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A pilot study into the use of FDG-mNP as an alternative approach in neuroblastoma cell hyperthermia

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Abstract

Purpose—Herein, we present a pilot study concerning the use of fluorodeoxy glucose conjugated magnetite nanoparticles as a potential agent in magnetic nanoparticle mediated neuroblastoma cancer cell hyperthermia. This approach makes use of the ‘Warburg effect’, utilising the fact that cancer cells have a higher metabolic rate than normal cells.

Materials and methods—FDG-mNP were synthesized, then applied to the SH-SY5Y neuroblastoma cancer cell line and exposed to an AC magnetic field. 3D Calorimetry was performed on the FDG-mNP compound. Simulations were performed using SEMCAD X software using Thelonious, (an anatomically correct male child model) in order to understand more about the end requirements with respect to cancer cell destruction.

Results—We investigated FDG-mNP mediated neuroblastoma cytotoxicity in conjunction with AC magnetic field exposure. Results are presented for 3D FDG-mNP SAR_{mnp} (10.86 ± 0.99 W/g of particles) using a therapeutic dose of 0.83 mg/mL. Human model simulations suggest that 43 W/kg SAR_{Theo} would be required to obtain 42 °C within the centre of a liver tumour (Tumour size, bounding box x=64, y=61, z=65 [mm]), and that the temperature distribution is inhomogeneous within the tumour.

Conclusion—Our study suggests that this approach could potentially be used to increase the temperature within cells that would result in cancer cell death due to hyperthermia. Further development of this research will also involve using whole tumours removed from living organisms in conjunction with magnetic resonance imaging and positron emission tomography.

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Keywords

FDG-mNP; Iron oxide nanoparticles; FDG; Hyperthermia; SH-SY5Y; Theranostics; Cancer; Tumours

Introduction

Despite years of research, financial costs attached to cancer treatments have continued to escalate, and in spite of the development of a plethora of anti-cancer drugs, mortality rates clearly continue to rise. Indeed it is estimated that 1 in 3 people in the UK alone will develop cancer during their lifetime. These disturbing facts demonstrate scope for an alternative treatment approach. Classically treatments have included chemotherapy and radiotherapy or combinations of these. However, such treatments are not without their side effects, which include nausea, hair loss, and other such unpleasant and undesirable associated occurrences, which remain problematic for patients. These side-effects arising from the toxic nature of the chemotherapeutic agents used in treatment, may also cause significant damage to healthy normal tissues adjacent to the cancerous tissue, and in addition, may cause mutations in the cancer itself potentially leading to the development of new cancer resistant cell lines.

Neuroblastoma is an extra cranial solid tumour arising from neuroblasts in infancy within the sympathetic nervous system. Cancer cell distribution depends upon the primary neuroblastoma location. Factors such as the age of patient, tumour stage, molecular biology, location of the tumour, histopathology, physiology and biochemical parameters of the neuroblastoma help in determination of the risk associated with a given treatment approach. Even after high dose chemotherapy, a 70 – 80 % mortality rate is found within high risk patients with neuroblastoma metastasis in liver, bone, bone marrow and lymph nodes [1]. New tumour cell lines may potentially expand and re-occur following treatment with the classic established treatments which include chemotherapy, radiotherapy and even surgical solutions [2]. Consequently, there are many reasons why a totally successful cancer treatment still remains elusive. To compound the issue, there are also a huge variety of cancer types; their aetiology and how they present clinically varies greatly, and therefore each cancer type may respond differently to different types of treatment therapies and regimes. The mechanisms for understanding tumour development and subsequent responses to any given treatment are not completely understood, however a property that all cancer cells appear to have in common is their rate of growth leading to the capacity to outnumber normal cells. This property common to all cancers is known as the Warburg effect. The consequence of the Warburg Effect is that cancer cells increase their glucose uptake relative to normal cells via glycolysis, to produce adenosine triphosphate through oxidative phosphorylation. And pediatric tumours such as neuroblastoma are no exception to this effect and consequently therapeutic strategies have been proposed around this effect [3].

