Journal of Controlled Release 155 (2011) 358–366

Contents lists available at ScienceDirect

Journal of Controlled Release

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

A novel nested liposome drug delivery vehicle capable of ultrasound triggered release of its payload

Stuart Ibsen [a,*,1], Michael Benchimol [b,1], Dmitri Simberg [c], Carolyn Schutt [a], Jason Steiner [d], Sadik Esener [e]

[a] Department of Bioengineering, Moores Cancer Center, University of California San Diego, 3855 Health Sciences Dr. # 0815, La Jolla, CA 92093–0815, USA
[b] Department of Electrical & Computer Engineering, Moores Cancer Center, University of California San Diego, La Jolla, CA 92093, USA
[c] Moores Cancer Center, University of California San Diego, La Jolla, CA 92093 USA
[d] Department of Material Science, Moores Cancer Center, University of California San Diego, La Jolla, CA 92093, USA
[e] Department of Nanoengineering, Moores Cancer Center, University of California at San Diego, La Jolla, CA 92093, USA

ARTICLE INFO

Article history:
Received 4 May 2011
Accepted 18 June 2011
Available online 2 July 2011

Keywords:
Burst release
Ultrasound triggered release
Focused ultrasound
Microbubbles
Triggered drug delivery

ABSTRACT

The use of focused ultrasound can be an effective method to locally highlight tumor tissue and specifically trigger the activation of echogenic drug delivery vehicles in an effort to reduce systemic chemotherapy side effects. Here we demonstrate a unique ultrasound triggered vehicle design and fabrication method where the payload and a perfluorocarbon gas microbubble are both encapsulated within the internal aqueous space of a liposome. This nested lipid shell geometry both stabilized the microbubble and ensured it was spatially close enough to interact with the liposome membrane at all times. The internal microbubble was shown to fragment the outer liposome membrane upon exposure to ultrasound at intensities of 1–1.5 MPa. The focused ultrasound allowed the release of the internal payload to localized regions within tissue phantoms. The vehicles showed high payload loading efficiency of 16%, stability in blood of several hours, and low level macrophage recognition in vitro. High speed fluorescent videos present the first optical images of such vehicles interacting with ultrasound. This ability to open the outer membrane in small regions of deep tissue could provide a second level of spatial and temporal control beyond biochemical targeting, making these particles promising for in vivo animal studies.

1. Introduction

Indiscriminate exposure of all cells in the body to a systemically administered chemotherapy drug is the main cause of harmful toxic side effects [1]. Certain drug delivery vehicles such as Abraxane for delivery of paclitaxel and liposomal Doxil for doxorubicin [2,3] reduce exposure of non-targeted cells to the drug while accumulating a therapeutic dose within the tumor. Passive accumulation in the tumor tissue due to the enhanced permeation and retention of the vasculature [2] coupled with slow drug release limits the bioavailability to non-tumor organs [4]. However, this slow release also limits the maximum levels of drug in the tumor [5], and nonspecific accumulation in healthy tissue remains a major hurdle [2].

The use of tumor targeting ligands has the potential to improve the preferential accumulation of these delivery vehicles in tumor tissue [6,7]. The delivery requires endocytosis of the targeted vehicle with subsequent endosomal escape [8,9]. However, saturation of the targetable receptors limits the targeting efficiency. Also, tumor "receptors" are rarely unique to the tumor [10] and the targeted particles accumulate in other healthy tissues, especially in the liver and spleen, causing local toxicity [11].

To address the difficulties of pure biochemical targeting, an independent non-biochemical trigger is required to cause instantaneous drug release only from the particles that have accumulated in the tumor tissue. Ultrasound is an attractive trigger candidate due to its low cost, wide availability, its generation external to the body, and its independence from biochemical or physical properties of the tumor. It can be focused to small volumes of deep tissue on the order of several cubic millimeters [12] to avoid healthy tissue. It is non-ionizing, and does not damage tissue as long as the exposure is kept below 720 mW/cm^2 [13,14].

The best particles to respond to ultrasound at safe exposure levels are gas-filled microbubbles [15] already approved for human use as ultrasound contrast agents [16,17]. Ultrasound causes large size fluctuations in microbubbles due to the large density difference between the compressible gas and the surrounding water, which induces microstreaming of fluid around the microbubble and disrupts nearby membranes [18]. Microbubbles can also adiabatically implode (cavitate) producing a shockwave and water jets which can penetrate nearby membranes. This causes sonoporation and can facilitate delivery of DNA or drugs into cells [17,19–21]. Significant work has been done to employ microbubbles as delivery vehicles in vivo [19,21]

* Corresponding author. Tel.: +1 858 534 9848; fax: +1 858 534 9830.
E-mail address: sibsen@ucsd.edu (S. Ibsen).
[1] These authors contributed equally to this work.

0168-3659/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2011.06.032

without much success [22]. This is likely attributed to extremely short circulation times of microbubbles in vivo (3–15 min half-life [22]) and to limited payload capacity.

Surface loading of a hydrophilic payload, such as DNA, is limited by the surface area of the microbubble [23–26] and leaves it exposed to degradation and potential immune system recognition. Hydrophobic payloads are carried in limited volumes of thickened lipid, polymer, or oil surrounding the microbubble [25,27] but when fragmented the hydrophobic drug will be contained in relatively large lipid particles reducing diffusion rates.

Drug loaded liposomes have been attached to the surface of microbubbles [28], however the points of attachment can concentrate shear stress during transport through the microvasculature and destabilize the entire particle. Separate drug-loaded liposomes and microbubbles can be targeted to the same tissue, but successful delivery of the drug depends on very close co-localization of both particles because the cavitation shockwave is only effective at disrupting membranes within a few tens of microns. It is unlikely that both particles would be present in sufficient proximity and concentration to deliver a therapeutic dose.

