

2. Methods

2.1. Nanoparticle synthesis

The PAA-coated, Fe_3O_4 nanoparticles were prepared by the polyol method as reported in a previous study. A 3 mM $FeCl_3 \cdot 6H_2O$ solution was completely dissolved in ethylene glycol and homogenized through ultrasonication and vigorous stirring. A solution of 4 mM poly(acrylic acid), deionized water, and 0.3 M urea was then added. The mixture was further ultrasonicated for 10 min and then sealed in a Teflon-lined, stainless-steel 50 ml autoclave vessel. The autoclave vessel was then heated to 200 °C for 12 h and then allowed to cool to room temperature. Newly formed PAA-Fe_3O_4 nanoparticles were isolated through magnetic separation and washed several times with water and ethanol to removed inorganic and organic impurities [22].

For UC-Fe_3O_4, 0.01 M $FeCl_2 \cdot 6H_2O$ and 0.02 M $FeCl_3 \cdot 6H_2O$ were dissolved in distilled $6H_2O$ at 80 °C under nitrogen through stirring. After 60 min, NaOH was added to the mixture. The mixture was then stirred for a further 2 h at 90 °C under nitrogen.

2.2. Characterization

UC-Fe_3O_4 and PAA-Fe_3O_4 nanoparticles were dispersed in complete-DMEM, HBSS and DI-water. Size was quantified through dynamic light scattering conducted on a Zetasizer Nano ZS (Malvern Instruments, Ltd.) using the nanoparticles dispersed in complete-DMEM to mimic *in vivo* conditions and in HBSS for stability comparison. Solutions were maintained at 37 °C for the duration of characterization. Zeta potential was measured by electrophoretic light scattering conducted by a Zetasizer Nano ZS (Malvern Instruments, Ltd.) using the nanoparticles dispersed in purified deionized water (DI-water). X-ray diffraction of UC-Fe_3O_4 and PAA-Fe_3O_4 nanoparticles has been previously conducted [22].

2.3. Growth media

High glucose Dulbecco's Modified Eagle's Medium (DMEM) (HyClone, Fisher Scientific) was obtained from the Fisher Scientific. Each container stored 500 ml of DMEM mixed with 7.00 mM L-glutamine and 2250 mg of glucose, without sodium pyruvate. Growth media were completed with 50 ml of fetal bovine serum (FBS) (Atlanta Biologicals), 5 ml of penicillin/streptomycin (HyClone, Fisher Scientific), 5 ml of minimum essential medium (MEM), non-essential amino acids 100× concentration (CellGro, Mediatech Inc.), and 5 ml of L-glutamine (CellGro, Mediatech Inc.).

2.4. Hank's balanced salt solution

500 ml of de-ionized water was heated in an autoclave in a glass jar for 30 min. After sterilization the glass jar was allowed to rest for a minimum of 24 h to achieve room temperature. The following components were massed to be added to 100 ml of the sterilized de-ionized water: 0.98 g of HBSS mixture (Sigma-Aldrich), 0.037 g sodium bicarbonate (Fisher Scientific), 10.50 g D(+) glucose (Sigma-Aldrich), and 8.59 g HEPES (Sigma-Aldrich). Two 50 ml conical vials were used in the creation of 100 ml of HBSS. After the addition of all solutes, each 50 ml conical vial was then adjusted to a pH of 7.2 through the addition of a 1 M sodium hydroxide solution. Aliquots were taken from the two vials and further purified before use.

2.5. Nanoparticle solutions

UC-Fe_3O_4 and PAA-Fe_3O_4 stock solutions of 13.8 and 11.2 mg/ml were respectively used to create 7.5, 5, 2.5, and 1 mg/ml solutions prepared with complete-DMEM. Serial dilutions were performed starting with the 1 mg/ml solution to create 0.5, 0.2, 0.1, 0.05, 0.025, 0.01, 0.005, and 0.001 mg/ml prepared with complete-DMEM.

2.6. Cell culture

MDA-MB-231 human mammary gland adenocarcinoma tissue samples were acquired from the American Type Culture Collection. Samples were cultured in T75CN vent cap tissue culture flasks (Sarstedt) and were maintained at a constant temperature of 37 °C in a 5% CO_2 environment during culturing and testing. Splitting was performed with 3 ml of trypsin–EDTA 1× concentration (HyClone, Fisher Scientific).

2.7. Innate toxicity

96 white well plates were seeded with 100 μl of 30,000 cells/μl. After seeding, the 96 white well plates were incubated at 37 °C in a 5% CO_2 environment for 24 h to allow for cell adhesion. This cellular density was chosen so to disallow complete confluency of any of the seeded wells by the end of the innate toxicity experiment. MDA-MB-231 cultures in the 96 well plates were subjected to 100 μl of UC-Fe_3O_4 or PAA-Fe_3O_4 suspended in complete-DMEM after this 24 hour period and further incubated for 48 h at 37 °C. Following the 48 hour incubation period, the viability of each well was assessed using the CellTiter-Glo® luminescent cell viability assay (Promega). The lysis buffer was created per the Promega protocol. The 96 well plates were placed on an orbital shaker for 10 min following the addition of 100 μl of the completed lysis buffer to each test well. The plate was further allowed to rest for another 10 min before the quantification of luminescent output.

2.8. Photothermal ablation

A standard power meter (Coherent Inc.) was used to determine the relation between the applied current and the optical power output. A liner function was fit to the data to allow calculation of the laser power and consequent intensity over an applied area. For each PAA-coated magnetite nanoparticle concentration, laser intensity and wavelength used during MDA-MD-231 irradiation trials, a heating and cooling curve was generated. An infrared camera (FLIR-T640) was oriented to monitor the temperature of the nanoparticle solutions. For determination of the heating curve, temperatures were recorded every minute for the first 8 min and every 2 min for the next 12 min. The cooling curve was determined by recording the temperature at 30 s, 1 min, and every following minute for the next 7 min after removal of the laser. After establishing the appropriate laser intensity, hyperthermic ablation trials were conducted on MDA-MB-231 cell cultures seeded in 48 well plates (BD Biosciences). Wells were initially seeded with 150 μl of approximately 6750 cells/well and incubated at 37 °C