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Thermosensitive gemcitabine-magnetoliposomes for combined hyperthermia and chemotherapy

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Abstract
The combination of magnetic hyperthermia therapy with the controlled release of chemotherapeutic agents in tumors may be an efficient therapeutic with few side effects because the bioavailability, tolerance and amount of the drug can be optimized. Here, we prepared magnetoliposomes consisting of magnetite nanoparticle cores and the anticancer drug gemcitabine encapsulated by a phospholipid bilayer. The potential of these magnetoliposomes for controlled drug release and cancer treatment via hyperthermic behavior was investigated. The magnetic nanoparticle encapsulation efficiency was dependent on the initial amount of magnetite nanoparticles present at the encapsulation stage; the best formulation was 66%. We chose this formulation to characterize the physicochemical properties of the magnetoliposomes and to encapsulate gemcitabine. The mean particle size and distribution were determined by dynamic light scattering (DLS), and the zeta potential was measured. The magnetoliposome formulations all had acceptable characteristics for systemic administration, with a mean size of approximately 150 nm and a polydispersity index <0.2. The magnetoliposomes were stable in aqueous suspension for at least one week, as determined by DLS. Temperature increases due to the dissipation energy of magnetoliposome suspensions subjected to an applied alternating magnetic field (AMF) were measured at different magnetic field intensities, and the values were appropriated for cancer treatments. The drug release profile at 37 °C showed that 17% of the gemcitabine was released after 72 h. Drug release from magnetoliposomes exposed to an AMF for 5 min reached 70%.

Keywords: Brazilian MRS, magnetite nanoparticles, magnetoliposome, magnetic hyperthermia, drug release

(Some figures may appear in colour only in the online journal)
1. Introduction

Gemcitabine is a cytotoxic drug that is commercially available as an aqueous solution of its salt, gemcitabine hydrochloride. Gemcitabine can be administered intravenously or as an infusion, and it has exhibited efficacy in the treatment of a wide variety of solid tumors and tumor cells [1, 2]. Gemcitabine is rapidly metabolized in the blood, liver, kidney and other tissues, with a short half-life of 8–17 min. However, only a small fraction of gemcitabine is converted to active metabolites; 91%–98% of the administered drug is converted to the inactive metabolite [1, 3]. Consequently, high gemcitabine dosages are required to achieve the appropriate therapeutic dose of the active metabolites, increasing the risks of toxicity and side effects [1, 3, 4].

Carrier systems are an interesting alternative for increasing the effectiveness of gemcitabine and reducing side effects. Various formulations, including aerosols [5–7], phospholipid conjugates [8–10] and encapsulation in polymeric nanoparticles [11], have yielded improvements in drug activity. Promising results have also been obtained for liposome formulations [9, 12].

The gemcitabine molecule is neutral at physiological pH and has a low molecular weight, enabling rapid diffusion through the liposome bilayer. Preparation techniques affect the encapsulation capacity of gemcitabine in liposomes. High encapsulation rates have been obtained for liposomes prepared by hydration of the lipid film and subsequent freeze/thaw cycles to yield unilamellar vesicles. In general, formulations in which gemcitabine is encapsulated in liposomes exhibit enhanced anticancer activity in vitro due to increased drug concentration within cells and prolonged half-life of gemcitabine in the plasma. In vivo evaluations of these formulations have also demonstrated improved drug efficacy [13–15].

In this work, we describe the preparation and characterization of gemcitabine-loaded magnetoliposomes as a combination of magnetic hyperthermia therapy and chemotherapy. These magnetoliposomes exhibit superior properties compared with similar systems described in the literature [13–15] because a minimal amount of organic solvent was used in the preparation to obtain an encapsulation efficiency of 54%. In addition, the properties of the magnetoliposomes, including particle size and polydispersity index (PDI), were appropriate for in vivo applications.