More recently however, experimental treatments have been tried in which magnetic nanoparticles (mNPs) have been used alone and in conjunction with chemotherapeutic agents [4, 5]. The use of magnetic nanoparticles (mNPs) mainly comprised of iron oxide due to biocompatibility and low toxicity has been investigated [6–10]. In addition, recent work

has been undertaken concerning the application of RF fields to magnetic nanoparticles bound to membrane receptors. Such work has indicated that signalling pathways are involved in apoptosis when the cancer cell temperature elevates to 42 °C and supports endosomal escape of internalized particles [7]. RF fields are known to be associated with the dissipation of heat via mNPs thus facilitating the destruction of tumour cells or the sensitisation of the cancer cells prior to radiotherapy – a procedure known as magnetic fluid hyperthermia [8–10]. FDG conjugated to iron oxide particles were synthesized and was used in our previous study, as a possible PET-MR hybrid particle for medical diagnosis. This previous study involved an evaluation of the effect of these particles on MCF 7 cells viability [6]. There are other important applications for such hybrid particles too, for example, uses in a Warburg effect mediated magnetic fluid hyperthermia. Hence herein we have synthesized magnetite nanoparticles conjugated to fluorodeoxy glucose. We characterise the hybrid nanoparticle, and evaluate its potential use in neuroblastoma cell hyperthermia when exposed to an AC magnetic field as schematized in figure 1. We also perform numerical modelling studies to further our understanding of the suitability of this conjugated compound as an alternative for neuroblastoma cancer cell hyperthermia treatment.

Material and Methods

Reagents

Dowex 50 cation exchange resin, Eosin Y, Azur A, Malachite green, methylene blue, sodium tetrahydroborate (NaBH_4), cysteamine (2-aminoethanethiol), were purchased from Sigma (Istanbul, Turkey). Iron(III) chloride, Amberlite anion exchange resin, Ambersep 900 OH ion exchange resin, potassium carbonate (K_2CO_3), mannose triflate (1,3,4,6-tetra-O-acetyl-2-O-triflate-beta-d-mannopyranose), sodium cyanoborohydride (NaCNBH_3) were supplied from Fluka. All other chemicals were purchased from Merck Chem. Co (Istanbul, Turkey).

Synthesis procedure

(A) Synthesis of mannose triflate-cysteamine—The synthesis was carried out according to Babu et al. [11]. Briefly, 19.21 mg of mannose triflate was dissolved in 200 μL of distilled water at 90 °C (solution A). 84.85 mg of cysteamine was dissolved in 70 μL of pure water in another test tube and the pH was adjusted to 7.5 with 1.0 M HCl. Then, 40 mg of NaCNBH_3 was added to the solution (solution B). Solution A was then mixed with solution B at 90 °C for 1 h. Then, the reaction product was precipitated and allowed to dry in the incubator at 65 °C over night and dissolved in dimethyl formamide (1.0 mL).

(B) NaF substitution of mannose triflate-cysteamine—The Fluorination reaction was performed according to Gillies et al. [12]. Briefly, 1.0 mL of 0.035M Man-CA solution which is prepared as described above, 100 μL of Kryptofix solution (2.0 mg/mL in DMF), 200 μL of K_2CO_3 aqueous solution (2.0 mg/mL), 100 μL of DMF, and 200 μL NaF (2.0 mg/mL) (16mM) were mixed in a reaction tube and heated at 90 °C in a water bath for 20 min.

When the reaction was completed, the product was purified by passing it through the following columns by eluting with DMF: Dowex 50 cation exchange resin column (is used for excess Kryptofix complex), Ambersep 900 OH (is used for neutralization following Dowex 50 column treatment), Amberlite anion exchange resin (is used to remove non-reacted free NaF), respectively. Finally, C-18 pre-cartridge was used to remove non-polar impurities.

(C) Synthesis of mNPs—The nanoparticles were prepared accordingly to Medine et al. [13]. Briefly, 12 mL of 2 M FeCl_3 solution in 2 M HCl was added to a 500 mL three necked glass flask. Fifty mL of a freshly prepared 0.08 M Na_2SO_3 solution was added to the flask under nitrogen gas by dropwise. 5 ml of NH_3 solution (25 %) was added to the mixture under nitrogen gas. The solution was incubated at 70 °C for 30 minutes and then cooled to below 45 °C. The black precipitate was recovered using an external magnetic field and washed several times with distilled water. The particles were then washed with a water–ethanol (2:1) mixture. The precipitate was dispersed into a mixture of 80 mL ethanol and 20 mL distilled water.

(D) Synthesis of FDG-mNPs—The purified NaF-CA from the previous step, was mixed with 25 mM MNP at room temperature. Then 50 mM of NHS (N-Hydroxysuccinimide) was gently added to the mixture. The reaction mixture was allowed to stand for mixing for 2 h in an orbital shaker.

