In Vitro Assessment of a Novel Cancer Therapy Combining Drug Loaded Magnetic Nanoparticles and Magnetic Hyperthermia

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ABSTRACT

We propose to develop a novel combination therapy for prostate/breast treatment by combining two treatment modalities (hyperthermia therapy and chemotherapy) into one using MCaP nanoparticles (NP) system. Our proposed MCaP NPs possess unique properties, including: 1) superparamagnetism: paramagnetic particles can generate heat (hyperthermia) that can kill cancer cells when a remote, medium, external inducting alternated magnetic field (AMF) is applied; 2) high surface area for drug loading and release; and 3) abundant surface functional groups for attachment of targeting/imaging moieties. In this study by using magnetic induction heating to characterize the properties of newly-formulated iron-doped CaP paramagnetic (Fe-n-CaP) based nanoparticles (NPs) *in vitro*. Particularly, our newly formulated nanoparticle Fe-n-CaP with dual therapy functions for cancer treatment can reach the required therapy temperature. The experiments show that under the selected field parameters, sufficient energy deposition is achieved which leads to a substantial temperature increase within a short-time frame, meanwhile control the drug release with the temperature change has been tested. And also inductive heating experiments on Fe-n-CaP water suspension injected into muscle tissue was successes at magnetic field with current 25A, frequency 330 kHz.

Keywords: Magnetic nanoparticles, Magnetic hyperthermia, Cancer therapy

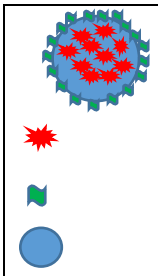
1 INTRODUCTION

In cancer treatment, the major dilemma of cancer therapy is that often the dose of systemically applied chemotherapeutics needed to annihilate all tumor cells will cause severe negative side effects in patients. Therefore new therapeutic methods need to be developed to improve the effectiveness of this therapy and to reduce adverse effects. The application of ferrofluids (e.g., Fe_3O_4 nanoparticles) for hyperthermia treatment has become of much interest in recent years. More recently, advances in the area of nanotechnology has contributed to the development of magnetic fluid hyperthermia. The new method applies paramagnetic nanoparticles (NPs) bound to chemotherapeutics and injection into the supplying vascular system of the respective tumor while focusing a strong external alternating magnetic field to this region. Both hyperthermia and locally released chemo drugs can kill cancer cells simultaneously [1-2]. Due to the dual kill mechanism (heat and drug), this cancer therapy method may result in a more effective cancer treatment with fewer side effects (allowing reduction in the amount of chemotherapeutics). In this study by using magnetic induction heating to characterize the properties of newly-formulated iron-doped CaP paramagnetic (Fe-n-CaP) based nanoparticles (NPs) *in vitro*. Here, we propose to develop and test a multifunctional nanoparticle drug delivery system that is able to kill cancer cells by both hyperthermia and chemotherapy in a controllable way. We expect this combination therapy using both hyperthermia and chemotherapy is more effective than either hyperthermia therapy or chemotherapy alone, due to a synergistic effect. This dual therapy should generate less toxic side effects and achieve high efficacy against prostate cancer or breast cancer. Particularly, our newly formulated nanoparticle Fe-n-CaP with dual therapy functions for cancer treatment can reach the required therapy temperature. The experiments show that under the selected field parameters, sufficient energy deposition is achieved which leads to a substantial temperature increase within a short-time frame, and control the drug release with the temperature change has been tested. And also inductive heating experiments on Fe-n-CaP water suspension injected into muscle tissue was successes at magnetic field with current 25A, frequency 330 kHz.

Very few studies exist for exploration of both hyperthermia and chemotherapy in cancer treatment at the same time. Recently, thermo-chemotherapy based on a liposome system has been tested *in vitro* [3]. The doxorubicin drug releases at least 50% when the media temperature increased to 43 °C. Other than this, literature regarding the application of combined hyperthermia and chemotherapy using a multifunctional nanoparticle system for breast cancer treatment is virtually nonexistent. Our idea is the change of the temperature profile could change or control the drug release rate; therefore, this combination therapy using both hyperthermia and chemotherapy will be more effective to kill the cancer cells.