2. Experimental details

2.1. Synthesis of the magnetic nanoparticles

The magnetite nanoparticles (Fe₃O₄) were synthesized using the coprecipitation method. A mixture of FeCl₃·6H₂O and FeCl₂·4H₂O with a stoichiometric ratio of 2:1 (Fe³⁺/Fe²⁺) was prepared in basic medium. Functionalization was performed by adding citric acid to the magnetite nanoparticle suspension in water. The suspension was then stirred for 30 min at 80 °C. The sample was washed with water to remove excess citric acid and was lyophilized. The resulting material was referred to as ca-Fe₃O₄, corresponding to magnetite nanoparticles functionalized with citric acid. To prepare an aqueous suspension of ca-Fe₃O₄ with a magnetite nanoparticle concentration of 1 g l⁻¹, powdered functionalized magnetite nanoparticles were resuspended in water at near-neutral pH (7.0–7.4) and sonicated for 6 h [16].

2.2. Magnetoliposome preparation

Magnetoliposomes-gemcitabine (gem-ML), magnetoliposomes (ML) and blank liposomes (L) were prepared using the thin film hydration method [16]. Briefly, DPPC/cholesterol (12:3 mg) was dissolved in 5 ml of chloroform, and the solvent was removed at 40 °C using a rotary vacuum evaporator to form a thin film. For the gem-ML sample, the ca-Fe₃O₄ aqueous suspension containing gemcitabine was hydrated at 50 °C for 4 h. The formulation, which had a molar ratio of 0.09/0.07/0.95 ca-Fe₃O₄/gem/DPPC, was further centrifuged to remove unencapsulated magnetic nanoparticles and gemcitabine. The pellet was then resuspended in water and extruded through polycarbonate membranes with 100 nm pores to obtain unilamellar liposomes. The samples were stored at 4 °C. To prepare the ML samples, only a ca-Fe₃O₄ aqueous suspension (no gemcitabine) was used in film hydration. For the L samples, hydration was performed using phosphate buffer.

2.3. Physicochemical characterization

X-ray diffraction (XRD) patterns were obtained using a Rigaku diffractometer (model Geigerflex) and Co(Kα) radiation by sweeping from 20° to 80° (2θ) at a rate of 4° per min. Standard silicon was used as the external standard. The XRD data were numerically refined using the least-squares method with the Fullprof fitting program (version January 2011).

The Mössbauer spectrum was collected in constant acceleration transmission mode with an ~15 mCi Co⁶⁷/Rh gamma-ray source. The spectrometer was equipped with a transducer unit (CMTE model MA250) controlled by a linear function driving unit (CMTE model MR351). The spectrum was obtained at 298 K. Values of isomer shifts are quoted relative to an αFe standard foil at room temperature. The experimental resonance lines were numerically analyzed according to model-independent hyperfine field distributions by least-squares fitting data using the algorithm in NORMOS-90™ software.

Fourier transform-infrared (FTIR) spectra were obtained in transmission mode on a Perkin-Elmer Spectrum GX spectrometer. Samples were ground, dispersed in KBr, and pressed as tablets. The spectra were obtained at an interval of 4000–400 cm⁻¹ with a resolution of 4 cm⁻¹.

The iron oxide in the ca-Fe₃O₄ sample was quantified by a colorimetric method with ortho-phenanthroline. The absorbance of the solutions was determined at 510 nm in a UV–Visible Shimadzu UVmini Spectrophotometer [17].
The mean hydrodynamic diameter and PDI of the iron oxide nanoparticle suspension were determined by dynamic light scattering (DLS; Nano Size ZS equipment). Intensity correlation functions were measured at a scattering angle of $\theta = 90^\circ$ and a wavelength of 633 nm. All reported hydrodynamic diameters were calculated using the Stokes–Einstein equation, $r_h = kT/(6\pi \eta D)$, where $k$ is Boltzmann’s constant, $T$ is temperature, $\eta$ is solvent viscosity, $D$ is a diffusion constant, and $r_h$ is the hydrodynamic radius of the spherical particle. The zeta potential of the iron oxide nanoparticle suspensions was determined by electrophoretic mobility of charges using phase analysis light scattering on a Nano Size ZS. For these measurements, the ca-Fe$_3$O$_4$ and Mag samples were dispersed in water and sonicated for 6 h. The pH of these solutions was adjusted with either aqueous $10^{-3}$ mol l$^{-1}$ KOH or $10^{-3}$ mol l$^{-1}$ HNO$_3$, and the particle sizes were determined as a function of pH.