Characterization of FDG-mNPs

The particle size and morphology of the MNP samples were assessed by using a scanning electron microscopy (SEM) (Phillips XL-30 S FEG, Aachen, Germany) (figure 2). The mean particle size was determined by dynamic light scattering (DLS) and the surface charge of the nanoparticles was investigated through zeta potential measurements using Zetasizer, (Malvern NanoZS Instruments, Worcestershire, UK) after dilution of the samples in distilled water. Elemental analysis was performed to evaluate the elemental composition using Energy-dispersive X-ray spectroscopy (EDX) (Shimadzu, Kyoto, Japan). EDX data confirmed that nanoparticles contain F and Cl, originating from impurities during the sample preparation (figure 2A). Transmission electron microscopy (TEM) (JEOL JEM 2100F HRTEM, Massachusetts, USA) was used for describing morphological characterization of the mNPs (figure 2B). The magnetite core was characterized by X-ray Diffraction (XRD) patterns, which were consistent with the crystal characteristic of magnetite (ICDD No. 75-1609). Fe_3O_4 crystals with a spinel structure have six diffraction peaks as seen in figure 3C. High performance liquid chromatography (HPLC) (Schimadzu, Kyoto, Japan) chromatograms were obtained to check the purity of nanoparticles using a Schimadzu HPLC system equipped with a LC-10ATvp quaternary pump, DAD detector, and 7.0 μm reversed-phase (RP) -C-18 column 250 $\times$ 21 mm I.D., (Macherey-Nagel). 60 % acetonitrile – 40 % water was used as an eluent. Other conditions included, flow rate: 0.7 mL/min; column temperature: 25 °C; wavelength: 210 nm.

Cell Culture

SH-SY5Y cells (from CRL-2266, American Type Culture Collection, Manassas, USA) were seeded onto tissue culture treated 35 mm² petri dishes (Corning, Birmingham, UK) in 1000 µL growth medium containing Ham's F12: MEM (1:1) appended with 2 mM L-glutamine, 10 % FBS, 0.1 mg/mL streptomycin and 5 mL 100 U penicillin (Cell culture reagents from Sigma, Dorset, UK). These cells were incubated at 37 °C, in 5 % CO₂.

Cytotoxicity Experiment

We investigated the capability of FDG-mNP to destroy neuroblastoma tumour cells. We used a cell line SH-SY5Y (a neuroblastoma cancer cell line). The FDG-mNP were produced using the technique described by Ozkaya et al [6]. SH-SY5Y cells were exposed to 218.7 kHz; 5.24 mT AC magnetic field ($H \times f = 1.145 \times 10^9 \text{ Am}^{-1} \text{ Hz}$) for 30 minutes with 0.19 mg/mL (50 µl), 0.36 mg/mL (100 µl), 0.83 mg/mL (250 µl) of concentrations of FDG-mNP using the LC-AMF setup. Post 30 minutes exposure, FDG-mNPs were replaced with fresh cell growth medium. After a 24 hour incubation period, the cells were stained with Hoechst stain (Sigma, Dorset, UK), propidium iodide (Sigma, Dorset, UK) and analyzed by microscopy using a fluorescent microscope (Leica, Newcastle Upon Tyne, UK). Microscopy images were obtained using an inverted fluorescent Leica microscope (Newcastle Upon Tyne, UK). Propidium iodide stains dead cells, Hoechst stains the nucleus, i.e. all the cells (figure 5B). Phase contrast shows the cells with FDG-mNPs under no filter. When we superimpose the Hoechst with the Propidium iodide results, we obtain the cytotoxicity percentage. It is a live cell, dead cell assay to calculate the percentage of SH-SY5Y cytotoxicity post ac magnetic field exposure to FDG-mNPs.

Calorimetric Experiment

2 mL of 4 mg/mL, 0.19 mg/mL, 0.36 mg/mL, 0.83 mg/mL concentrations of FDG-mNP with (include a 30 minute incubation step in 1 ml SHSY5Y media) and without cells were used for calorimetric experiments. Same type of container was used for all the calorimetric experiments. FDG-mNPs were subjected to vortex and ultra-sonication prior to calorimetry. Pico M with OTG-MPK 5 optical sensor system (Opsens, Quebec, Canada) was used for real-time temperature measurement, while subjecting the sample to a Live Cell AC Magnetic Field setup (LC-AMF; nanoTherics, Staffordshire, UK) attached to the mageTherm system (nanoTherics, Staffordshire, UK). Our apparatus is shown in figure 5. Specific absorption rate (W/g of particles) were calculated using the following equation.

$$SAR = \left(\frac{C}{\phi} \right) \left(\frac{\Delta T}{\Delta t} \right) \quad (1)$$

Where specific heat capacity of the mNP (J/Kml) is C , concentration of mNP (mg/mL) is ϕ , and the rate of change of temperature over time is T/ t ; $t = 25 \text{ ms}$. An appropriate region of the graph was used for calculations by using the corrected slope method [14].