Compared to other tumors (e.g. lung, melanoma), breast tumors and prostate tumors have a low perfusion rate [4]. This results in the possibility that a breast tumor is particularly suited for hyperthermia treatment, where temperature increases between 4 and 7 °C, and the ambient *in vivo* temperature can cause tumor hyperthermia. Moreover, due to the vasculature in breast tumor, magnetic

nanoparticles can easily accumulate inside tumor through an "enhanced permeability and retention (EPR) effect"[5]. Due to the location of breast tumors, it is also convenient to use a static external MF to target the magnetic nanoparticles in the breast tumor by using the concept of magnetic drug targeting (MDT) [6]. To significantly improve the outcome of conventional chemotherapy treatment, we propose to develop a novel synergistic therapy for breast cancer treatment. Chemotherapy drug and magnetic nanoparticles is encapsulated and integrated as a new nanoparticle which is described in Figure 1.



Outline of drug-loaded Fe-n-CaP nanoparticle

Fe^{3+} doping

Chemo Drug

CaP nanoparticle

Figure 1. Schematic representation showing the regarding development of combinational cancer therapy using MCaP nanoparticles.

2 METHODS AND EXPERIMENTS

2.1 Synthesize and Characterize MCaP NPs and Investigate Drug Loading and Release

Magnetic calcium phosphate NP fabrication

The way that the MCaP NPs prepared have the following key characterized and shown in Figure 1: 1) Iron oxide (Fe_3O_4) is trapped inside CaP. The chemical composition and crystal structure has been confirmed by Fourier transform infrared spectroscopy (FTIR), Energy Dispersive X-ray spectroscopy (EDX), and X-ray Diffraction (XRD) (Data not shown here): 2) NP surface is enriched with chitosan. The NH_2 of NPs allow for surface conjugation with drug or fluorescent labeling: 3) The NP suspension is colloidally stable due to highly positive charge and steric effect of chitosan biopolymer; and (4)The resultant MCaP NPs is highly magnetic.

MCaP NP formulation optimization

We have improved the McaP NP formulation by fine tuning the amount CaP encapsulating the iron oxide magnetic NPs. As shown in Figure 2. At similar NP size (~180 nm) of formulations A; B, C, and D, has decreasing amount of CaP and increasing loading of iron oxide, respectively. By reducing the amount of CaP, more iron oxide was encapsulated inside and thus the magnetic heating capability will be increased.

