Ultrasound in Med. & Biol., Vol. ■■, No. ■■, pp. ■■–■■, 2018

0301-5629/$ - see front matter

https://doi.org/10.1016/j.ultrasmedbio.2018.03.009

● *Original Contribution*

MACROPHAGES AS DRUG DELIVERY CARRIERS FOR ACOUSTIC PHASE-CHANGE DROPLETS

Ching-Hsiang Fan, Ya-Hsuan Lee, Yi-Ju Ho, Chung-Hsin Wang, Shih-Tsung Kang, and Chih-Kuang Yeh

Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu, Taiwan

(*Received* 5 *August* 2017; *revised* 6 *March* 2018; *in final form* 14 *March* 2018)

Abstract—The major challenges in treating malignant tumors are transport of therapeutic agents to hypoxic regions and real-time assessment of successful drug release *via* medical imaging modalities. In this study, we propose the use of macrophages (RAW 264.7 cells) as carriers of drug-loaded phase-change droplets to penetrate ischemic or hypoxic regions within tumors. The droplets consist of perfluoropentane, lipid and the chemotherapeutic drug doxorubicin (DOX, DOX-droplets). The efficiency of DOX-droplet uptake, migration mobility and viability of DOX-droplet-loaded macrophages (DLMs) were measured using a transmembrane cell migration assay, the alamarBlue assay and flow cytometric analysis, respectively. Our results indicate the feasibility of utilizing macrophages as DOX-droplet carriers (DOX payload of DOX-droplets: 459.3 ± 35.8 μg/mL, efficiency of cell uptake DOX-droplets: 88.8 ± 3.5%). The migration mobility (total number of migrated microphages) of DLMs decreased to 32.3% compared with that of healthy macrophages, but the DLMs provided contrast-enhanced ultrasound imaging (1.7-fold enhancement) and anti-tumor effect (70.9% cell viability) after acoustic droplet vaporization, suggesting the potential theranostic applications of DLMs. Future work will assess the tumor penetration ability of DLMs, mechanical effect of droplet vaporization on *in vivo* anti-tumor therapy and the release of the carried drug by ultrasound-triggered vaporization. (E-mail: ckyeh@mx.nthu.edu.tw) **© 2018 World Federation for Ultrasound in Medicine & Biology. All rights reserved.**

Key Words: **Cell-mediated drug delivery, Macrophages, Acoustic droplets, Acoustic droplet vaporization, Tumor therapy.**

INTRODUCTION

Current chemotherapies for malignant solid tumor have delivered limited gains in therapeutic overcome (Minchinton and Tannock 2006). One of the main issues is that their efficiency is structurally limited by the heterogeneous tumor microenvironment, which prevents drugs from entering deep into the extravascular tumor tissue. Malignant solid tumors consist of dense tumor cells that restrict the space for angiogenesis, resulting in tortuous and dysfunctional vasculatures (Danquah et al. 2011; Nakamura et al. 2016) that produce irregular blood supply, high interstitial fluid pressure (IFP, 10–100 mm Hg) (Lunt et al. 2009; Nakamura et al. 2016; Padera et al. 2004) and compact extracellular matrix within tumor tissue. These conditions limit the penetration of chemo drugs across the tumor microvasculature into tumor avascular cores. Because the resulting dose of chemo drugs is insufficient, cells within the tumor core exhibit poor treatment outcome, drug resistance and local recurrence after therapeutic treatments (Minchinton and Tannock 2006). To increase the drug distribution within a tumor, a cell-based delivery system using macrophages was developed. Several recent reports have indicated that many therapeutic substances can be carried by macrophages for subsequent systemic trafficking and targeting of therapeutic substances to tumor sites (Choi et al. 2012; Ikehara et al. 2006; Madsen et al. 2012).

A variety of chemokines and factors secreted from tumor cells have been reported to promote migration of macrophages to tumor sites (Murdoch et al. 2004; Muthana et al. 2011). Furthermore, the number of infiltrated macrophages in tumors can be viewed as an indicator of the degree of tumor malignancy; macrophages accumulate at high levels in hypoxic regions of the tumor. In this regard, many groups have reported the feasibility of using

Address correspondence to: Chih-Kuang Yeh, Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, No. 101, Section 2, Kuang-Fu Road, Hsinchu 30013, Taiwan. E-mail: ckyeh@mx.nthu.edu.tw

macrophages to assist as a cellular Trojan horse to promote the distribution of therapeutic substances (such as genes, viruses, gold nanoparticles and liposomes) within the tumor (Choi et al. 2007, 2012; Huang et al. 2015). Although macrophages have shown promise in cell-based delivery, it is still critical that therapeutic cargos can be accurately targeted for effective tumor therapy *via* external trigger, while the drug-releasing process can be non-invasively monitored with medical imaging tools.

