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Effects of the microbubble shell physicochemical properties on ultrasound-mediated drug delivery to the brain

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ABSTRACT

Lipid-shelled microbubbles have been used in ultrasound-mediated drug delivery. The physicochemical properties of the microbubble shell could affect the delivery efficiency since they determine the microbubble mechanical properties, circulation persistence, and dissolution behavior during cavitation. Therefore, the aim of this study was to investigate the shell effects on drug delivery efficiency in the brain via blood–brain barrier (BBB) opening in vivo using monodisperse microbubbles with different phospholipid shell components. The physicochemical properties of the monolayer were varied by using phospholipids with different hydrophobic chain lengths (C16, C18, and C24). The dependence on the molecular size and acoustic energy (both pressure and pulse length) were investigated. Our results showed that a relatively small increase in the microbubble shell rigidity resulted in a significant increase in the delivery of 40-kDa dextran, especially at higher pressures. Smaller (3 kDa) dextran did not show significant difference in the delivery amount, suggesting that the observed shell effect was molecular size-dependent. In studying the impact of acoustic energy on the shell effects, it was found that they occurred most significantly at pressures causing microbubble destruction (450 kPa and 600 kPa); by increasing the pulse length to deliver the 40-kDa dextran, the difference between C16 and C18 disappeared while C24 still achieved the highest delivery efficiency. These indicated that the acoustic energy could be used to modulate the shell effects. The acoustic cavitation emission revealed the physical mechanisms associated with different shells. Overall, lipid-shelled microbubbles with long hydrophobic chain length could achieve high delivery efficiency for larger molecules especially with high acoustic energy. Our study, for the first time, offered evidence directly linking the microbubble monolayer shell with their efficacy for drug delivery in vivo.

1. Introduction

Microbubbles, gas-filled microspheres (1–10 μm) initially used merely as contrast agents for ultrasound imaging, have recently been shown critical in ultrasound-mediated therapeutic applications such as sonothrombolysis [1,2], molecular delivery to the cell via sonoporation [3,4] and/or endocytosis [5,6], and to the brain parenchyma via blood–brain barrier (BBB) opening [7,8] paracellularly or transcellularly. For molecular delivery purposes, although the biological mechanisms may vary, sonoporation and/or endocytosis and BBB opening share the same physical mechanism that cavitation increases the permeability of the cell membranes. In all the cases, the microbubble properties play important roles in determining the delivery efficiency. For example, larger microbubbles (4–5 μm in diameter) induce larger BBB opening and delivery efficiency than smaller microbubbles (1–2 μm in diameter) [9,10]; soft-shelled (lipid or protein) microbubbles gave higher cell viability and transfection rate of gene delivery than hard-shelled (polymer) microbubbles [11].

Overall, the main goal of drug delivery is to achieve high efficiency without causing cell damage, and with the use of lipid-coated microbubbles it is achievable. In fact, with lipid-coated microbubbles the overall drug delivery efficiency could be influenced by changing the lipid hydrophobic chain length that modulates the overall physicochemical properties of the monolayer shell. Borden et al. have shown that increasing the lipid hydrophobic chain length increased the gas permeation resistance to the environment [12], decreased the acoustic dissolution rate while enhancing the lipid-shedding phenomenon during insonification [13]. Kwan et al. have reported that bubbles with longer lipid hydrophobic chains required longer re-stabilization following shell rupture, and longer to dissolve after the onset of collapse due to stronger attractive intermolecular forces [14,15]. Longer acyl chains can also increase the lipid monolayer thickness [16] and microbubble mechanical properties such as in-plane rigidity [17], thereby modulating cavitation response and the shear stress applied on the cell membrane [18,19]. Those results suggest that the physicochemical

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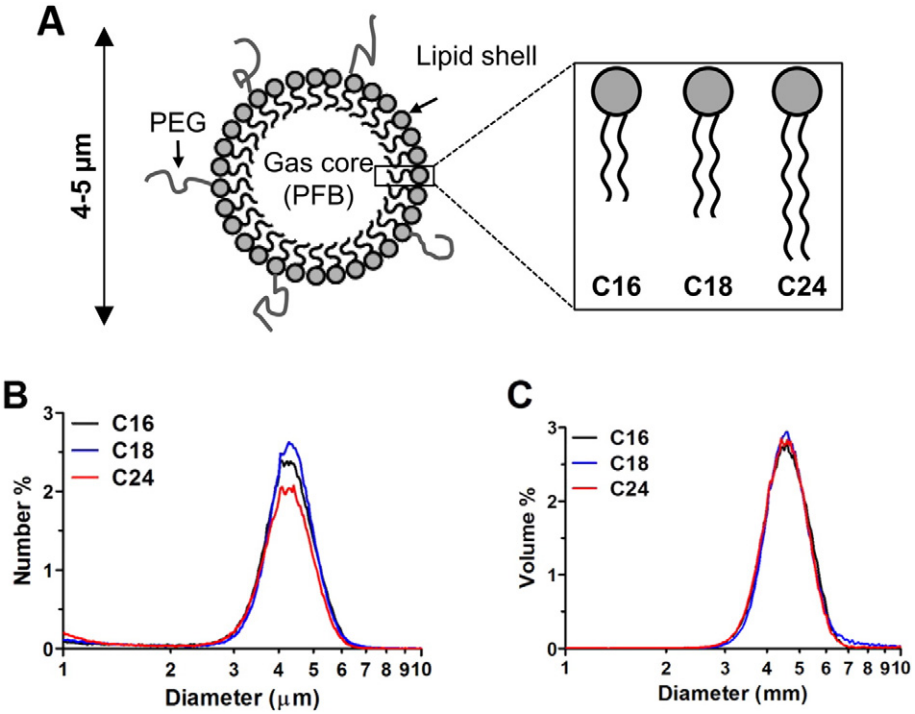

