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Magneto low-density nanoemulsion (MLDE): A potential vehicle for combined hyperthermia and photodynamic therapy to treat cancer selectively

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ABSTRACT

In this paper, we introduce a new drug delivery system (DDS) called magneto low-density nanoemulsion (MLDE), which can carry maghemite nanoparticles and Chlorin e6 as an active photosensitizer drug. This design can enhance tumor damage after minor heat dissipation and/or minimum visible light photosensitization doses by classical magnetic hyperthermia (MHT) and photodynamic therapy (PDT), respectively. We establish protocols to prepare the MLDE and to load the drug combination onto it. The MLDE prepared herein is nanometric (< 200 nm), has high encapsulation efficiency, and is stable for at least 12 months in water dispersions. Flow cytometry results demonstrated that MLDE presents targeted selectivity toward the MCF-7 breast cancer cell line but not in NHI-3T3 mouse fibroblast cell lines, because the MCF-7 cancer cell surface contains overexpressed low density lipoprotein (LDL) receptors. Despite this targeted effect, MHT or PDT alone does not prompt significant antiproliferative outcomes. On the other hand, MHT and PDT in combination induce a strong and synergic action on MCF-7 cells and reduce the cell viability. In conclusion, the developed MLDE deserves further investigation because it is biocompatible, displays good encapsulation efficiency, and is highly stable. Moreover, it is selectively taken up by cancer cell surfaces with receptor recognition based on LDL receptor overexpression, which potentiates the action of combined MHT and PDT.

1. Introduction

Recent interest in the engineering and synthesis nanosized biocompatible magnetic materials has offered new perspectives on their biological and medicinal applications. Magnetic fluids are a crucial platform to develop active magnetic materials for diagnostic and therapeutic purposes [1, 2]. In the beginning, these materials were mainly used as contrast agents for magnetic resonance imaging. More recently, magnetic fluids have been successfully tested as key materials for local magnetic-induced hyperthermia (MHT). In fact, application of an external AC magnetic field raises the local temperature and causes different kinds of tissue injury [1, 3, 4]. Several studies have demonstrated that mild hyperthermia (temperature rise to about 41–43 °C) triggers cell degeneration primarily by apoptotic and immune-mediated mechanisms, which makes this protocol a valuable option for cancer treatment [4, 5]. These systems have recently been used as multifunctional tools that control drug release rate by magnetic hyperthermia and generate heat also by a photothermal effect [6–9]. Furthermore, magnetic particles can target tumors: application of an external magnetic field can drive the material to a specific site in the body [10].

The many advantages of magnetic particles have motivated their combination with different anticancer therapies. The so-called “combination therapy” consists in administering two or more drugs/treatment modalities with different mechanisms of action in an attempt to overcome resistance mechanisms, to lower the required drug dose and hence the side effects, and to increase the therapeutic efficacy through a synergic effect [11, 12]. In this scenario, mild hyperthermia has been successfully combined with photo-responsive therapies [13], especially photodynamic therapy (PDT), which employs non-ionizing light [14].

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PDT is a therapeutic procedure that uses a light-activated photosensitizer (PS) to produce reactive oxygen species, chiefly singlet oxygen ($^1O_2$), to trigger tumor cell destruction, tumor vasculature damage, and severe inflammatory action [15, 16]. Dereski and collaborators demonstrated that mild MHT inhibits sub-lethal damage repair, thereby making tumor cells more sensitive to PDT [17]. This effect is corroborated by recent research showing that combined PDT and MHT exert highly cytotoxic effects at low drug doses [14, 18, 19]. Our research group demonstrated that PDT and MHT in combination elicit a synergic tumor response in glioblastoma and mesenchymal stem cell lines [20, 21]. On the other hand, Hirschberg et al. reported that PDT and MHT alone is not effective against human or rat glioma spheroids as cancer models [22].

PSs and magnetic fluids are insoluble in aqueous media, which highlights the importance of developing new drug delivery systems (DDSs) that allow drug co-encapsulation. Another important issue is to achieve selective and controlled delivery of the optimum drug ratio in the same area of the tumor site, to obtain enormous clinical advantages [23, 24]. Selective targeting therapy is a promising approach; it requires a nanosized DDS that is stable enough in the plasma circulation and that possesses the ability to reach the targeted cells [11, 25]. Some years ago, our research group introduced a new DDS called magneto low-density nanoemulsion (MLDE) [26, 27]. LDEs are biocompatible cholesterol-rich nanoemulsions. In the body, they bind to low-density lipoprotein (LDL) receptors, which are normally overexpressed on the cancer cell surface, to deliver the drug directly to the target cancer cells [28]. This new biocompatible material was engineered to entrap both the PS and the magnetic fluid in its oil-rich core, to enable the synergic action of the PDT and MHT therapies. The LDE design enhances tumor damage after minor heat dissipation and/or minimum visible light photosensitization doses, which provides a new perspective on the combination of magnetic materials and photodynamic-based processes [21].

The present study represents a step forward as it introduces a new class of multifunctional nanomaterials that combine the PDT and MHT actions. More specifically, we use citrate-coated maghemite (γ–Fe_2O_3) nanoparticles and the PS molecule chlorin E6 (Chl_{e6}), a second-generation PS with strong red absorbance and $^1O_2$ formation yield [29]. We define the DDS preparation procedures and describe its characterization and stability features. We determine the PS photophysical properties by steady-state and time-resolved optical spectroscopy. We investigate the DDS cytotoxicity during combined MHT/PDT to the MCF-7 human breast cancer and the NHI-3T3 mouse fibroblast cell lines by flow cytometry.

