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Spatial and temporal observation of phase-shift nano-emulsions assisted cavitation and ablation during focused ultrasound exposure

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ABSTRACT

Background: Phase-shift nano-emulsions (PSNEs) with a small initial diameter in nanoscale have the potential to leak out of the blood vessels and to accumulate at the target point of tissue. At desired location, PSNEs can undergo acoustic droplet vaporization (ADV) process, change into gas bubbles and enhance focused ultrasound efficiency. The threshold of droplet vaporization and influence of acoustic parameters have always been research hotspots in order to spatially control the potential of bioeffects and optimize experimental conditions. However, when the pressure is much higher than PSNEs' vaporization threshold, there were little reports on their cavitation and thermal effects.

Object: In this study, PSNEs induced cavitation and ablation effects during pulsed high-intensity focused ultrasound (HIFU) exposure were investigated, including the spatial and temporal information and the influence of acoustic parameters.

Methods: Two kinds of tissue-mimicking phantoms with uniform PSNEs were prepared because of their optical transparency. The Sonoluminescence (SL) method was employed to visualize the cavitation activities. And the ablation process was observed as the heat deposition could produce white lesion.

Results: Precisely controlled HIFU cavitation and ablation can be realized at a relatively low input power. But when the input power was high, PSNEs can accelerate cavitation and ablation in pre-focal region. The cavitation happened layer by layer advancing the transducer. While the lesion appeared to be separated into two parts, one in pre-focal region stemmed from one point and grew quickly, the other in focal region grew much more slowly. The influence of duty cycle has also been examined. Longer pulse off time would cause heat transfer to the surrounding media, and generate smaller lesion. On the other hand, this would give outer layer bubbles enough time to dissolve, and inner bubbles can undergo violent collapse and emit bright light.

1. Introduction

Ultrasound contrast agents (UCAs) are microbubbles typically ranging in diameter from 1 to 10 μm. They were firstly introduced into diagnostic application to enhance the echogenicity in 1968 [1]. However, nowadays their therapeutic applications have been exploited a lot: such as tissue occlusion [2], drug/gene delivery [3], transformation of cell membrane and vascular permeability [4,5]. It is believed that both the thermal and nonthermal effects of therapeutic ultrasound can be enhanced by the presence of UCAs. However, some problems exist in utilizing UCAs assisted therapy: one is their size distribution, which is in the micron range, limiting them leaking into tissue from blood vessels. The other is the circulation time, which may not be long enough and constrains UCAs accumulation in target region. Furthermore, injected UCAs flowing through the whole body may cause some side effects, such as high osmotic pressure and blood vessel dilation. To overcome these limitations, phase-shift nano-emulsions (PSNEs) are possible candidates.

PSNEs are usually composed of perfluorocarbons (PFCs) and coated materials. Among PFCs, most used are dodecafluoropentane (PPFP) and perfluorohaxane (PFH), whose boiling temperatures are 29 °C and 60 °C respectively. At room temperature, they are in liquid state, and can be manufactured to droplets as small as 200 nm [6], having the potential to leak out of the blood vessel into the interstitial tissue [7]. When coated by bovine serum albumin, lipid or other kinds of shells, PFCs become more stable against coalescence and spontaneous evaporation at body temperature. Therefore, they have longer circulation time to accumulate at target point of tissue. At desired location, PSNEs can undergo a phase shift into gas bubbles under intense acoustic exposure,

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which is termed as acoustic droplet vaporization (ADV). ADV bubbles can act like normal UCAs, enhancing *in situ* thermal and non-thermal effects, reducing cavitation threshold, and providing ultrasonic imaging feedback. These unique characteristics of PSNEs have triggered a large number of researches on its mechanisms and applications.

Kripfgans et al. have proposed ADV process early in 2000 [8], and applied it to tissue occlusion and phase aberration [9], as it can provide gas bodies lasting long enough and emitting strong backscatter signals. Miller et al. [10] have proposed stabilized PSNEs droplet as a nucleation-promoting agent which can greatly enhance therapeutically useful nonthermal bioeffects, and proved it has higher nucleation efficiency than the Optison™. Besides, many researchers combined PSNEs with high-intensity focused ultrasound (HIFU). Zhang et al. [11] have demonstrated that microbubbles generated by PSNEs could enhance thermal ablation by controlling and increasing local energy absorption. With gel phantom, Kawabata et al. [12] studied the optical change and acoustic emission, and suggested that PSNEs enhanced cavitation activities is responsible for the temperature elevation. They also delivered the PSNEs into tumor tissues, and found that HIFU expose can make an obvious hole first at the surface and then expanding into tumor while the focus of the ultrasound was set inside the tissue. Schad et al. [7] have investigated the HIFU parameters for bubble production from droplet vaporization and its dependence of the acoustic exposure conditions and droplet parameters. Singh et al. [13] have studied the physics and chemistry of phase transition of PFC_5 and PFC_6. In the same condition, PFC_5 may commence phase-transition much earlier and for a longer time during the acoustic cycle.