Ultrasound provides real-time tissue imaging, which has been extensively applied in clinical diagnosis for kidney, liver and myocardial diseases. (Berzigotti et al. 2018; Hansen et al. 2015; Inaba and Lindner 2012; Ismail et al. 2015; Klauser et al. 2011; Lindner 2010). Currently, ultrasound has been raising interest in the field of controlled drug delivery because of the development of ultrasound theranostic agents that can package and release payloads under monitoring *via* ultrasound (Fan et al. 2013). Recent studies have indicated that ultrasound can convert acoustic liquid perfluorocarbon (PFC) droplets into gaseous bubbles *via* liquid-to-gas transition. This process produces an instantaneous expansion of volume (three- to fivefold in diameter) within the time scale of microseconds, referred to as acoustic droplet vaporization (ADV) (Kang et al. 2014a, 2014b; Lo et al. 2007). The ADV-generated bubbles can serve as contrast agent for ultrasound imaging, as well as a gas embolism for vascular occlusion (Chen et al. 2015; Ho et al. 2016; Reznik et al. 2011; Zhang et al. 2010b). The violent bubble production results in extensive mechanical stress that not only destroys the droplet shells (*e.g.,* albumin or phospholipids), but also the surrounding tissues, permitting the cargo to be released and transported into the deep tissues (Ho and Yeh 2017; Ho et al. 2016). Because of these features, acoustic droplets can be modified as a multifunctional theranostic platform, including multiple therapeutic effects, drug carrier and ultrasound contrast agents.

A previous report explored the feasibility of drug-loaded macrophages for chemotherapy in tumor hypoxic regions with ultrasound (Huang et al. 2015). The therapeutic macrophages in the study were prepared *via* co-incubation with macrophages and the mixture of acoustic polymer/C_5F_{12} bubbles and doxorubicin (DOX)-loaded polymer vesicles. However, the drug payload and ultrasound-responsible capability of the macrophages may have been limited because the particles might have a distinct cellular uptake efficiency, and it was difficult to ensure if the cells ingested these two kinds of particles. In the present study, we investigated the feasibility of utilizing macrophages to load DOX-loaded acoustic droplets (DOX-droplets). The inner shell of the surfactant monolayer of droplets serves as a suitable carrier for DOX because of the extreme lipophobicity and hydrophobicity of DOX (Chokshi et al. 2011). Macrophages appear to be a suitable drug vesicle for tumor treatment because they can actively migrate toward tumors and penetrate hypoxic regions. First, we characterized the properties of DOX-droplets. Second, we investigated the influence of DOX-droplets on macrophages, including migration mobility and cellular viability. Finally, we assessed the ultrasound contrast-enhanced ability of DOX-droplets and ultrasound-medicated DOX release from DOX-droplet-loaded macrophages (DLMs) *in vitro*. Our results suggest that DLMs are useful agents for tumor theranostics.

METHODS

Fabrication of DOX droplets

The lipid shells of the droplets consist of 1.5 mol% distearoyl-glycerophosphoethanolamine-poly(ethylene glycol) 2000 (DSPE-PEG2000, Avanti Polar Lipids Inc., Alabaster, AL, USA), 40 mol% cholesterol (Sigma Aldrich Co., St. Louis, MO, USA), 29 mol% distearoyl-glycerophosphoglycerol (DSPG, Avanti Polar Lipids Inc.) and 29.5 mol% distearoyl-glycerophosphocholine (DSPC, Avanti Polar Lipids Inc.). The lipid mixtures were blended into chloroform. The chloroform was eliminated by evaporation over 24 h. The dried lipid film was then hydrated with 0.5 vol% glycerol phosphate-buffered saline (PBS). Subsequently, DOX (Seedchem Co. PTY LTD, Melbourne, Australia) and perfluoropentane were dissolved in the lipid solution, and droplet emulsions formed when the vial was immersed in a sonication bath at room temperature for 5 min. The prepared droplets were centrifuged for 3 min (2000*g*) to eliminate unloaded drug. The prepared DOX-droplets were resuspended in PBS at 4 °C. To visualize drug release from droplets, a large amount of DiI-C18 (8 mol%) was labeled in the lipid shell of droplets (DiI-droplets) without DOX addition as hydrophobic model drugs. DiI-C18 is a fluorescent and photostable dye and is a reliable probe for fluorescence imaging (Luan et al. 2014). In addition, the lipophilic property of DiI-C18 enabled incorporation into a lipid membrane, just as with DOX (Tinkov et al. 2010).

Characterization of DOX-droplets

The structure of the prepared droplets/DOX-droplets was observed using an inverted microscope (IX71, Olympus Corp., Tokyo, Japan). The concentration and size distribution of droplets were measured with a Coulter counter (Multisizer 3, Beckman Coulter Inc., Carlsbad, CA, USA). The DOX payload of the DOX-droplets was also determined. The prepared DOX-droplets were collected and sonicated (Model 2510, Branson Ultrasonics, Danbury, CT, USA) to completely destroy the droplets. The final precipitate was resuspended with de-gassed, de-ionized water. The encapsulated DOX was measured with a fluorescence spectrophotometer (Safire, Tecan, AG, Switzerland;