3D Calorimetric Experiment

In order to evaluate the heat dissipation property of FDG-mNP within a tissue mass, 3D calorimetry was performed. Peptide gel solution (Biogelx, Lanarkshire, United Kingdom) was gently mixed using a pipette and sonicated for 30 seconds to produce a homogeneous solution. 250 μ l from a 4 mg/mL FDG-mNP preparation was centrifuged for 5 minutes at 1500 rpm. The supernatant was discarded and the FDG-mNP pellet was disturbed and thoroughly mixed with 1 mL of peptide gel solution to obtain a concentration of 0.83 mg/mL. FDG-mNP peptide gel solution was incubated in a cell culture incubator at 37 °C with a humidified atmosphere of 5 % CO₂ for 1 hour and allowed to settle down to form a gel that should mimic evenly dispersed magnetic nanoparticles embedded within a tissue mass.

The 3D FDG-mNP peptide gel was subjected to frequency $f = 218.7$ kHz; flux density $B = 5.24$ k. A/m using LC-AMF setup. A Fibre Optic temperature probe was positioned at the centre and later on the surface of the 3D FDG-mNP peptide gel matrix to measure the real time temperature for calculating SAR_{mnp} (W/g of particles) using equation 1.

Thermal problem

In order to examine the potential of MFH to liver cancer treatment and to better understand the mechanism of heat transfer in human tissues mixed with mNPs, we have undertaken a numerical simulation using SEMCAD X software [SPEAG, Schmid and Partner Engineering, Zurich, Switzerland].

The thermal problem was solved using a Pennes bioheat equation [15]

$$\rho c \frac{\partial T}{\partial t} = \nabla \cdot (\kappa \nabla T) - \rho_b c_b \rho \omega (T - T_b) + \rho Q + \rho SAR \quad (2)$$

where κ is the thermal conductivity, SAR_{Theo} is the specific absorption rate, ω is the perfusion rate, Q is the metabolic heat generation rate, ρ is the density of medium, ρ_b , c_b , and T_b are the density, specific heat capacity and the temperature of blood. The parameters of the tissues were considered constant during the simulations. The boundary condition was imposed in the form of mixed one, which represents the cooling associated with heat exchange with environment

$$-\frac{k \partial T}{\partial n} = h(T_{ext} - T) \quad (3)$$

where h is the convection coefficient and in our case was chosen to be 9.5 W/m²/C, T_{ext} is the external temperature (25 °C). Moreover, we have assumed that the SAR_{mnp} is known from the experiments and its scaled value was used as a source of the heat in equation (2). It is worthy of note that the Pennes heat equation is limited to tissues with high degree of perfusion, such as liver. This means that blood perfusion is assumed to be uniform throughout the tissue, and it is considered that all the heat leaving the artery is absorbed by

the local tissue, and there is no venous rewarming. Furthermore, the Pennes model is limited to the perfusion source term, i.e. the arterial temperature is assumed to be equal to the body core temperature [14–16]. In order to predict the temperature distribution in an anatomically correct human model, we used Thelonious, a male child model (age 6, height 1.15 m, weight 18.6 kg) from Virtual Population [17]. The dielectric and thermal parameters of the model were taken from IT'IS [18] and, were calculated for the frequency $f = 218.7$ kHz, that is, the same frequency that was in the case of the SAR_{mnp} measurements that were conducted on the magneTherm system. This enabled us to model the most realistic case using a stage 4 neuroblastoma of irregular shape. The Tumour dimensions modelled were, bounding box $x=64, y=61, z=65$ [mm]; Liver dimensions, bounding box $x=128, y=122, z=130$ [mm]. The dielectric and thermal properties of the liver and the tumour are given in Table 1. The same parameters for perfusion were assigned to both tissues [19]. The blood heat capacity (c_b) was 4.05×10^6 J/ m³/ K and the heat generation rate (Q) was 10.4122 W/ kg. The mass of tumor is 0.075 kg and its volume is 6.957×10^{-5} m³. And theoretical SAR, i.e., SAR_{Theo} was calculated with regard to the mass of the tumor.

