Ultrasonics Sonochemistry 28 (2016) 31–38

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

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

Ultrasound mediated destruction of multifunctional microbubbles for image guided delivery of oxygen and drugs

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ARTICLE INFO

Article history:
Received 6 January 2015
Received in revised form 18 May 2015
Accepted 24 June 2015
Available online 25 June 2015

Keywords:
Oxygen
Perfluorocarbon
Multifunctional microbubbles
Fluorescence imaging
Ultrasound targeted microbubble destruction
Drug delivery

ABSTRACT

We synthesized multifunctional activatible microbubbles (MAMs) for ultrasound mediated delivery of oxygen and drugs with both ultrasound and fluorescence imaging guidance. Oxygen enriched perfluorocarbon (PFC) compound was encapsulated in liposome microbubbles (MBs) by a modified emulsification process. DiI dye was loaded as a model drug. The ultrasound targeted microbubble destruction (UTMD) process was guided by both ultrasonography and fluorescence imaging modalities. The process was validated in both a dialysis membrane tube model and a porcine carotid artery model. Our experiment results show that the UTMD process effectively facilitates the controlled delivery of oxygen and drug at the disease site and that the MAM agent enables ultrasound and fluorescence imaging guidance of the UTMD process. The proposed MAM agent can be potentially used for UTMD-mediated combination therapy in hypoxic ovarian cancer.

1. Introduction

Ovarian cancer is the ninth most common cancer and ranks fifth as the cause of cancer death in women [1]. Patients commonly develop ovarian cancer asymptomatically and present with peritoneal metastasis at the time of diagnosis. Since most often the disease has spread from the ovaries at presentation, the overall outcome is poor, with less than 30% survival in 5 years [2]. Currently, the standard first-line therapy for advanced ovarian cancer includes surgical staging or cytoreductive surgery, followed by intravenous (i.v.) or intraperitoneal (i.p.) chemotherapy with paclitaxel (PTX) and carboplatin administered every three weeks. However, 80% of patients will relapse the treatment and ultimately develop the resistant disease [3]. The development of drug resistance in ovarian cancer is closely associated with tumor hypoxia [4]. Hypoxia comprises therapeutic effectiveness of many anticancer drugs through multiple mechanisms such as increased acidity, increased hypoxia-induced factor 1α (HIF-1α), generation of free radicals, and cellular adaptation [5–7]. Therefore, hyperoxic treatments, such as oxygen respiration and hyperbaric chamber, are commonly prescribed before radiotherapy or chemotherapy in order to boost tumor oxygenation, improve drug uptake, and enhance tumor response [8–10]. However, these systemic treatment options have limited specificity and efficacy.

The emergence of the microbubbles (MBs) and the ultrasound targeted microbubble destruction (UTMD) technique offers a new opportunity for delivering oxygen and anti-cancer drugs to the hypoxic tumor site simultaneously in order to overcome the hypoxia-induced chemoresistance. Originally used as a contrast enhancement agent in ultrasonography, MBs have attracted more and more research interests as a disease-targeting agent for molecular imaging [11,12] and as a biocompatible carrier for drug delivery [13]. We have encapsulated perflourocarbon compounds (PFC) in liposome MBs and loaded the MBs with paclitaxel (PTX) for UTMD-mediated drug delivery. Our previous in vitro and in vivo studies have demonstrated that the UTMD process can effectively enhance the PTX deposition at the disease site and improve the therapeutic outcome [14,15]. We have also delivered oxygen and PTX simultaneously for combination therapy in hypoxic ovarian

Abbreviation: MAMs, multifunctional activatible microbubbles.

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http://dx.doi.org/10.1016/j.ultsonch.2015.06.024
1350-4177/

cancer cells. Our in vitro study showed that simultaneous delivery of oxygen and PTX significantly enhanced the local oxygen release, the anti-proliferative activities, and the cell apoptosis ratio for hypoxic ovarian cancer cells, superior to other treatment groups such as applying PTX only or applying PTX-loaded microbubbles with or without ultrasound mediation (unpublished data). Our in vivo study in ovarian cancer xenograft mice showed that intravenous injection of oxygen and PTX-loaded MBs followed by UTMD yielded the increased tumor apoptosis, the reduced VEGF expression, the decreased expressions of HIF-1α and P-gp, and the significant inhibition of tumor growth [16]. These studies suggest that the oxygen and PTX-loaded MBs and the UTMD process may provide a promising drug delivery strategy for the combination treatment of hypoxic ovarian cancer. These studies also suggest the need of quantitative imaging techniques for reliable control of the UTMD-mediated drug delivery process. Currently, the UTMD process is commonly guided by ultrasonography. However, the imaging sensitivity and specificity of ultrasonography is sub-optimal owing to hyperechoic artifacts and non-specific gas bubbles. We have encapsulated fluorescence agents in MBs for potential use of fluorescence imaging guided drug delivery [17,18]. Considering the high molecular sensitivity of fluorescence imaging and the deep imaging depth of ultrasonography, it is rationale to load oxygen, anti-cancer drugs, and fluorescence dyes in liposome MBs so that the UTMD-mediated drug delivery process can be guided by both ultrasonography and fluorescence imaging modalities simultaneously.

In this paper, we demonstrated simultaneous delivery of oxygen and therapeutics by an image-guided UTMD process. To simplify the experiment design, a commonly used fluorescence dye, [1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine iodide] (DiI), was used as the model drug. To minimize the unnecessary use of the lab animals, we validated the image-guided UTMD process in a benchtop phantom and an ex vivo tissue model.