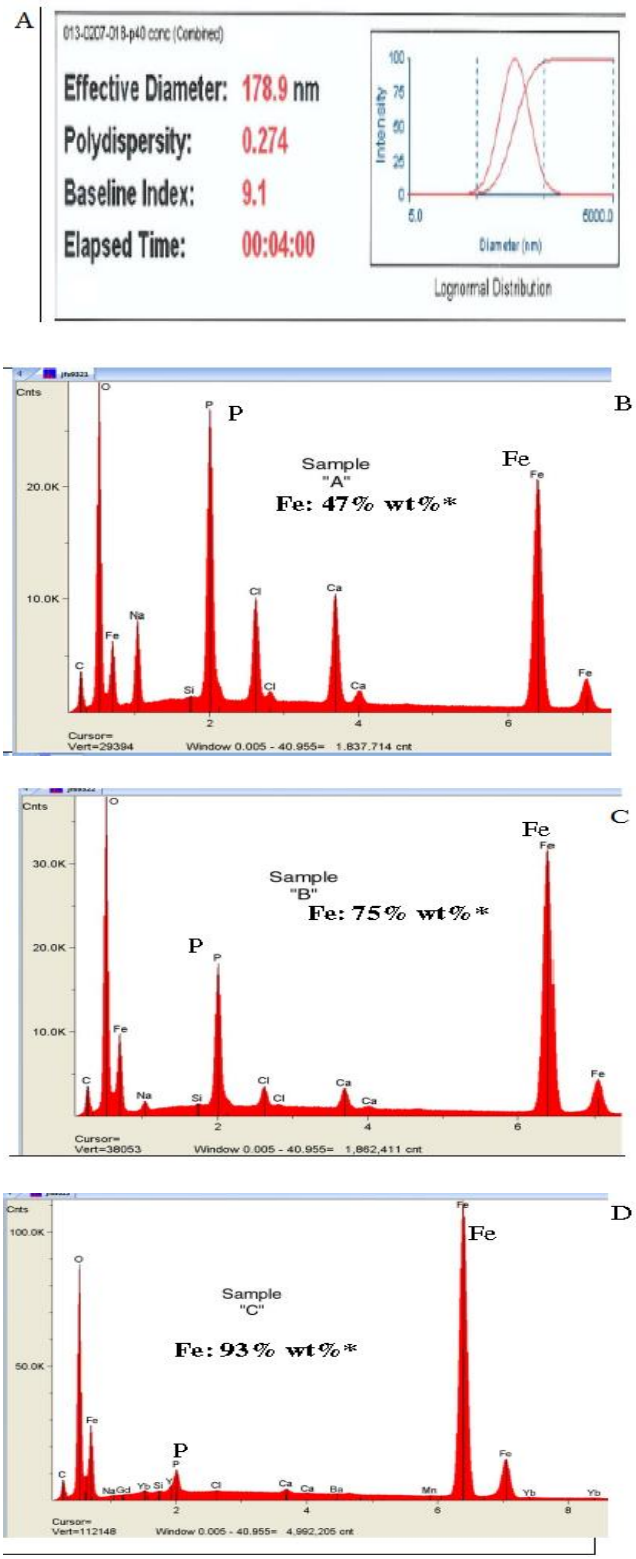


Figure 2. Particle size and EDX analysis of three different formulations of MCaP NPs. A) Particles of formulation C; B-D) EDX analysis of MCaP NPs. *Note: results do not include elements with Z<11 (Na).

Drug-loading and release of MCaP NPs

CaP particles are widely used for drug conjugation due to electrostatic interaction of the surface with small molecules. Docetaxel has four hydroxyl groups and an ester group in its chemical structure. We expect that this chemotherapy drug can be readily attached to the MCaP NP surface due to electrostatic interactions at pH value higher than 6.5 (Chitosan amine pKa 6.5, docetaxel pKa 12.02) [7]. A series of experiments (changing the buffer pH will change the charge of docetaxel) is aimed at attaching docetaxel to NPs, followed by characterization of drug loading. 1 gram of Docetaxel is resuspended in 10 mL of 100% EtOH and mixed quickly for 10 minutes. 3 mL of this100 mg/mL Docetaxel solution is added drop wise to 3 mL of MCaP NP (18.6 mg/mL) solution. We noticed that the particle size gradually increase, indicating the loading of docetaxol on MCaP NPs surface. Drug-loaded NPs are magnetically separated and analyzed by UV-vis. The absorbance in the EtOH supernatant is recorded at 230 nm. It is concluded that 8 mg of Taxel can be loaded onto 9.3 mg of P40 MCaP NPs.

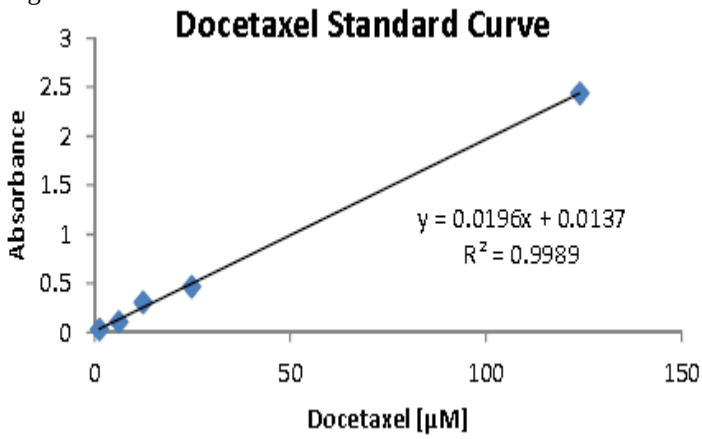


Figure 3. Standard calibration curve of Docetaxel in EtOH (Absorbance at 230 nm v.s. concentration).