Transmission electron microscopy (TEM) micrographs of the samples were acquired with a Tecnai G2-20 Super Twin FEI microscope at 200 kV. Several drops of the nanoparticle suspension were deposited on the microscope grid, and images were obtained after solvent evaporation.

Magnetization measurements were performed using a vibrating sample magnetometer (Lake Shore 7404) with a noise base of $1 \times 10^{-6}$ emu, a time constant of 300 ms at room temperature, and a maximum magnetic field of 2 T.

2.4. Drug release studies

2.4.1. Drug release from magnetoliposomes at physiological temperature. Drug release studies were conducted under incubation at physiological temperature (37 °C). Briefly, 0.5 ml of the gem-ML suspension was placed in a 15 ml tube containing 0.5 ml of phosphate-buffered saline (PBS, pH 7.4). The magnetoliposome solution was centrifuged at 5000 rpm for 5 min at 4°C after 24, 48 and 72 h; then, the supernatant was removed and lyophilized. Gemcitabine was quantified using a Varian model Pro Star 210 with a 5 µl loop injection valve. The chromatographic system was equipped with a UV detector, which was set at the maximum absorption of gemcitabine.

2.4.2. Drug release from magnetoliposomes under an AC magnetic field. Magnetoliposome samples (0.5 ml) were placed in vials and exposed to AMF using the same procedure as described for the heat dissipation study. After being exposed to an AC magnetic field for specific periods of time (30–300 s), gem-ML samples were dispersed and centrifuged at 5000 rpm for 5 min. The supernatant was collected and lyophilized. The residue was reconstituted with water/ acetonitrile (40:60 v/v), and 20 µl was analyzed by HPLC.

2.5. Heat dissipation measurements

Heat dissipation was measured using EASYHEAT equipment (Ambrell). The nanoparticles were suspended in water, placed inside plastic tubes, and positioned in the middle of an induction coil with a frequency of 356 kHz. An alternating magnetic field (AMF) was applied initially for 30 s. The temperature increase after AMF exposure was measured with a digital thermometer (WT-1). The procedure was repeated after increasing the exposure time in increments of 30 s.

2.6. Mesenchymal stem cell (MSC) isolation and culture

MSCs were isolated from adipose tissue depots of Lewis rats. Ingual adipose tissue was collected from 6 week old rats, washed with PBS and digested with 0.1% collagenase type I (Sigma-Aldrich) in PBS for 1 h under agitation at 37 °C. Collagenase activity was inhibited by the addition of fetal bovine serum (FBS; Gibco), and the digested tissue was centrifuged at 330 g for 10 min. The cell pellet was resuspended and plated in culture flasks containing high-glucose Dulbecco’s modified Eagle’s medium (DMEM; Sigma-Aldrich) supplemented with 10% FBS and 100 U ml$^{-1}$ penicillin/streptomycin (Life Technologies). The cell cultures were incubated in a humidified atmosphere with 5% CO$_2$ at 37 °C for 24 h before the first medium change; thereafter, the medium was changed every 2 days. The mesenchymal population was isolated based on its ability to adhere to the culture plate. At 80%–90% confluence, the cells were detached using 0.25% trypsin-EDTA (Gibco) and replated in new flasks at a 1:3 ratio. MSCs at passage 3 were used in all experiments. The experiments were approved by the local ethics committee on animal experimentation (CEUA/UFMG, protocol 168/2013).