Results and Discussion

The FDG-mNP mediated hyperthermia approach is shown in figure 1. Halkes' method has been applied for cysteamine conjugation to mannose triflate with minor modifications in our work [20]. Reaction yields were calculated as 92.39 ± 7.4 % ($n = 3$) for mannose triflate thiol amide ((3,4,6-tri-O-acetyl-N-(2-mercaptoethyl)-2-O-[(trifluoromethyl)sulfonyl]-d-erythro-hexopyranosylamine-3,4,6-tri-O-acetyl-2-O-[(difluoromethyl)sulfonyl]-d-erythro-hexopyranose (1:1) and 100 % ($n = 3$) for the inactive fluorinated derivative of mannose triflate thiol amide (3,4-di-O-acetyl-2-deoxy-2-fluoro-N-(2-mercaptoethyl)hexopyranuronosylamine) according to HPLC chromatograms similar to our previous results [6]. EDX analyses confirmed the elemental composition (figure 2A). SEM and TEM images demonstrated that mNPs were nano sized and in the range 10 to 20 nm (figure 2B, 3A). While hydrodynamic diameters of mNPs were 156 nm, FDG-mNPs was 321 nm. The surface potential of the mNPs and FDG-mNPs were found to be -42.8 mV and 2.8 mV, respectively. Conjugating with FDG resulted in a decrease of negative zeta-potential, while increasing the size of mNPs.

The sample as powder was characterized by means of X-ray diffraction. XRD analysis shows that the most intense peak corresponds to the (311) crystallographic orientation of the spinel phase of Fe_3O_4 magnetic nanoparticles. The mean size of the nanoparticles was determined from the X-ray diffraction pattern by using the Scherrer approximation and confirmed the results found by TEM, SEM images (10–20 nm). Additionally XRD data showed that the nanoparticles had a cubic spinel structure (figure 3B). HPLC analyses confirmed that synthesized FDG-mNPs were pure (figure 3C). Synthesized FDG-mNP had an iron oxide concentration of 4 mg/mL. When exposed to an AC magnetic field, FDG-mNPs did dissipate heat efficiently as shown in figure 5A.

Our experiments showed that 250 μ L of 4 mg/mL FDG-mNPs in 1 mL of cell culture media, i.e. about 0.83 mg/mL FDG-mNPs in SH-SY5Y resulted in overall 89 % cytotoxicity. Although the particles (mNPs) are toxic on their own as can be seen from the graph (even

when they are not exposed to an AC magnetic field, - since they are killing 70% of the cells), the data showed that exposure to an AC magnetic field appears to enhance cell death by up to 17% (** $P < 0.05$). Our results (figure 5C) clearly show that there is increased destruction of tumour cells with the use of FDG-mNPs together with the application of an AC magnetic field. To understand why we observed the enhanced cell death in the cell line, FDG-mNP calorimetry with cells was performed. Figure 6B shows that the SAR_{mnp} value of 0.83 mg/mL FDG-mNP was statistically significant (** $P < 0.05$) over 0.36 mg/mL and 0.19 mg/mL. So the amount of heat dissipated by the nanoparticles should have induced intracellular and extracellular hyperthermia in the test condition. Some hypotheses, however, state that internalized mNPs could effectively kill cancer cells and activate apoptotic pathways as the cell membrane would insulate the cell leading to escalated intercellular hyperthermia. Moreover dextran coated magnetic nanoparticles taken up by cancer cells have been observed to localize on the cell membrane and within lysosomes, leading to structural disruption in cancer cells upon exposure to AMF. This in turn is thought to amplify the generation of reactive oxygen species and the killing of cells. Similarly prior research in magnetic fluid hyperthermia on cell viability suggests that there would be a significant increase in membrane fluidity and permeability which may be due to irreversible lipid and protein denaturation [35].

Increased uptake of FDG-mNPs via endocytic pathways of the cells may be responsible for the cytotoxicity in the FDG-mNPs only control [25]. Alternatively, this high toxicity observed in the no AMF condition would be due to either one or a combination of the following: i) Free fluorine and chlorine resulting from the coating procedure; ii) increased nanoparticle to cell concentration; iii) the sensitive nature of the semi-adherent SH-SY5Y neuroblastoma cell line. However a better FDG coating procedure results in mono dispersity and will allow significant uptake of nanoparticles by cells due to their biocompatible glucose shell and would lead to a lower cytotoxicity under controlled conditions. We note, however, that effects of FDG-mNPs on healthy tissue requires evaluation, and would be the subject of future research.