A known amount of docetaxel is dissolved in 13% EtOH in saline buffer and added to a MCaP suspension. After mixing in an orbital shaker for 2 hrs, the NP suspension is ultracentrifuged at 12000 rpm for 30 min. The drug concentration in the supernatant is determined by HPLC method. Using this data, the drug bound to the CaP NPs can be calculated. Polysorbate 80 (Tween 80) is added due to its use for preparation of docetaxel clinically. A known amount of lyophilized NP powder is suspended in phosphate buffer saline (PBS) solution. After designated time point (10 min, 30 min, 1 hr, 1 day), the solution is ultra-centrifuged at 20000 for 30 min. Meanwhile, the drug released in the supernatant has been determined. Under the AMF, several samples were heated to specified temperatures (37, 39, 41, and 43°C) and the cumulative drug release was measured.

Investigate drug release of MCaP NPs at various temperature and time points

Aliquots of drug-loaded MCaP were exposed to varying conditions for 30 minutes each to observe the release of docetaxel as a function of temperature. To achieve 37°C, a standard incubator was used. Thermal induction by an alternating magnetic field (AMF) was used to yield the 43°C temperature. Prior to experimentation, the nanoparticles were centrifuged at 20,000 rpm and washed with water to ensure there was no free docetaxel in solution. A similar method to quantify docetaxel as shown in previous report [7] was used to determine the amount of docetaxel released. After exposure to each experimental condition, magnetic separation was used to separate drug loaded MCaP from the supernatant containing released docetaxel. The supernatant was analyzed at 230 nm for absorption of Docetaxel using UV-vis spectrometer. The percentage of drug release from MCaP NPs was shown in **Table 1**.

Table 1: Drug release from MCaP NPs at various temperature for 30 min..

P90 experiment	Docetaxel released [µM]	Released docetaxel (%)
Room temp	0.202	38.6
37°C	0.243	43.5
43°C	0.429	57.0

Intraperitoneally injection of docetaxel at 4 mg/kg dose in a 100 µl volume has been shown to induce partial inhibition of human prostate xenograft growth. For a mice (about 25 gram), only 4*25= 100 µg of docetaxel is needed in 100 µl NP suspension. Assuming chosen NP concentration is about10 mg/mL, we only needs to achieve loading at 100 µg docetaxel /1 mg of NPs. If we chosen NP concentration at 100 mg/mL, the loading only needs to be 10 µg docetaxel /1 mg of NPs. Our previous research suggested that both loading is easily achievable [7].

2.2 Heating Characterization on Drug Loaded MCaP NPs

NP hyperthermia by induction heating

NP formulation C (Figure 2) with the higher Fe loading was heated up using AMF. As shown in Figure 4, at two different concentrations, MCaP NPs can be heated up to 60 °C within 6 minutes. Based on the initial slope of Figure 4 (within 60 seconds the temperature increase 7 °C), heat capacity of water (4187J/(kg*K), iron concentration (12 mg of Fe in 3 mL volume), and equation below, it was determined the SAR value of MCaP NPs is 41 W/g(Fe).

$$SAR = C \times \frac{\Delta T}{\Delta t} \times \frac{1}{M(Fe)}$$

loading at 100 µg docetaxel /1 mg of NPs. If we chosen NP concentration at 100 mg/mL, the loading only needs to be 10 µg docetaxel /1 mg of NPs.

MCaP NP hyperthermia by using AMF that is the high frequency heat system (IHG061A) with maximum current

30A, 220V voltage, output frequency 100-500 KHz, and maximum. oscillating power 6.6 KW that was purchased from Across International Company (NJ).

