Colloids and Surfaces B: Biointerfaces 148 (2016) 157–164

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Doxorubicin-loaded photosensitive magnetic liposomes for multi-modal cancer therapy

Saqlain A. Shah[a,b,*], M.U. Aslam Khan[c], M. Arshad[d], S.U. Awan[e], M.U. Hashmi[f], N. Ahmad[g]

[a] Department of Physics, Forman Christian College (University), Lahore, Pakistan
[b] Materials Science & Engineering, University of Washington, Seattle, WA, USA
[c] Department of Polymer Engineering and Technology, University of the Punjab, Lahore, Pakistan
[d] Nanoscience Division, National Center for Physics, Islamabad, Pakistan
[e] Department of Physics, COMSATS institute of information technology, Islamabad, Pakistan
[f] Department of Applied Sciences, Superior University, Lahore, Pakistan
[g] Department of Physics, Islamic International University, Islamabad, Pakistan

ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form 5 August 2016
Accepted 30 August 2016

Keywords:
Magnetic hyperthermia
Superparamagnetic
Liposomes
Doxorubicin
HeLa cells
MTT assay
Proteome analysis

ABSTRACT

Multifunctional magnetic nanosystems have attracted an enormous attention of researchers for their potential applications in cancer diagnostics and therapy. The localized nanotherapies triggered by the external stimuli, like magnetic fields and visible light, are significant in clinical applications. We report a liposomal system that aims to treat cancer by magnetic hyperthermia, photodynamic therapy and chemotherapy simultaneously. The liposomes enclose clinically used photosensitizer m-THPC (Foscan) and anti-cancer drug doxorubicin, in its hydrophobic lipid bilayers, and contains magnetite nanoparticles in hydrophilic core. Three different sizes of magnetic nanoparticles (10, 22 and 30 nm) and liposomes (40, 70 and 110 nm) were used in this study. Magnetite single domain nanoparticles forming the magnetic core were superparamagnetic but liposomes expressed slight coercivity and hysteresis due to the clustering of nanoparticles in the core. This enhanced the heating efficiency (specific power loss) of the liposomes under an AC field (375 kHz, 170 Oe). Cell viability and toxicity were studied on HeLa cells using MTT assay and proteomic analysis. Confocal and fluorescence microscopy were used to study the photosensitizer's profile and cells response to combined therapy. It revealed that combined therapy almost completely eliminated the cancer cells as opposed to the separate treatments. Magnetic hyperthermia and photodynamic therapies were almost equally effective whereas chemotherapy showed the least effect.

1. Introduction

Magnetic nanosystems have attracted an enormous attention of researchers due to their potential applications in imaging, diagnostics & therapy [1,2]. Cancer is one of the most lethal diseases that kill millions of humans every year. Treatment of cancer via chemotherapy has always been challenging due to the toxic effects of drugs on healthy tissues. Anticancer drugs usually kill the rapidly dividing cells and also kill healthy cells as a collateral damage. Methods are being developed to target the cancer cells selectively thus avoiding the healthy cells. Magnetic nanoparticles are being considered future materials for controlled drug delivery, hyperthermia therapy, magnetic resonance imaging (MRI) and magnetic particle imaging (MPI) [1,2] etc. When magnetic nanoparticles are subjected to oscillating magnetic fields, it generates heat due to hysteresis and Néel losses [3,4]. In case of ferro-fluids, Brownian relaxations may also contribute in heating. But the hydrophobic and toxic nature of most magnetic particles limits their prolonged blood circulation. Toxicity of magnetic nanoparticles can be reduced by surface coatings with biocompatible compounds like polymers, proteins and albumins etc. Iron-oxide nanoparticles are the best candidates for biomedical applications due to their noteworthy magnetic properties and remarkable biocompatibility. Surface coated nanoparticles have been quite promising in biomedical imaging, e.g., MRI and MPI etc [1]. But using magnetic nanoparticles as drug carriers for controlled drug delivery is still a challenge.

* Corresponding author at: Department of Physics, Forman Christian College (University), Lahore, Pakistan.
E-mail addresses: saqlainshah@fccollege.edu.pk, saqlain007pk@hotmail.com (S.A. Shah).

http://dx.doi.org/10.1016/j.colsurfb.2016.08.055
0927-7765/

Liposomes provide a platform to safely enclose and transport different therapeutic agents across the human body with sufficient blood circulation time [5]. Lipid bilayers of liposomes can contain hydrophobic agents whereas aqueous cores may enclose hydrophilic substances simultaneously [6]. Liposomes are non-toxic and biodegradable, and they can transport large amounts of therapeutic agents without being intercepted by the immune system [5]. Their biocompatibility may be further enhanced by surface coatings with polymers like polyethylene glycol (PEG) [7,8] etc. Liposomes enclosing doxorubicin is already in clinical practice [9].

Triggering therapy and drug release using external stimuli like magnetic field and light, may solve various problems related to efficacy of drugs and their toxicity. Like magnetic hyperthermia therapy (MHT) triggered by external magnetic field, photodynamic therapy (PDT) is activated by visible light. There are various photosensitizers that respond to the particular wavelengths of light by releasing reacting oxygen species (ROS), e.g., singlet oxygen, which are highly toxic for biomolecules [10]. One such photosensitizer is m-THPC (Foscan, Foslip) which is one of the most effective second generation drugs for PDT [11].