It was noted that FDG-mNPs became hot within 10 minutes (figure 5A) with a SAR_{mnp} value of 49.85 ± 1.08 W/g of particles, but we need to consider that nanoparticles are in a suspension when we perform calorimetry. Once injected into a tumour and when the magnetic nanoparticles become internalized, the SAR_{mnp} value is expected to drop significantly [25]. Hence we considered a 3D calorimetry option. FDG-mNP peptide gel 3D calorimetry data for the therapeutic dosage 0.83 mg/mL is shown in figure 7. The SAR (W/g of particles) did drop when FDG-mNPs were embedded within peptide gel as shown in figure 7C. This does therefore indicate that when the movement of FDG-mNP is restricted, the SAR (W/g of particles) is expected to drop. Additionally it was observed that the change of temperature and SAR (W/g of particles) was high in the centre of the 3D FDG-mNP peptide gel when compared to the surface. This phenomenon tends to indicate that localized FDG-mNP mediated hyperthermia is possible by adjusting the concentration of the FDG-mNP dosage.

In order to investigate the successful tumour reduction of a stage 4 neuroblastoma in liver, we modelled an anatomically correct human model simulations to show how such modelling

could be used to understand and assist the requirements for engineering FDG-mNPs for our future tumour tissue experiments. In figure 8 the temperature distribution in the tumour model is shown after 1800 secs for $SAR_{Theo} = 43$ W/kg (figure 8B) and $SAR_{Theo} = 93$ W/kg (figure 8C). It is worthy of note that the temperature (applied to the area in question for this tumour) of about 42 °C covers the whole tumour for $SAR_{Theo} = 93$ W/kg while for $SAR_{Theo} = 43$ W/kg it only applies to the centre of the tumour mass. The same situation is shown in figure 9A, B where the temperature distribution is pictured as an iso-surface. Based on figure 9C, we note that a SAR_{Theo} increment of 10 W/kg gives rise to a temperature rise of 1 °C in the case of liver cancer. Moreover, raising the SAR_{Theo} to 93 W/kg causes the temperature to rise to 47 °C in the middle of the tumour. Hyperthermia generally involves reaching and maintaining a temperature of 42 to 45 °C in the tumour for several minutes (usually 30 minutes). At the same time, the surrounding regions of the body must be kept cool enough so that damage to such surrounding regions will be minimal. That is why we established a minimum SAR which resulted in a temperature rise in the liver tumour close to 42 °C ($SAR = 43$ W/kg in this case). Moreover, it has already been shown that a temperature rise in a body increases the effectiveness of some types of chemotherapy [31].

In Figure 8B it has been assumed that the SAR_{Theo} (equation 2) was constant during the time (1800 seconds). Figure 8D depicts a temperature rise over time in selected points of the tumour. Numerical modelling indicated that the highest temperature (42 °C) was found in the middle of tumour, (while the temperature decreases towards the edges). This modelling therefore indicated that the temperature in the tumour is not likely to be homogeneous within the tumour. This inhomogeneity of the temperature in the tumour can be seen in figure 8E. The size of the tumour and its location within a human therefore appears to play a crucial rule regarding potential hyperthermia treatment of a given tumour mass, in terms of optimizing the effectiveness of treatment, and there may be substantial discrepancies here between actual living cancer tissue and numerical modelling. Our numerical modelling study clearly shows that the temperature distribution is highly inhomogeneous in the tumour tissue, even when the heat source, that is, the SAR_{Theo} is assumed to be constant, which does correlate with our 3D calorimetric results figure 7.

An optimal SAR_{Theo} should be reached for accepted frequencies (f), magnetic field strengths (H) and average particle concentrations. The aforementioned parameters are essential to optimize heat transfer. In our case the SAR_{mnp} obtained from experiments is related to the SAR_{Theo} (with the usual meaning of power absorbed per mass of human tissue) via the expression:

$$SAR_{mnp} = [\rho_{tumour} / (\rho_{NP} / \phi)] * SAR \quad (4)$$

Where ρ_{tumour} is the density of tumour, ρ_{NP} is the density of magnetic nanoparticles (magnetite with density 5180 kg/m³) and ϕ is the volume fraction defined as

$$\phi = V_{NP} / (V_{NP} + V_{tumour}) \quad (5)$$

Taking into account $SAR_{mnp} = 10860 \text{ W/kg}$ and numerical $SAR_{Theo} = 43 \text{ W/kg}$ one can calculate the volume fraction needed to receive the temperatures from 42 to 47 °C (figure 9C). The calculated data are shown in Table 2.