2.7. Biocompatibility and cytotoxicity assays

The biocompatibility of ca-Fe$_3$O$_4$, ML, L, and gem-ML was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Invitrogen) as previously described [18]. The MTT assay measures mitochondrial activity based on the reduction of tetrazolium salt to formazan crystals by live cells. Briefly, 1 x 10$^5$ cells/well were seeded into 24-well plates. After incubation for 24 h, the cells were treated with 210 µl of freshly prepared sample solutions (ca-Fe$_3$O$_4$, ML, L or gem-ML). At each time point (24, 48, and 72 h), the medium was removed, and 170 µl of MTT solution (5 mg ml$^{-1}$) and 210 µl of new basal medium were added to each well. After 2 h, the formazan crystals were dissolved in 210 µl of 10% SDS in 0.01 M HCl. After 18 h, 100 µl of the solution was transferred to a 96-well plate, and the optical density was measured at 595 nm.

3. Results and discussion

3.1. Characterization of magnetite nanoparticles

Comparison of the XRD patterns (figure 1) with the iron oxide standards (PDF 19-629) confirmed the formation of the spinel structure of Fe$_3$O$_4$ nanoparticles. The crystal size obtained for the Fe$_3$O$_4$ sample using Scherer’s formula was approximately 9.0 nm.

The experimental Mössbauer spectrum (figure 2) for the Fe$_3$O$_4$ sample was fitted using the least squares method with
two blocks of model-independent hyperfine field distribution (figure 3): one for the A-sites (Fe$^{3+}$–O in tetrahedral coordination sites) and the other for the B-sites (electronically coupled Fe$^{3+}$–O and Fe$^{2+}$–O in octahedral sites) of the magnetite structure. The resonance lines were rather asymmetrically broadened compared with the typical symmetrical Lorentzian shape, as expected for pure bulk magnetite. Using this model, the isomer shift values were made to vary linearly with the hyperfine field values. Regarding the values of the Mössbauer parameters (table 1), the hyperfine field at maximum probability, $B_{hf}^{max}$, was at 48(1) T with a corresponding isomer shift relative to $\alpha$Fe, $\delta/\alpha$Fe, of 0.29(3) mm s$^{-1}$ for the tetrahedral site and at $B_{hf}^{max} = 46(1)$ T and $\delta/\alpha$Fe = 0.57 (3) mm s$^{-1}$ for the octahedral site. These data, together with the XRD data, confirmed the presence of magnetite in the Fe$_3$O$_4$ sample. According to this model, a satellite (RA = 5.3% of the whole distribution for this distribution block; table 1) maximum appears on the probabilities profile. The corresponding $\delta/\alpha$Fe = 1.01 mm s$^{-1}$ is rather indicative of ferrous instead of mixed valence iron, but this assignment cannot be confirmed from these data only.

The observed differences between the relatively complex Mössbauer spectrum obtained for this magnetite sample and the expected characteristic spectrum for pure bulk magnetite could have resulted for several reasons. According to published data [19, 20], a narrow particle size distribution may lead to inhomogeneous hyperfine coupling and asymmetric line broadening. Magnetic relaxation effects in an ensemble of small particles with a given size distribution may cause line broadening and asymmetry of the resonance lines relative to the theoretical Lorentzian shape [19].

The adsorption of citrate on magnetite nanoparticle surfaces was investigated using FTIR spectroscopy (figure 3). The characteristic peaks at 587 cm$^{-1}$ and 481 cm$^{-1}$ correspond to the symmetric and asymmetric stretch of the Fe–O bond of iron oxide. The characteristic peak at 1722 cm$^{-1}$ for citric acid (C–O vibration) was not observed in the ca-Fe$_3$O$_4$ spectra, indicating that citric acid binds the magnetite surface of the nanoparticles by adsorption of citrate anions. After citrate binding to the magnetite surface, the asymmetric (1581 cm$^{-1}$) and symmetric (1390 cm$^{-1}$) C–O stretching
shifted to 1622 cm\(^{-1}\) and 1401 cm\(^{-1}\), respectively, due to the C–O interaction with the Fe ions of the magnetite nanoparticle surface. The intense band at 3414 cm\(^{-1}\) is attributable to the stretching of hydroxyl groups, including those from residual water molecules adsorbed on the surface of the iron oxide structure, and the carboxylate of the carboxylic acid groups. The peak observed at 2924 cm\(^{-1}\) is related to stretching of the –CH\(_3\) group.