Core shell nanosystems have the potential to enclose multiple therapeutic agents for multimodal therapies. Liposomes are particularly significant because they can encapsulate larger amounts of hydrophobic as well as hydrophilic substances. There have been various studies combining photodynamic therapy with photothermal therapy (PTT) mainly using plasmonic nanoparticles [12–14]. Mostly gold nanoparticle systems have been focused in this regard. Quite a few studies are reported in literature that combined MHT with PDT. Oliveira et al. performed spectroscopic studies of a ferrofluid combined with phthalocyanine [15]. Corr et al. [16] designed a nanocomposite of magnetite with porphyrin, thus combining magnetism with photo-responsiveness, but they did not study cellular response of these composites. Corato et al. [17–19] gave latest contributions to the field. They developed ultra magnetic liposomes bigger in size (average diameter 150 nm), having dense magnetic cores and they reported interesting results. We have an opinion that smaller liposomes are more favorable for clinical applications because they can be uniformly distributed across the tumor. Their therapeutic effects, e.g., MHT and PDT, are delivered more evenly across the treatment region which may not be the case with the bigger liposomes. Smaller liposomes may be less susceptible to any possible invasion by the immune system. Furthermore, Corato et al. [19] prepared dense magnetic cores by encapsulating magnetite particles of average diameter of 9 nm with a purpose to enhance magnetic heating. We think that this does not make any remarkable difference to the magnetic heating profile unless there are strong inter-particle interactions within the core. Instead, if bigger magnetite particles in superparamagnetic domain are encapsulated in smaller aqueous cores, we get better performance in vitro and in vivo. We present a multifunctional liposomal system that encloses doxorubicin and m-THPC in its hydrophobic lipid bilayers and contains magnetite nanoparticles of larger superparamagnetic sizes in aqueous cores, thus combining chemotherapy, PDT and MHT in a single liposomal system.

2. Materials and methods

2.1. Magnetite nanoparticles

Magnetite nanoparticles were prepared by alkaline co-precipitation of $FeCl_2$ and $FeCl_3$ [20,21]. Nitric acid was used for the size sorting of nanoparticles after co-precipitation. Particles were collected using a permanent magnet. This process was repeated several times till the required size was achieved. Magnetic nanoparticles (MNPs) were mixed with sodium citrate and heated at 80 °C for 30 min to allow the absorption of citrate anions on their surfaces [19]. Three sizes of MNPs were prepared: MNP10 (10 nm), MNP22 (22 nm) and MNP30 (30 nm).

2.2. Magnetic photosensitive liposomes (MPLs)

Magnetic photosensitive liposomes (MPL) were prepared using the methods reported earlier [17–19] with some modifications. Chloroform solutions of 1,2-dipalmitoyl-*sn*-*glycero*-3-phosphocholine (DPPC), 1,2-distearoyl-*sn*-*glycero*-3-phosphocholine (DSPC), and 1,2-distearoyl-*sn*-*glycero*-3-phosphoethanolamine-*N*-[(carboxy(polyethylene glycol)2000] (ammonium salt) (DSPE-PEG2000), were mixed in 85, 10 & 5% (molar) ratio. This solution was diluted with 5 mL of diethyl ether and with 1.2 mL of chloroform. 1 mL MNPs dispersed in sodium citrate buffer was added to the above solution during sonication to allow the hydrophilic MNPs to settle down in the aqueous cores of liposomes. Afterwards, 3.5 mg/mL solution of m-THPC and 2 mg/mL solution of hydrophobic doxorubicin in chloroform were mixed in above solution to transfer m-THPC & doxorubicin in lipid bilayers. Organic solvents were then evaporated at around 30 °C till the gel disappeared. Any possible traces of bigger liposomes were filtered out using a 200 nm filter. Sizes of liposomes were controlled by tuning various reaction parameters, e.g., solutions concentrations, sonication, stirring and reaction time, etc. Experiments were repeated plenty of times to acquire the required sizes. The unsettled MNPs were separated using a permanent magnet. The resulting liposomes were re-suspended in sodium citrate buffer.

2.3. Characterization

Magnetic nanoparticles and liposomes were analyzed using a transmission electron microscope (TEM)(FEI Tecnai G2F20) to evaluate particle morphology and measure the average particle diameter and size distribution. To understand the colloidal properties of MNPs, hydrodynamic diameter, and distribution (polydispersity index) were measured using dynamic light scattering (DLS) (ZetasizerNano, Malvern). Differential scanning calorimetry (DSC) was used to study the heat flow profile of liposomes with and without its constituent drugs in a temperature range 25–250 °C. Absorbance and fluorescence spectra of MPLs were recorded using Perkin Elmer spectrometer (λ_{exc} 430 nm, λ_{em} 650 nm). Confocal microscopy system (Leica Microsystems) operating at 650 nm was used to acquire fluorescence images of liposomes. Magnetization behavior was studied using vibrating sample magnetometer (VSM) (Lakeshore) operating at 25 Oe, 200 Oe and 2000 Oe. VSM curves of MNPs fluid were fit to the Langevin function to obtain the median magnetic core diameter, and distribution [22]. Release profile of doxorubicin was investigated by heating liposomes in water at 37 °C, 45 °C & 55 °C for 30 min each and using UV–vis spectrophotometer. Drug release was measured with respect to control sample that was heated at 80 °C for 30 min.

2.4. In vitro study

The in vitro study of liposomes was carried out on HeLa cells. Cell viability of liposomes was determined using MTT assay [23]. 5 mg MPLs were added to HeLa cells having concentration of 10^5 cells/mL and incubated for 24 h at an atmosphere of 5% CO_2 and 37 °C, to allow the nanoparticles to bind to cells. After 24 h, the suspensions were subjected to different combinations of therapies, i.e. magnetic hyperthermia therapy (MHT), chemotherapy (DOX) and photodynamic therapy (PDT). Magnetic hyperthermia of suspension was performed using magnetherm system (Nanotherics) operating at