Moreover, this SAR_{Theo} value of 43 W/kg, that is required to reach 42 °C temperature in the case of liver tumour (Bounding box for the tumour $x=64, y=61, z=65$ [mm]) resulting from numerical modelling could be different in reality, and would have to be determined in future studies. However, such modelling may be viewed as a useful adjunct in setting a lower limit for the temperatures reached. Clearly the applied AC magnetic field is also important when considering therapeutic applications as some studies show that the SAR value is directly proportional to an increase in field amplitude, field intensity and applied frequency [32]. Herein we used the magneTherm-a dedicated instrument attached with the LC-AMF system to expose the cells with time varying magnetic field as shown in figure 4. Such a limit for magnetic fluid hyperthermia was suggested about 30 years ago, and was based on patient discomfort i.e. $H \times f = 4.85 \times 10^8 \text{ Am}^{-1}\text{Hz}$ [26–30]. However a new limit was later determined to be $H \times f = 5.10 \times 10^9 \text{ Am}^{-1}\text{Hz}$ [28]. These limits have become the broadly accepted norms for *in vitro*, *in vivo* and clinical applications.

The alternating magnetic field used in this study was $1.134 \times 10^9 \text{ Am}^{-1}\text{Hz}$, which falls within the acceptable limits. Moreover new limit might be proposed in the near future based on on-going clinical trials throughout the world. The use of mNPs with magnetic hyperthermia has the potential to solve some of the major issues associated with current cancer treatments. Specifically, it removes the potential problem of resistance, which can be a problem when certain types of chemotherapy drugs are used, since heat cannot offer chemical resistance. It also has the advantage that mNPs are non-specific and therefore may provide a potential universal treatment.

The rational of Magnetic Fluid Hyperthermia relies on exploiting tumour characteristics. The increased metabolic activity of tumour cells is related to the over expression of glucose transporters in the cell membrane, which enables an increased uptake of glucose. There are several types of glucose transporter. However, the GLUT-1 receptor has been found to be expressed in nearly all cancer cell lines. These GLUT-1 receptors in tumour cells have been imaged by the use of MRI scanning, utilizing superparamagnetic iron oxide Mbps as a contrast agent [24]. These mNPs are taken up by the GLUT-1 receptors by using a glucose analogue, called 2-deoxy-D-glucose (2-DG). This analogue enables the uptake of the mNPs within the cancer cells, and also decreases the rate at which the mNPs are removed from cells and therefore from the body. This arises on account of the phosphorylation of this glucose analogue by the enzyme hexokinase. The phosphorylation of this glucose analogue means that it is therefore not a substrate for the glycolytic pathway and as a result, it cannot be broken down further. This consequently results in the retention of the mNPs within cancer cells [6]. It was proposed that gold nanoparticles (GNPs) (1–1,000 nm) modified by glucose have been considered to increase the toxicity of radiotherapy in human malignant cells [22]. There are other reports indicating that thiolamines enhance the radiation-induced apoptosis [23] We note that 2-mercaptoethyl derivative of FDG conjugated-Fe₃O₄ NPs has less apoptotic effect comparing to 2-mercaptoethyl derivative of FDG.

The magnetic heating parameters (time of exposure to heating, frequency, amplitude, intensity) may vary for different tumour cell lines, but our pilot experiments indicate that this technique of using FDG-mNPs could have potential uses in the treatment of neuroblastoma. Furthermore we note that improved coated iron oxide nanoparticle linked to FDG would most likely also have to be established [21]. This will be the subject of further research.

Futuristically, in order to determine the effectiveness of potential FDG-mNPs mediated hyperthermia, we suggest that this synthesized ^{19}F FDG-mNP can be used in combination with magnetic resonance imaging to track the injected nanoparticles within a three dimensional space as ^{19}F nuclei has an increased magnetogyric ratio when compared to ^{31}P and ^1H with potential in vivo tracking [33]. Or ^{18}F FDG could be infused post-AMF exposure to indicate any remaining tumour hotspots at the exposure site. We could therefore confirm shrinkage or destruction of the tumor itself (thus providing a means of real-time visualized post treatment). IV delivered radioactive ^{18}F FDG alone (in conjunction with PET scanning) is already extensively used in patients in the diagnosis of breast cancer.