The TEM micrographs presented in figure 4 indicate that the ca-Fe\(_3\)O\(_4\) nanoparticles had a nearly spherical shape and uniform size and were not agglomerated. The size distribution obtained from the TEM image analysis is presented in the inset of figure 4(a). The histogram shows that the diameter of ca-Fe\(_3\)O\(_4\) ranged from 2 to 15 nm, with an average mean size of 7 ± 2 nm according to a log-normal distribution. This size range is desirable for superparamagnetic particles.

Figure 5 shows the virtual absence of hysteresis loss in the magnetization curve. At the applied field of 1.2 T, the sample presented a saturation magnetization of 61 emu g\(^{-1}\), which is somewhat lower than the reported value of 92–100 emu g\(^{-1}\) for magnetite nanoparticles. This behavior may be attributed to the nanocrystalline magnetic particle size; the particles may be single domain and thus exhibit some magnetic relaxation. Based on the TEM image, these ca-Fe\(_3\)O\(_4\) nanoparticles have an average diameter of 7 nm.

### 3.2. Characterization of magnetoliposomes

The formulations were characterized by DLS and zeta potential measurements. The hydrodynamic diameters of ML and gem-ML were adequate for a drug delivery system. DLS analysis demonstrated that ML and gem-ML exhibited monomodal distributions centered at hydrodynamic diameters of 142 nm and 145 nm, respectively. The PDI was approximately 0.10 for both samples. The zeta potentials of ML and gem-ML were −10 mV and −9 mV, respectively.

The size and zeta potential of gem-ML were followed over 14 days. The average hydrodynamic diameter and PDI of gem-ML remained stable for at least 4 days (between 140 and 145 nm), suggesting that the gem-ML remained monodispersed and did not appreciably aggregate (figure 6). Between days 4 and 7, the hydrodynamic diameter increased to 179 nm. After day 7, a new peak with a much larger diameter (\(D = 896\) nm) was observed and was attributed to cluster formation. This new peak was accompanied by an increased PDI from 0.16 to 0.18. The change in the zeta potential (−3.20 mV) may indicate drug release into the medium.

The stability of gem-ML was evaluated as a function of temperature. As shown in figures 7(a) and (b), at physiological temperature, the size and zeta potential remained unchanged; however, at 42 °C and 56 °C, the size increased, and the zeta potential (modulo value) decreased, indicating drug release into the medium. The data obtained for the ML sample, which are also presented in figures 7(a) and (b), demonstrated that the zeta potential and size of ML and gem-ML differed only at temperatures greater than 42 °C, supporting the proposed drug release.

The encapsulation efficiencies of the gemcitabine and ca-Fe\(_3\)O\(_4\) nanoparticle magnetoliposomes were 54% and 36%, respectively. These encapsulation efficiencies were superior to those previously reported for gemcitabine-encapsulated magnetoliposomes. An encapsulation efficiency of approximately 10% was reported for gemcitabine magnetoliposomes encapsulated by reverse phase evaporation [7, 8].

### 3.3. Heating efficiency

The magnetic heating characteristics of the magnetoliposomes were determined in an AMF (amplitude, 30 kAm\(^{-1}\); frequency, 356 kHz). The resulting heating profile is presented in figure 8.

For hyperthermia applications, the temperature of cancerous tissue must reach 42 °C–45 °C for effective therapy. As shown in figure 8, the samples are appropriate for effective hyperthermia treatments. When the samples were exposed to an AMF for 5 min, the temperature ranged from 32 °C to approximately 56 °C. The curve revealed relatively rapid temperature increases at intervals of 30 s. After 3 min, an approximate steady state was established. These results indicate that the magnetite can sufficiently elevate the temperature of the environment to induce hyperthermia. Efficient heat dissipation can be attributed to the superparamagnetic nature of magnetite nanoparticles, as shown by the magnetization curve (figure 5).

