International Journal of Pharmaceutics 514 (2016) 133–141

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Engineering thermosensitive liposome-nanoparticle hybrids loaded with doxorubicin for heat-triggered drug release

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ARTICLE INFO

Article history:
Received 30 April 2016
Received in revised form 1 September 2016
Accepted 2 September 2016

Keywords:
Liposomes
Nanoparticles
Doxorubicin
Nanomedicine

ABSTRACT

The engineering of responsive multifunctional delivery systems that combine therapeutic and diagnostic (theranostic) capabilities holds great promise and interest. We describe the design of thermosensitive liposome-nanoparticle (NP) hybrids that can modulate drug release in response to external heating stimulus. These hybrid systems were successfully engineered by the incorporation of gold, silver, and iron oxide NPs into the lipid bilayer of lysolipid-containing thermosensitive liposomes (LTSL). Structural characterization of LTSL-NP hybrids using cryo-EM and AFM revealed the incorporation of metallic NPs into the lipid membranes without compromising doxorubicin loading and retention capability. The presence of metallic NPs in the lipid bilayer reinforced bilayer retention and offered a nanoparticle concentration-dependent modulation of drug release in response to external heating. In conclusion, LTSL-NP hybrids represent a promising versatile platform based on LTSL liposomes that could further utilize the properties of the embedded NPs for multifunctional theranostic applications.

1. Introduction

Liposomes are self-assembled phospholipid vesicles that have been clinically approved as a nanoscale delivery system for various therapeutic applications (Allen and Cullis, 2013; Barenholz, 2012). Liposomes can effectively entrap both hydrophilic (Lozano et al., 2015; Li et al., 1998) and hydrophobic compounds (Chen et al., 2010; Koudelka and Turánek, 2012; Lozano et al., 2015) in their aqueous core or the lipid bilayer, respectively. Liposomes are biocompatible, biodegradable and can effectively modify the pharmacokinetic profile of their loaded drugs (Sawant and Torchilin, 2012). Stealth liposomes can passively accumulate at the tumor site as they leak through malformed blood vessels while avoiding healthy tissues and organs (Gabizon et al., 1994; Gabizon and Martin, 1997). Next-generation liposomes that can trigger-release their drug content in response to external stimuli have been developed to improve drug bioavailability and overall therapeutic efficacy (Al-Ahmady et al., 2014, 2015). The most clinically advanced such vesicle system is commercially known as ThermoDox®, a lysolipid-containing temperature-responsive liposome (LTSL) that is currently in clinical trials for treatment of solid tumors in combination with mild hyperthermia (HT) (Al-Ahmady and Kostarelos, 2016).

Over the last few years, advances in nanotechnology have dramatically increased interest in developing metallic nanoparticles (NPs) for a wide range of applications (Grzelczak and Liz-Marzan, 2013; Hormeno et al., 2014; Johannsen et al., 2010; Liu et al., 2012; Maestro et al., 2014; Visaria et al., 2006; Wong and Liu, 2010). The nanoscale size of metallic NPs such as gold (AuNPs), silver (AgNPs), superparamagnetic iron oxides (SPIO) and their novel optical and magnetic properties enable their use for both imaging and therapeutic purposes. For example, the enhanced surface plasmon resonance of AuNPs makes them particularly attractive for imaging and photo-thermal applications (Maestro et al., 2014). The absorption of light at certain wavelengths causes the oscillation of surface electrons and subsequent local heat generation, that can be controlled by the intensity of the laser

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http://dx.doi.org/10.1016/j.ijpharm.2016.09.009
0378-5173/

beam irradiation, duration of NP exposure and the concentration of AuNPs (Alkilany et al., 2012).

Similarly, SPIO NPs have been used extensively as a contrast agent for magnetic resonance imaging (MRI). The magnetic properties of SPIO NPs can also be exploited for the generation of magnetically-mediated hyperthermia (Chanda et al., 2010). The exposure of SPIO NPs to an alternating magnetic field can generate high local heating by Neel fluctuations which have showed potential in cancer therapy (Jordan et al., 2006; Maier-Hauff et al., 2007). NP-induced hyperthermia has the potential to overcome some of the limitations of conventional heating techniques when treating deep or non-accessible tumors (Preiss and Bothun, 2011). However, the inherent hydrophobic surface character of metallic NPs and their limited colloidal stability can significantly affect their biological performance.

We and others have previously shown the possibility of using liposome technology as a delivery platform that could allow the incorporation of metallic NPs (Al-Jamal et al., 2008; Jain et al., 2003; Lozano et al., 2012). Liposomal incorporation of metallic NPs has proved to be an effective way to increase nanoparticle stability and biocompatibility in biological conditions (Lozano et al., 2012; Preiss and Bothun, 2011). This can be achieved by either the incorporation of hydrophobic NPs within the lipid membrane or encapsulation of the hydrophilic NPs in the liposomal aqueous core (Tai et al., 2009). Alternatively, metallic NPs can be functionalized onto the liposomal surface by either physical or chemical conjugation (Chithrani et al., 2010; Pornpattananangkul et al., 2010). To date several examples of liposome-NP hybrids have been designed for diagnostic (Soga et al., 2010), or simply for colloidal stabilization (Kojima et al., 2008; Lozano et al., 2012) purposes, with some interesting examples of smart hybrid systems that can trigger release in response to external stimuli. Such cases of thermosensitive liposome-NP hybrids were designed to utilize the photonic and electromagnetic properties of NPs for local heat generation and triggered content release (Paasonen et al., 2010; Tai et al., 2009; Wu, 2008). As a proof of concept, several studies have demonstrated the release of encapsulated dye molecules from such hybrid systems (Bealle et al., 2012; Chen et al., 2010; Paasonen et al., 2010). However, only a few studies have reported the potential of combining thermosensitive liposome-NP hybrids with therapeutic agents for combinatory therapeutic and diagnostic applications (Tian et al., 2011).