2. Experimental

2.1. Materials

Cholesteryl oleate (> 98%), egg phosphatidylcholine (99%), triolein (> 98%), fetal serum bovine (FBS), streptomycin, and penicillin G (all from Sigma) were purchased from Sigma-Aldrich (St. Louis, Missouri - USA). Cholesterol (> 99%) was acquired from Avanti polar lipids (Alabaster, Alabama - USA). The DMEM cell culture medium was purchased from Gibco (Thermo Fisher Scientific, Waltham, MA - USA). Chl_{e6} was obtained from Porphyrin Products Inc. (Logan, Utah – USA). Citrate-coated iron oxide nanoparticles (γ–Fe_2O_3) were supplied by professor Paulo Cesar de Maorais All the reagents were analytical grade and were used without prior treatment or purification.

2.2. LDE preparation

The LDEs were prepared by using a lipidic mixture consisting of 20 mg of cholesteryl oleate, 40 mg of egg phosphatidylcholine, 1 mg of triolein, and 0.5 mg of cholesterol dissolved in chloroform/methanol (2:1). The mixture was dried under N_2 for film formation and hydrated with phosphate-buffered saline (PBS, pH 7.4), as described by Silva et al. [30]. Lipid emulsification was carried out by ultrasonic irradiation followed by centrifugation with a density gradient form by Optprep®, (Optprep® 40%) in PBS. The LDE·Chl_{e6} nanoemulsion was prepared at a 10:1 ratio from a Chl_{e6} stock solution (50 μmol L^{-1}) in anhydrous ethanol. This solution was stirred on a magnetic stirrer at 56 °C for 6 h (Corning Model: Stirrer/Hotplate-Speed 9). A final volume of 200 μL of PBS was added to the empty LDE, to obtain a final Chl_{e6} concentration of 5.0 μmol L^{-1}. The LDE formulations (LDE and LDE.Chl_{e6}) associated with the magnetic fluid (γ–Fe_2O_3) were prepared by adding 2 μL of the citrate-coated γ–Fe_2O_3 to give a final concentration of 1.5×10^{13} particles mL^{-1}. The mixtures were shaken at 45 °C for 2 h, to afford MLDE and MLDE·Chl_{e6}. All the formulations were sterilized by passage through 0.22-μm pore polycarbonate filter (Millipore, Billerica, MA, USA) and stored at 4 °C until use.

2.3. LDEs: characterization and stability studies

The LDE average size, polydispersity index (PDI), and zeta potential were investigated by dynamic light scattering (Malvern Instruments®, Nano ZS ZEN3600, UK). The analyses were performed with a He—Ne laser operating at 633 nm, scattering angle of 173°, and temperature of 25.0 ± 0.1 °C. The LDE·Chl_{e6} stability in PBS was measured by dynamic light scattering and Chl_{e6} quantification for 30 days. The efficiency of Chl_{e6} encapsulation into the LDE was estimated spectrophotometrically; $\varepsilon_{402} = 1.22 \times 10^5\ M^{-1}\ cm^{-1}$ was used. All the results are reported as the mean of three separate measurements ($n = 3$) ± SD.

2.4. Accelerated stability

The accelerated stability studies were carried out on the shelf life analyzer Lumisizer® (612 LumiSizer, LUM GmbH, Berlin, Germany). For this analysis, 400 μL of the LDE formulation was placed in cuvettes with maximum radial position of 129.5 mm. The sample was then centrifuged at 3000 rpm and constant temperature of 25 °C for 4.56 h. A transmission profile was obtained at a wavelength of 880 nm every 65 s. These parameters allowed us to predict that the formulations were stable for 12 months. The data were obtained with a centrifuge coupled with an optical system working in the NIR region, which recorded transmission profiles between the top and the bottom of a cuvette. The differences between the transmission profiles as a function of time enabled us to determine quantifying parameters such as the sedimentation velocity and the formulation stability and to estimate the product shelf life. The results were analyzed with the software V.5.1 SEPView (LUMGmbH, Berlin, Germany).

2.5. Chl_{e6} photophysical properties

The free Chl_{e6} and the MLDE·Chl_{e6} absorption and fluorescence spectra were recorded at room temperature (25 °C) and under constant stirring by using a Hitachi U-3000 spectrophotometer and a Spex Fluorog 3 (Jobin-Ivon) spectrofluorimeter, respectively. For the fluorescence spectra, the samples were excited at 400 nm, and the fluorescence signal was registered between 600 and 750 nm.

The excitation source used to measure the Chl_{e6} fluorescence lifetime consisted of a diode laser that pumps a solid-state laser (Nd:YVO4–Millenia Xs–Spectra Physics) that later goes through a crystal frequency folder, to give the final bunch with potency of 10 W and emission wavelength of 532 nm. This equipment pumps a titanium-sapphire laser (Tsunami–Spectra Physics). The titanium-sapphire crystal generates laser pulses (5-ps width) with maximum frequency repetition of 82 MHz. The fluorescence decline curves were analyzed with the operational software of the instrument itself. The adjustments were validated through the minimum value in the chi-square function (χ^2).