PSNEs are promising agents for therapeutic application. They can be vaporized by HIFU in a localized manner, and greatly reduce the pressure for cavitation and thermal ablation. The threshold of droplet vaporization and the influence of acoustic parameters have always been research hotspots in order to spatially control the potential of bioeffects and optimize experimental conditions. We also observed point lesion at a relatively low input power. However, when the pressure is much higher than PSNEs' vaporization threshold, there are little reports on their cavitation and thermal effects. In this study, PSNEs were uniformly buried in tissue-mimicking gel phantoms, which have similar acoustical parameters to ordinary tissue. Their cavitation activities were assessed by SL distribution and intensity, which is a direct indicator of inertial cavitation. Ablation process was observed as the heat deposition could denature the albumin in phantom and produce white lesion. Spatial and temporal informations on PSNEs induced cavitation and ablation during pulse HIFU were provided. PSNEs were easily evaporated into bubbles during continuous HIFU exposure, limiting the acoustic waves delivered into the phantom. However, pulsed HIFU may ease this kind of shielding effect.

2. Materials and methods

2.1. System set-ups

Fig. 1 shows the schematic of experimental set-ups. One channel of a double-channel arbitrary wave generator (AWG420, Tektronix) was used to generate tone bursts of 10 μs in duration at different duty cycle (T_{on}/T_{total} = 1:10, 1:20, 1:30, 1:40, 1:50), which was amplified by a power amplifier (AG1017, T&G Power Conversion, Inc., Rochester, NY) and fed to a concave focused transducer (Imasonic, Besancon, France). The transducer was a 1.2 MHz single-element transducer, with an active diameter of 156 mm, and a geometrical focal length of 120 mm. The acoustic power to electric power transmission efficiency of the transducer was 65%.

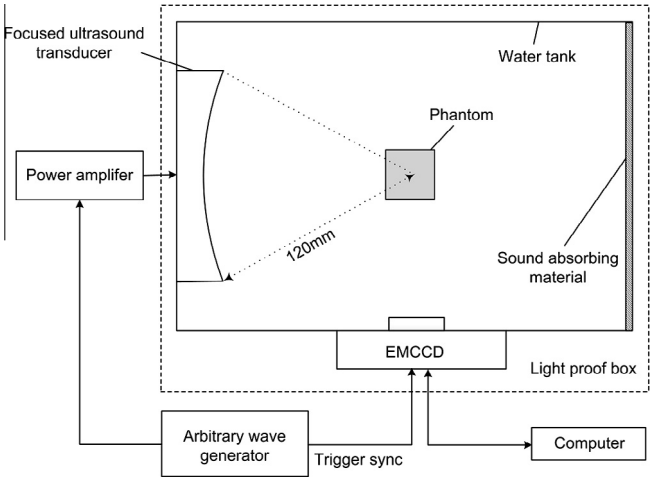

Fig. 1. System set-ups.

Characteristic of the acoustic pressure distribution of the focused ultrasound field has been described in detail elsewhere [14]. The transducer was mounted on one side of an acrylic tank 50 cm (long) × 28 cm (high) × 28 cm (wide), which was filled with degassed water as medium. On the back of the tank, there was an acoustic absorber to reduce the reflect signals. A phantom with buried PSNEs was held in the tank and precisely located by an *X*–*Y*–*Z* positioning stage. SL images were used to quantify cavitation activities and captured by an EMCCD camera (iXon3 897, Andor technology PLC, Northern Ireland, UK), which was triggered by the other channel of the arbitrary wave generator. The exposure times of EMCCD were changed from 20 s to 100 s to ensure an equality total on time of 2 s. The SL images were post-processed using Andor SOLTS software (Andor technology PLC, Northern Ireland, UK). The light generation system was in a light-proof box to reduce the background noise. All the results reported were recorded at the room temperature of about 20 °C.

2.2. Materials

2.2.1. Preparation of phase-shift nano-emulsions

Phase-shift nano-emulsions were composed of PFH and bovine serum albumin. According to our preliminary experiments, PFH with higher stability is more proper to be used in HIFU field. It can generate sparsely distributed bubble cloud and bright SL in good accordance with each other. So PFH was used as liquid core in our study. The preparation process was the same as reported by Zhang et al. [15]: 0.3 ml PFH (Sigma–Aldrich Co. LLC., UK) and 9.7 ml degassed, deionized water were mixed and cooled by an external ice bath to avoid evaporation. Then the solution was emulsified with an ultrasonic liquid processor (VC 705, Sonic & Materials, Newton, USA) for 1 min to produce more uniform distribution. The resulting emulsion was poured slowly into a 5 ml 2% (v/v) albumin solution to coat droplets with an albumin shell. There have already been a lot of researches demonstrating the inverse relationship between ADV threshold and droplet size. The smaller the droplet is, the more stable it is. However, the inertial cavitation (IC) threshold is not significantly dependent on the droplet size [16]. As SL is a direct indicator of IC, it can be assumed that within limits, droplet size would not change SL result qualitatively. Our considerations for size of droplets are like the following: (1) it should be within the range of 100–750 nm, which enables them to extravasate into the interstitial space in solid tumors [17]. (2) It should be small enough to avoid disturbing the ultrasound waves' propagation before vaporization. (3) It should