Fig. 1. Schematic of the lipid-shelled microbubble used in this study (A) and their representative size distribution in number (B) and volume (C). Three different lipid acyl chain lengths (C16, C18, C24) were used to generate microbubbles of different physicochemical properties, while the emulsifier (DSPE-PEG2000), the molar ratio between the main lipid and the emulsifier (9:1), the gas core (PFB), and the size of the microbubbles (4–5 μm) were kept the same in order to focus on the effects of lipid hydrophobic chain length. The size of the different-shelled microbubbles was statistically the same (Table 2). All microbubble suspensions were diluted to the same concentration (8×10^8 particles/mL) immediately prior to injection.

properties of the lipid-shelled microbubbles may play a role in affecting the drug delivery efficiency, but the exact effects remain to be discovered.

This study aimed at investigating the shell effect of lipid-coated microbubbles on the drug delivery efficiency in vivo. We hypothesize that increasing the lipid hydrophobic chain length would enhance the drug delivery efficiency after focused ultrasound (FUS)-induced BBB opening. The microbubbles used were coated with phosphatidylcholine (PC) lipids of various acyl chains (C16, C18, C24), and the phospholipid:lipopolymer ratio was fixed in order to isolate the effect of the PC acyl chain length. In addition, the diameter of the microbubble samples was kept constant at 4–5 μm in all experiments in order to exclude the influence of the microbubble size. Both molecular size (3 kDa and 40 kDa dextran been delivered), acoustic pressure (225–600 kPa), and pulse length (100 cycles and 1000 cycles) dependences were investigated in order to fully assess the microbubble shell effects on the drug delivery efficiency.

The different shelled microbubble dynamics in vivo were also captured during insonification using passive acoustic cavitation detection (PCD) in order to potentially uncover the physical mechanisms affecting the delivery efficiency such as micro-streaming and micro-jetting. The signal recorded by PCD is the acoustic emission from the cavitating bubbles, which represents the cavitation intensity with the signature of stable and/or inertial cavitation. We assume that the detected harmonics and ultraharmonics relate to stable cavitation (low and high amplitude bubble pulsation, or decaying oscillation) resulting in micro-streaming in a short or long period, and the detected broadband emission to inertial cavitation (violent bubble oscillation, bubble breakup or rebound) causing micro-jetting or shock wave emission, based on the bubble activities categorized by Leighton [20]. Both types of cavitation are thought to contribute to ultrasound-mediated drug delivery.

2. Materials and methods

2.1. Microbubble generation

All the lipids were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA), including 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC or C16), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC or C18), 1,2-dilignoceroyl-sn-glycero-3-phosphocholine (DLiPC or C24) and 1,2-distearoyl-sn-glycero-3phosphoethanolamine-N-[methoxy(polyethylene glycol)2000] (DSPE-PEG2000). The perfluorobutane gas (PFB, 99 wt.% purity) used for microbubble generation was obtained from FluoroMed (Round Rock, TX, USA).

The lipid-coated microbubbles as shown in Fig. 1A were prepared at a 9:1 molar ratio of lipids and lipopolymers (DSPE-PEG2000). They were generated using the probe sonication method and size selected to 4–5 μm in diameter using differential centrifugation, as described elsewhere [21]. A Multisizer III particle counter (Beckman Coulter Inc., Opa Locka, FL, USA) with a 30-μm aperture was used to measure the microbubble size distribution (Fig. 1B–C) and concentration. The final size-isolated (4–5 μm) microbubble suspension was stored at 4 °C till the time of injection. All the microbubble samples used for this study were freshly prepared within 24 h to ensure experimental consistency.

2.2. Animal and drug preparation

All animal experiments were conducted in accordance with procedures approved by the Columbia University Institutional Animal Care and Use Committee. A total of 123 male C57BL/6 mice (Harlan Laboratories, Indianapolis, IN, USA) weighing 20–25 g were used for this study. The animals were divided into five experimental groups depending on the ultrasound applied and the dextran molecule delivered during the FUS-induced BBB opening. The groups were further divided into 38 cohorts based on the experimental protocol as listed in Table 1. Before