



pH- and thermosensitive thin lipid layer coated mesoporous magnetic nanoassemblies as a dual drug delivery system towards thermochemotherapy of cancer



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ABSTRACT

A new pH-sensitive and thermosensitive dual drug delivery system consisting of thin lipid layer encapsulated mesoporous magnetite nanoassemblies (MMNA) has been developed which can deliver two anticancer drugs simultaneously. The formulation of lipid layer used is 5:2:2 w/w, DPPC: cholesterol:DSPE-PEG₂₀₀₀:MMNA. The structure, morphology and magnetic properties of MMNA and lipid coated MMNA (LMMNA) were thoroughly characterized. This hybrid system was investigated for its ability to carry two anticancer drugs as well as its ability to provide heat under an alternating current magnetic field (ACMF). A very high loading efficiency of up to ~81% of doxorubicin hydrochloride (DOX) with an ~0.02 mg mg⁻¹ loading capacity and ~60% of paclitaxel (TXL) with an ~0.03 mg mg⁻¹ loading capacity are obtained with LMMNA. A sustained release of drug is observed over a period of 172 h, with better release, of ~88:53% (DOX:TXL), at pH 4.3 compared to the ~28:26% (DOX:TXL) in physiological conditions (pH 7.4). An enhanced release of ~72 and ~68% is recorded for DOX and TXL, respectively, during the first hour with the application of an ACMF (~43 °C). A greater in vitro cytotoxic effect is observed with the two drugs compared to them individually in HeLa, MCF-7 and HepG2 cancer cells. With the application of an ACMF for 10 min, the cell killing efficiency is improved substantially due to simultaneous thermo- and chemotherapy. Confocal microscopy confirms the internalization of drug loaded MMNA and LMMNA by cells and their morphological changes during thermochemotherapy.

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1. Introduction

During the past decade, dual drug delivery systems (DDDS) have invoked remarkable interest for use in a broad range of therapeutic applications. This is because certain limitations, like poor solubility of hydrophobic drugs [1,2], limited loading capacity of drug and poor colloidal stability, can be overcome by using DDDS, while they also have other advantages, including biodegradability [3], the minimization of side effects [4–7] and increased circulation time in vivo [8,9]. Researchers have investigated a number of DDDS, including poly(lactic-co-glycolic acid) (PLGA)–mesoporous silica nanoparticles [10], chitosan-containing PLGA nanoparticles [11], polymersomes [12], lipid–polymer hybrids [13], polymeric magnetic nanoparticles [14], nanoparticle–aptamer bioconjugates [1], magnetic mesoporous silica nanoparticles [15] and mesoporous

silica nanoparticles [16]. It has also been recently shown that the combination of two drugs shows synergistic effects, prevents more disease recurrence [1,17,18] and increases tumor regression capabilities compared to individual drugs in clinical studies [19,20]. Additionally, there are reports on synergistic effects towards cancer treatment using chemotherapy (using a single drug) along with thermal therapy [5,21]. It is known that drug release can be triggered by external stimuli, such as an AC magnetic field, an electric field, ultrasound, temperature and pH [21–24], which can enhance the efficacy of simultaneously released drugs in a synergistic manner. Superparamagnetic particles under an applied alternating current magnetic field (ACMF) can produce local heat that may further enhance drug release from its carrier. In addition, the heat so produced can simultaneously help in more cancer cell death due to thermal therapy. Thus, treatment with drugs in conjunction with heat can act as a combinatorial thermochemotherapy. With thermochemotherapy in mind, we have designed a new drug delivery system comprising a thin lipid layer encapsulating mesoporous magnetite (Fe₃O₄) nanoassemblies

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(MMNA) and demonstrated its use as a dual drug carrier where the drug release can be triggered by a change either in pH or in temperature by the application of an ACMF (Fig. 1).

The thin lipid layer is advantageous for drug delivery in that it: (i) prevents the agglomeration of particles; (ii) increases colloidal stability; (iii) is able to encapsulate both hydrophilic and hydrophobic drugs; and (iv) can control the drug release efficiency. The MMNA is encapsulated with a lipid formulation consisting of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), cholesterol (Chol) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino (polyethylene glycol)-2000] (DSPE-PEG₂₀₀₀). The formulation of LMMNA is made such that it is sensitive to both temperature and pH. Moreover, the functional PEG groups of DSPE-PEG₂₀₀₀ increase the circulation time and stability. The lipid layer protects the surface, improves the biocompatibility and prevents the agglomeration of MMNA in its aqueous phase [4,5,9]. These nanoassemblies, by virtue of their thermo- and pH sensitivity, may be used as stimuli responsive drug release systems in tumor tissue. In addition, these may be investigated not only as dual drug carriers but also as dual therapeutic agents [25–27]. They also have the potential to be used as contrast agents in magnetic resonance imaging [28].