To protect the microbubble and address the challenges described above, the microbubble and the payload can be encapsulated together within a protective outer liposome membrane shell. Previous efforts to incorporate gas bubbles into liposomes have used freeze drying techniques [29] or chemical reactions that create CO_2 microbubbles [30], but have very low yields. They also lack sufficient control over gas and payload entrapment, stability, and internal geometry, resulting in a large distribution of properties. Such distributions reduce the effectiveness of ultrasound to activate the entire population. Premade microbubbles stabilized with a lipid monolayer can be made independently using standard probe sonication techniques which increases bubble half-life in storage and in vivo. Microbubbles of desired size ranges can be collected and subsequently encapsulated in liposomes.

The most common methods of liposome encapsulation involve exposure to vacuum, sonication, heating, and/or extrusion, all of which destroy microbubbles. Ethanol injection is gentle enough to allow the microbubbles to survive the encapsulation process but produces liposomes too small to encapsulate a microbubble [31]. Detergent dialysis methods [32] can make liposomes large enough to encapsulate microbubbles and are gentle enough to not destroy them in the process.

Here we demonstrate a new manufacturing method to reproducibly encapsulate and protect premade microbubbles in a liposome as shown schematically in Fig. 1a. This method is similar to detergent dialysis but uses organic solvents to dissolve the lipids. A slow diffusive introduction of water allows the lipid membranes to seal and encapsulate the large microbubbles. We refer to these malleable nested structures as SHockwavE-Ruptured nanoPayload cArriers (SHERPAs).

2. Materials and methods

2.1. Materials

L-α-phosphatidylcholine (EPC) from chicken eggs, distearoyl phosphatidylcholine (DSPC), distearoyl phosphatidylethanolamine-methoxy(polyethylene glycol) MW5000 (mPEG-DSPE), and cholesterol were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). 1,2-propanediol, glycerol, ethanol, and perfluorohexane were purchased from Sigma-Aldrich. All water was purified using the Milli-Q Plus System (Millipore Corporation, Bedford, USA). DiO was purchased from Biotium, Inc. (Hayward, CA). The PBS was purchased from Hyclone Laboratories Inc. (Logan, UT).

2.2. SHERPA production

2.2.1. Lipid preparation

The SHERPAs were manufactured in a two step procedure with the microbubbles being formed through a probe sonication process and subsequently encapsulated in the outer liposome. The desired payload of nanoparticles or water soluble drug can be introduced in Solution 1, Solution 2, or in the PBS used for the final encapsulation step.

Solution 1: Outer liposome lipid solution

A 1.5 mL eppendorf tube was filled with 76 μL of EPC in chloroform (26 mM) (20 mg mL^{-1}) and 10 μL of cholesterol in chloroform (100 mM) (387 mg mL^{-1}). The chloroform was removed by evaporation while vortexing under an argon stream. 125 μL of ethanol was then added and the solution was vortexed at 3200 rpm for 30 s. To visualize lipid membranes, 5 μL of 1 mM DiO (Biotium, Hayward, CA) in ethanol was added.

Solution 2: Microbubble solution

A 1.5 mL eppendorf tube was filled with 25 μL of DSPC in chloroform (51 mM) (40 mg mL^{-1}) and 20 μL mPEG5000-DSPE in chloroform (8.6 mM) (50 mg mL^{-1}). The chloroform was removed by evaporation while vortexing under an argon stream. Then 450 μL of 1,2-propanediol was added. The solution was vortexed at 3200 rpm for 30 s, and then placed in a heating block at 60 °C.

After 10 min, the solution was vortexed at 3200 rpm for 10 s, and 150 μL glycerol was added. The solution was gently vortexed for 30 s, and then placed back into the 60 °C heating block. The heating, vortexing cycle was repeated until the glycerol was fully mixed in and the solution was

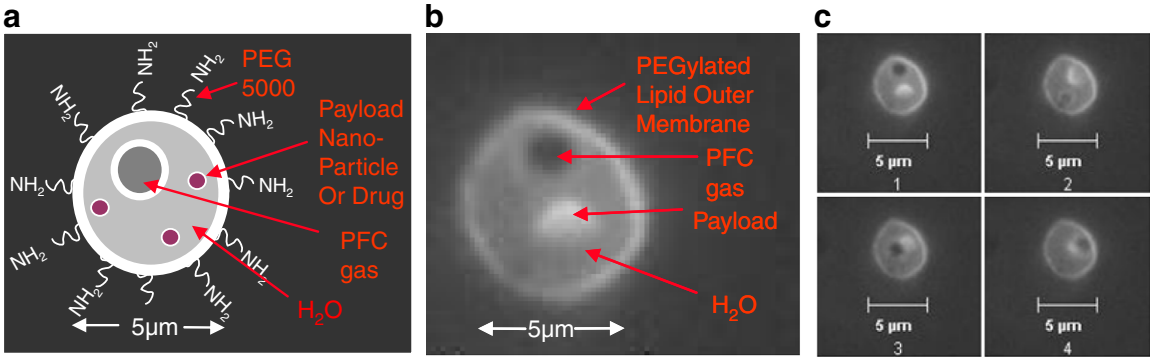

Fig. 1. SHERPA nested structural design (a) A schematic of the nested liposome SHERPA design. (b) Fluorescent image of a SHERPA resulting from the described manufacturing process. The payload is a small fluorescently labeled lipid membrane. (c) A series of sequential pictures taken of the SHERPA showing the microbubble and fluorescent lipid payload moving around inside due to Brownian motion. This confirms that the microbubble and payload were internal to the outer membrane and not just attached to the outside.