The aim of the present work was to engineer and characterize doxorubicin loaded-thermosensitive, liposome-NP hybrids as multimodal smart systems to control drug release. Drug (doxorubicin) loaded, LTSL were chosen for this study as the most advanced TSL that are currently in clinical trials (Clesion.com, 2016; Landon et al., 2011). The incorporation of three different types of metallic NPs into LTSL was studied and the capacity of the hybrid systems to modulate drug release was investigated.

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (MSPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG_{2000}) were kind gifts from Lipoid GmbH (Ludwigshafen, Germany). Ammonium sulfate, sodium hydroxide, chloroform and methanol were purchased from Fisher Scientific. 1,6-Diphenyl-l,3,5-hexatriene (DPH) was purchased from Invitrogen. Diethyl ether, doxorubicin hydrochloride (DOX), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), sodium chloride (NaCl), Sephadex® G-50, oleic acid-functionalized magnetic SPIO NPs solution 5 mg/ml in toluene, octanethiol-functionalized AuNPs 2% solution in toluene, decanethiol-functionalized AgNPs 0.1% (w/v) in hexane, 8-anilino-1-naphthalenesulfonic acid (ANS), and tetrahydrofuran were purchased from Sigma.

2.2. Preparation and characterization of liposomes and liposome-NP hybrids

Lysolipid containing temperature-sensitive liposomes (LTSL) composed of DPPC:MSPC:DSPE-PEG_{2000} 86:10:4 (molar ratio) were prepared using the reverse phase evaporation method [RP] and the lipid film-reverse phase evaporation method [F-RP] (Fig. 1A). For the RP method the lipids dissolved in chloroform/methanol mixture were mixed in 25 ml round bottom flask and then 6 ml of chloroform/diethylether (1:1, v/v) and 1.5 ml of 240 mM ammonium sulfate buffer (pH 5.4) were added at 1:4 v/v (aqueous/organic). In order to form w/o emulsion, the mixture was sonicated for 15 min in a bath sonicator at 40 °C. Organic solvents were then evaporated using rotary evaporator (BÜCHI, Switzerland) at 460 mbar and 40 °C for 2 h resulting in the formation of a gel-like phase followed by aqueous phase formation. Large unilamellar liposomes (LUVs) formed by this process were then reduced in size by 30 min sonication at 60 °C using a bath sonicator.

To prepare the liposomes by F-RP method, the same procedure described above was applied with the exception that a lipid film was first prepared by evaporating chloroform:methanol from the lipid mixture. This then followed by re-dissolving the lipid film in the chloroform/diethylether/ammonium sulfate mixture as described earlier.

For the preparation of liposome-NP hybrids, F-RP method was applied. First, the exact amount of hydrophobic NPs dispersion (5 μg or 10 μg) was added to 25 ml round bottom flask. The organic solvent in which the NPs were dispersed was removed using rotary evaporator at 6 mbar and 40 °C for 1 h. The lipids mixture of DPPC:MSPC:DSPE-PEG_{2000} 86:10:4 (molar ratio) dissolved in chloroform/methanol (4:1) were then added and the organic solvents were evaporated to form the lipid film containing the NPs. The resulting lipid film was then re-dissolved the lipid film in 6 ml of chloroform/diethylether (1:1). Following this step 1.5 ml of 240 mM ammonium sulfate buffer (pH5.4) was added and the mixture was sonicated for 15 min in a bath sonicator at 40 °C. Organic solvents were then evaporated using rotary evaporator at 460 mbar and 40 °C for 2 h and the size of LUVs of LTSL-Np hybrids was then reduced by bath sonication as described earlier.

Liposome size and surface charge were measured by using Zetasizer Nano ZS (Malvern, Instruments, UK). For size measurement samples were diluted with HEPES buffer saline (HBS) composed of 20 mM HEPES, 150 mM NaCl, pH 7.4 and measured in 1 ml cuvettes. Zeta potential was measured in disposable Zetasizer cuvettes and sample dilution was performed with distilled water.

2.3. Cryo-electron microscopy (Cryo-EM)

Visualization of samples with Cryo-EM was performed to study the morphology of LTSL-NPs hybrids and to confirm the incorporation of metallic NPs. Sample preparation was carried out in a temperature- and humidity-controlled chamber using a fully automated (PC-controlled) vitrification robot (Vitrobot). 46 A specimen grid was dipped into a suspension and withdrawn, and excess liquid was blotted away. Thin films were formed between the bars of the grids. To vitrify these thin films, the grid was shot into melting ethane. The grids with vitrified thin films were analyzed in a CM-12 transmission microscope (Philips, Eindhoven, The Netherlands) at 170 °C using a Gatan-626 cryo-specimen holder and cryotransfer system (Gatan, Warrendale, PA).