2. Materials and methods

Iron(III) chloride hexahydrate (FeCl₃·6H₂O) and iron(II) chloride tetrahydrate (FeCl₂·4H₂O) were purchased from Sigma–Aldrich. Ethylene glycol (EG), ethylenediamine (EDA) and anhydrous ethanol were from Merck and sodium acetate was from Himedia. Doxorubicin hydrochloride (DOX) and paclitaxel (TXL) were obtained from Sigma Aldrich. DPPC, Chol, (DSPE-PEG₂₀₀₀) and *N*-(NBD-aminohexanoyl)-1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine sodium salt (*N*-NBD) were purchased from Sigma Aldrich. MilliQ water was used in all the experiments.

2.1. Synthesis of aqueous stable MMNA

Aqueous stable MMNA were prepared by a solvothermal method [2,3,29]. In brief, 1 g of FeCl₃·6H₂O and FeCl₂·4H₂O (2:1

wt ratio) was dissolved in 30 ml of EG. The mixture was stirred vigorously for 1 h at 80 °C to obtain a homogeneous solution, followed by the addition of 2 g of sodium acetate and 7 ml of ethylenediamine (EDA). The solution temperature was slowly increased and maintained at 160 °C for 1 h. The temperature was then raised up to 180 °C and maintained for 6 h, before being allowed to cool down to room temperature. The solids were separated from the black solutions with a magnet and washed several times with water and ethanol (50:50 v/v) simultaneously.

2.2. Characterization

X-ray diffraction (XRD) analysis was carried out on a Philips 40 powder diffractometer PW3040/60 with Cu K_α (1.5406 Å) radiation. The surface charges of MMNA and LMMNA were measured using a zeta plus zeta potential analyzer (Brookhaven Instruments) at room temperature. The size and morphology of the MMNA were analyzed using high-resolution transmission electron microscopy (JEOL JAM- 2100F, 200 kV). Further, the surface morphology of LMMNA was characterized by atomic force microscopy (AFM; Digital Instruments, Nanoscope IV). The magnetic properties of MMNA and LMMNA were carried out using a vibrating sample magnetometer (VSM, Model 7410, Lake Shore) at room temperature. The Brunauer–Emmett–Teller (BET) surface area of the MMNA was measured on an ASAP 2020 analyzer (Micromeritics, USA). The thermal ability of MMNA and LMMNA was measured by applying an ACMF (EASY HEAT, EZLI5060, Ambrell, UK). The transmission electron microscopy (TEM) image was collected with an FEI Tecnai G2 BioTwin D312 microscope. Laser scanning confocal microscopy images were recorded using an Olympus Model IX 81 inverted confocal microscope. Flow cytometry analysis was carried out using BD FACSAria instrument.

2.3. Preparation of DOX loaded MMNA

To prepare the MMNA loaded with the anticancer drug DOX, a total of 100 mg of MMNA particles was dispersed in 5 ml of MilliQ water and sonicated for 10 min. Different amounts (0.5, 1, 2, 4, 6, 8 and 10 mg ml⁻¹ concentrations) of MMNA particles were then prepared from the stock (100 mg in 5 ml) in Eppendorf tubes (all the

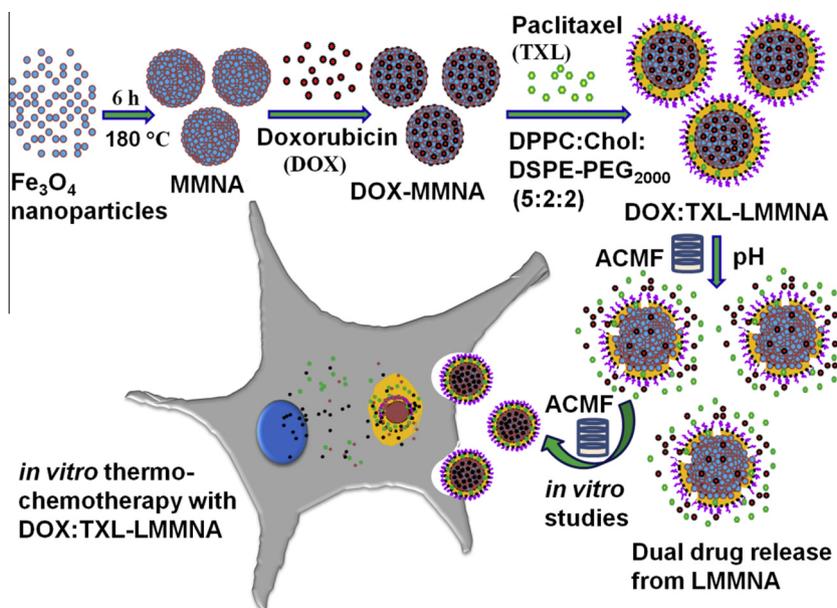


Fig. 1. The overall concept of the present study: the pH-sensitive and thermosensitive LMMNA is a dual drug delivery system containing the drugs doxorubicin (DOX, in mesopores) and paclitaxel (TXL, in lipid layer). Drug release is triggered by an ACMF applied to the tumor cells. In this paper, the formulation and in vitro characterization of dual drug loaded LMMNA with an ACMF are reported for the thermochemotherapy of cancer.

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