2. Materials and methods

2.1. Materials

Hydrogenated L-α-phosphatidylcholine, polyethylene glycol 40 (PEG-40) stearate, glycerol, Pluronic® F-68 (F68), [4′,6-Diamidino-2-Phenylindole] (DAPI) and propane-1,3-diol were obtained from Sigma–Aldrich Co. Ltd (St. Louis, MO, USA). Methylene chloride (CH_2Cl_2), [1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine iodide] (DiI) and dimethyl sulfoxide (DMSO) were obtained from Fisher Scientific (Fairlawn, NJ USA). 1,1,1,2,3,4,4,5,5,5-decafluoropentane (a PFC compound with boiling point of 55 °C) was obtained from Fluka (St. Louis, MO, USA). Sodium hydrosulfite was obtained from ACROS (New Jersey, USA).

2.2. Synthesis of oxygen and DiI loaded MAMs

Oxygen and DiI loaded MAMs comprising a PFC core and a lipid shell were fabricated by a modified emulsification process [19], as illustrated in Fig. 1. The lipid suspension was prepared in advance by mixing 50 mg hydrogenated L-α-phosphatidylcholine, 14 mg PEG-40 stearate, 1 ml glycerol (density 1.25 g ml^{-1}), 5 ml propane-1,3-diol (density 1.053 g ml^{-1}), and 50 ml distilled water (mass ratio 1:0.28:25:105:1000) in a conical flask. The mixture was placed in a 42 °C water bath, gently stirred for 30 min, and then cooled down to room temperature. After 100 μl DiI (1 mg/ml) was added to the 18 ml lipid mixture with gentle stirring, the lipid suspension was bubbled with oxygen for 5 min in the dark. After that, 2 ml PFC was bubbled with oxygen gas till saturation and added to DiI lipid suspension. The mixture was homogenized in the dark by an Omni Sonic Ruptor 250 ultrasonic homogenizer (Omni international, Waterbury, CT) at 50 W for 200 s. The above mixture was then added to 20 ml F68 solution (2% w/v) and emulsified in the dark for one minute. The obtained emulsion was centrifuged by Centrifuge 5810R (Eppendorf, Hamburg, Germany) at 800 rpm for 5 min. After centrifugation, the supernatant was discarded and the precipitate was re-dispersed in 40 ml distilled water enriched with oxygen for further use.

The number of the MAMs was counted by a hemacytometer. The MAM concentration was calculated by the following formula: microbubble concentration per milliliter = total microbubble count in 5 squares/80 × 400 × 10^4 × dilution factor. The size distribution and zeta potential were determined by a 90Plus Particle Size Analyzer (Brookhaven Instruments, Holtsville, NY, USA). The MAMs were diluted 100-fold with double-distilled water for size and surface charge measurements. Triplicated measurements were made by averaging 6 data points per sample for three independent samples. The encapsulation efficiency of DiI in MAMs were evaluated by an USB4000-FL fluorescence spectrometer (Ocean Optics Inc., Dunedin, FL, USA) at an excitation wavelength of 550 nm and an emission wavelength of 571 nm. To characterize the encapsulation efficiency, a calibration curve was first obtained by measuring fluorescence intensities at various DiI concentrations. After that, 1 ml DiI loaded MAMs were dissolved in 4 ml DMSO and the fluorescence intensity of the solution was compared with the calibration curve to determine the actual amount of the loaded DiI. The morphology of the DiI loaded activatible MBs was determined by a TCS SL confocal laser scanning microscope (Leica, Heidelberg, Germany) at an excitation wavelength of 543 nm and an emission wavelength of 590 nm.

2.3. Image-guided UTMD process for oxygen delivery

Oxygen and DiI loaded MAMs were fabricated for UTMD-mediated fragmentation and controlled delivery of oxygen. The process was demonstrated in a benchtop setup as sketched in Fig. 2. The setup consists of a Terason 2000 ultrasound imaging system equipped with a 12L5 5–12 MHz linear array probe (Teratech Corp., Burlington, MA, USA), an Omnisound 3000C ultrasound therapy system equipped with a 3 MHz therapeutic ultrasound probe (Physio Technology, Nevada,USA), a 5 MHz immersion focused ultrasound transducer (Olympus NDT Inc., Waltham, MA,USA) connected through a Panametrics 5900PR computer controlled pulse/receiver to a TDS 2024c digital storage oscilloscope (Tektronix, Beaverton, OR, USA), and a plastic tank filled with degassed water. A Spectra/Por dialysis membrane tube (Spectrum Laboratories Inc., Laguna Hills, CA, USA) was mounted at the center of the tank, with both ends connected to an external circulation system (marked as red in Fig. 2). During the experiment, microbubble suspension (concentration: 3 × 10^9 /ml, flow rate: 30 ml/h) was diluted by degassed water (flow rate: 50 ml/h), flowed through the dialysis tube, and collected by a waste collection flask. The therapeutic ultrasound probe was placed five millimeter above the dialysis membrane tube to activate the MAMs at a power density of 1 W/cm^2 and a duty cycle of 20%. A broadband needle hydrophone (ONDA, Sunnyvale, CA, USA) was used to calibrate the transmitted acoustic pressure.

As the ultrasound pulses were applied to the MAMs flowing through the dialysis membrane tube, the dissolved oxygen in the flow and the B-mode image of the tube were acquired by a USB4000 oxygen sensor (Ocean Optics, Dunedin, FL, USA) and the Terason ultrasound probe, respectively. The test results were compared with the control where only degassed water flew through the dialysis membrane tube. Ultrasound mediated fragmentation of the MAMs was also monitored by fluorescence imaging. For this study, the therapeutic ultrasound probe was placed underneath