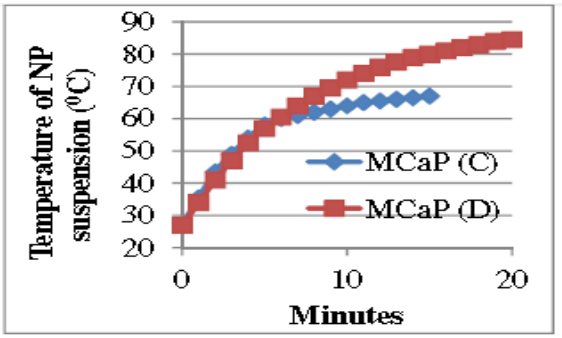


Figure 4. The induction heating capabilities of our prepared MCaP NP suspensions at two concentrations in water (water has higher heat capacity compared to human tissue, more difficult to heat up). The MCaP NPs can be heated to 60 °C within 6 mins. MCaP (D) has a concentration of 18.6 mg/mL. MCaP (C) has a concentration of 12.4 mg/mL.

NP accumulation at minimal concentration inside tumor:

Due to the difficulty to obtain soli, dead tumor tussies from tiseue banks, we have chosen to use tumor phantom as a tumor substitute for this subtask. A similar procdure as described inliterature was adopted [8]. Briefly, various amounts of dried magnetic MCaP NPs were mixed with 3 mL of sodium alginate (1.8 w/v %, Inotech AG, Switzerland, Cat# IE- 1080), then 0.8 mL of 1M $CaCl_2$ solution was injected into each of the above mixture to form a solid cylinder. These hydrogel cylinders were used as tumor phantoms. Also a piece of porcine meat cut into squares of 2mm thickness of skin, 7 mm of subcutaneous fat tissue, and 24 mm of muscle layers was used as tissue phantom. Each tumor phantom loaded with different concentration of MCaP NPs (e.g., 16.9 mg/mL or 19mg/mL) was placed inside the tissue phantom similar to the literature, mimicking subcutaneous tumor [8]. A thermometer was placed inside to monitor the temperature in real time. In the meantime, an IR thermo imager was also used for thermo imaging of the tumor phantom.

As shown in Figure 5, A minimum concentration at 16.9 mg/mL of MCaP NP inside solid tumor phantom is needed so that the temperature within tumor can reach 44 ℃ or higher. The higher the NP accumulation inside the tumor phantom, the higher temperature can be achieved by AMF.

3. CONCLUSIONS

Based on the preliminary tests on nanoparticle of Fe-n-CaP, there are further studies are underway so such to reduce the size of the nanoparticle size as possible; and determine whether intrsvenously injected MCaP NPs can accumulate inside the solid tumor tissue in mice at the minimal concentration.

Acknowledgement

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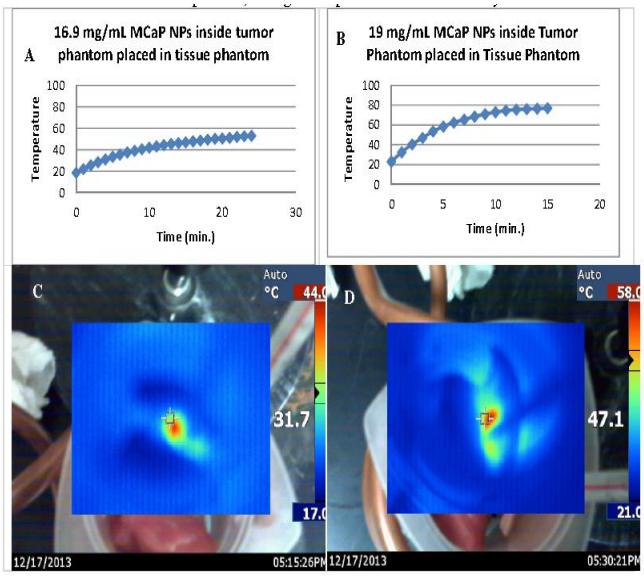

Figure54. A-B) heating curve of tumor phantoms placed inside tissue phantom. C-D) IR thermal images of the tumor phantom placed inside the tissue phantom.

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