### 3.4. Drug release from magnetoliposomes under an AC magnetic field

The thermosensitivity and drug release profile of the magnetoliposomes were evaluated in the presence of an AMF and at physiological temperature (37 °C).

Drug release from the magnetoliposomes under an AMF was 70% after 5 min of exposure (figure 9(a)). During the first minute, the heat produced from the magnetic nanoparticles increased the temperature by 7 °C, which was sufficient to
Figure 4. TEM micrograph of a ca-Fe₃O₄ nanoparticle. (a) Scale bar, 50 nm (inset: particle size and size distribution). (b) Scale bar, 5 nm.

Figure 5. Magnetization curve for this nanosized ca-Fe₃O₄ sample at room temperature.

Figure 6. Variation in the hydrodynamic diameter and zeta potential of the gem-ML sample as a function of time.

Figure 7. (a) Variation in the hydrodynamic diameter of ML and gem-ML as a function of temperature. (b) Variation in the zeta potential of ML and gem-ML as a function of temperature.
induce a phase transition of the lipid and release 40% of the encapsulated drug.

Measurements of drug release at physiological temperature (37 °C) after 24, 48 or 72 h (inset figures 9(a) and (b)) revealed that the maximum drug release during the maximum period (72 h) was only 17% of the encapsulated gemcitabine.

3.5. Cell viability studies

MTT assays were performed to examine the effects of ca-Fe₃O₄, ML and gem-ML on cell viability. As shown in figure 10, high cell viability (greater than 80%) was observed after 24 h of incubation for all samples. A time-dependent cytotoxic effect of all samples on MSCs was observed.

The observed cell viability was approximately 90%, 90% and 80% after 24 h of incubation with ca-Fe₃O₄, ML and gem-ML, respectively. The viability of cells incubated with ca-Fe₃O₄ and ML did not differ significantly at 48 h; however, the viability of cells incubated with ML decreased significantly at 72 h (60%). This decrease in viability may be attributable to the lipid components rather than to iron oxide.

The viability of cells incubated with gem-ML gradually decreased with incubation time, with values of 80%, 60% and 40% at 24 h, 48 h and 72 h, respectively. This decrease in viability indicates a significant cytotoxic effect of the drug in this formulation; the drug is released as demonstrated in the drug release studies (inset figure 9(a)). The magnetoliposomes were designed to deliver gemcitabine only to the tumor region upon AMF stimulation. Accordingly, at 37 °C without AFM exposure, only a very small amount of drug was released; this drug release was not sufficient to significantly decrease cell viability during the first 24 h.
4. Conclusions

Thermosensitive magnetoliposomes containing the hydrophilic drug gemcitabine were prepared. The evaluation of gemcitabine magnetoliposome encapsulation indicated that the properties of the formulation were suitable for combination hyperthermia and chemotherapy treatments.

The methodology was effective and produced thermosensitive magnetoliposomes containing both magnetic nanoparticles and gemcitabine. The physicochemical properties of the magnetoliposomes, such as hydrodynamic diameter, zeta potential and PDI, were appropriate for cancer treatment involving hyperthermia.

The effect of gemcitabine on the magnetic nanoparticle encapsulation efficiency was negligible when high concentrations of magnetic nanoparticles were used.

Heat generation by the gem-ML sample increased the temperature of the medium to 53 °C, which is sufficient to induce hyperthermia.

The effectiveness of magnetoliposomes as a gemcitabine carrier was also demonstrated, with an encapsulation efficiency of 55%. During the first 24 h at physiological temperature, a very small amount of the drug was released (5%) with no increase in toxicity.

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References

[1] Gravem H 2006 Gemcitabine-containing liposomes MS Dissertation Tromso University, Tromso, Norway