Conclusions

Our experiments indicate that FDG-mNPs can be used to destroy SH-SY5Y tumour cells (a neuroblastoma cancer cell line). Herein in SH-SY5Y, 0.83 mg/mL is the optimum dose of FDG-mNPs since it has killed up to 89 % cells. Although it is noted that the particles (mNPs) are toxic in their own right (even when they are not exposed to an AC magnetic field, since they are killing of 70 % of the cells), the data shows that exposure to an AC magnetic field appears to enhance cell death by up to 17 %. Suitable experimental conditions should involve a tumor surrounded by health tissue or a transplantable murine mouse model such as C1300 which possess human neuroblastoma characteristics [36]. Volume, concentration, stability, dispersity along with other physical and chemical parameters should be taken into account to determine the lethal concentration, lethal dosage, effective concentration and effective dosage of FDG-mNP in neuroblastoma cells. However, SH-SY5Y is an immortalized clonal cell line derived from human neuroblastoma, and when differentiated they behave like neurons, hence even in an undifferentiated state, they are semi-adherent and sensitive.

So these experimental conditions and the resulting numbers will not provide a decisive cytotoxicity percentage, but nevertheless demonstrate a proof of principle experiment which indicates that the surface of mNPs heat up rapidly in 10 minutes at low field amplitude within a 3D matrix, thus indicating that there is potential for destroying tumor cells within a tissue mass. Our simulations form a useful adjunct in predicting the behaviour of tumours under given conditions within the limits posed by such numerical modelling. However we conclude that our pilot study using FDG-mNPs in conjunction with magnetic heating concerning the destruction of neuroblastoma tumour cells and our simulations are worthy of further investigation.

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Figure 1.
Scheme for FDG-mNP disturb to cancerous cells.

Figure 2.
(A) EDX Analyses with atomic composition (B) Transmission electron microscope images of FDG-mNP.

Figure 3.

(A) Scanning electron microscope image of FDG-mNP (B) High performance liquid chromatography data. (C) X-ray diffraction data.

Figure 4. magneTherm instrument attached with the LC-AMF system which we used for the cell biology – ac magnetic field experiments.

Figure 5.

(A) 4 mg/mL FDG-mNP Calorimetry experiment (B) FDG-mNP cytotoxicity experiment microscopy (C) FDG-mNP cytotoxicity experiment: No ac magnetic field (AMF) control (I) Phase contrast (II) Hoechst (III) Propidium Iodide; 0.83 mg/mL (IV) Phase contrast (V) Hoechst (VI) Propidium Iodide (B) Graph showing cytotoxicity for different conditions with and without 218.7kHz; 5,24 k. A/m ac magnetic field (AMF) along with controls.

Figure 6.

(A) Change of temperature over time graph for FDG-mNP with neuroblastoma cells (B) SAR_{mnp} (W/g of particles) for FDG-mNP with neuroblastoma cells.

Figure 7. 3D FDG-mNP calorimetry (A) FDG-mNP with peptide gel change of temperature over time (B) FDG-mNP within peptide Gel (C) SAR_{mnp} (W/g of particles) for FDG-mNP embedded within peptide gel.

Figure 8.

(A) Thelonious with liver tumour (B) Temperature distribution contour plot for SAR_{Theo} 43 W/kg within the tumour after 1800 seconds (C) Temperature distribution contour plot for SAR_{Theo} 93 W/kg within the tumour after 1800 seconds (D) SAR_{Theo} 43 W/kg - temperature over time of the tumour from centre to edge (E) SAR_{Theo} 43 W/kg - temperature inhomogeneity within tumour, solid line (Blue) represents temperature along the vertical axis, while dashed line (Red) represents the horizontal one.

Figure 9.

The iso-surface for temperature 42°C indicating its volumetric distribution for $SAR_{Theo} = 43 \text{ W/kg}$ (A) and for $SAR_{Theo} = 93 \text{ W/kg}$ (B) in the liver tumour model. (C) Temperature over time in the middle of the liver tumour model as the function of SAR_{Theo} .

Table 1

Dielectric and thermal properties of background tissues (218 kHz).

Tissue	Conductivity (S/m)	Density (kg/m ³)	Heat capacity (J/kg/C)	Thermal conductivity (W/m/C)	Perfusion (ml/min/kg)
Liver	0.1086	1078.75	3540.2	0.5191	902.163
Liver tumour	0.1086	1078.75	3540.2	0.5191	111

Table 2

The volume fraction (ϕ) relation to the SAR_{Theo} and the temperature in the middle of the tumour for $SAR_{mnp} = 10860$ W/kg.

Temperature (°C)	Numerical SAR (W/kg)	Volume fraction (ϕ)
42	43	0.000824574
43	53	0.001016335
44	63	0.001208
45	73	0.0014
45.8	83	0.001592
47	93	0.001783