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● *Original Contribution*

SIMULTANEOUS EVALUATION OF THERMAL AND NON-THERMAL EFFECTS OF HIGH-INTENSITY FOCUSED ULTRASOUND ON A TISSUE-MIMICKING PHANTOM

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Abstract—Physiologically relevant phantoms with high reliability are essential for extending the therapeutic applications of high-intensity therapeutic ultrasound. Here we describe a tissue-mimicking phantom capable of quantifying temperature changes and observing non-thermal phenomena by high-intensity therapeutic ultrasound. Using polydiacetylene liposomes, we fabricated agar-based polydiacetylene hydrogel phantoms (PHPs) that not only respond to temperature, but also have acoustic properties similar to those of human liver tissue. The color of PHPs changed from blue to red depending on the temperature in the range 40°C–70°C, where the red/blue ratio of PHP had a good linearity of 99.06% for the temperature changes. Furthermore, repeated high-intensity focused ultrasound led to histotripsy on the PHP with liquefied and damaged areas measuring 0.7 and 4.0 cm^2, respectively, at the signal generator amplitude setting voltage of 80 mV. Our results indicate not only the usability of the thermochromic phantom, but also its potential for evaluating non-thermal phenomena in various high-intensity focused ultrasound therapies. (E-mail: shkim@kriss.re.kr)

Key Words: **High-intensity focused ultrasound, Phantom, Liposome, Tissue-mimicking.**

INTRODUCTION

The minimally invasive nature of high-intensity focused ultrasound (HIFU) surgery offers a significant advantage over other therapeutic tumor treatments (Choi et al. 2013). The major mechanism underlying HIFU therapy heating of the tumor above a temperature threshold sufficient to cause coagulative necrosis (target temperature >65°C). To ensure patient safety and to maintain clinical efficacy in HIFU surgery, it is important to accurately predict the temperature elevation inside tissues during HIFU sonication. Current non-invasive methods used to measure tissue temperature include infrared thermography (Mital and Scott 2007), ultrasound imaging (Varghese et al. 2002) and magnetic resonance thermometry (Le et al. 2011). However, it is not easy to directly measure the temperature inside target tissue.

Tissue-mimicking phantoms (Casciaro et al. 2008, 2009; Christian et al. 2008) have been widely used for evaluation, optimization and performance characterization of such ultrasound-based medical devices. Thermochromic tissue-mimicking phantoms are tissue-mimicking materials that can change color in response to a change in temperature. Recently, thermochromic phantom materials have been developed to identify heating patterns during thermal therapy (Butterworth et al. 2012; Qureshi et al. 2015). Dabbagh et al. (2014) described reusable heat-sensitive tissue-mimicking phantoms that change from blue to colorless on heating and change in reverse on cooling. However, the color change threshold was relatively low (50°C) for thermal ablation. Because the color change is reversible, measurement should be performed promptly before color reversal to record an accurate temperature. In addition, the main limitation of this phantom is the loss of transparency during the construction of a large volume of phantoms. Negussie et al. (2016) described another thermochromic tissue-mimicking phantom to which was added commercially available thermochromic ink (Kromagen Magenta MB60-NH). However, the threshold temperature was also limited to around 60°C–65°C, and the phantom could not be used to measure the precise temperature elevation after heating. Although various biomaterials including bovine serum albumin (Choi et al.

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2013; Zhang et al. 2008; Zhou and Gao 2013) and egg white (Divkovic et al. 2007; Wang et al. 2014) have been used to indicate temperature changes, the temperature of the target point inside the phantom could not be accurately measured.

Polydiacetylene (PDA), a biocompatible polymer, has been extensively investigated and utilized as an attractive platform for sensing applications because of its unique optical properties (Reppy and Pindzola 2007; Sun et al. 2010). The monomers of PDA can self-assemble into liposomal structures in an aqueous medium. On ultraviolet light irradiation, blue PDA can be readily obtained through rapid 1,4-addition polymerization of diacetylenes in the monomers. PDA can undergo a blue-to-red color change as well as fluorescence change in response to external stimuli such as heat, organic solvents and analyte–receptor interactions (Wang et al. 2015). Therefore, to detect temperature changes, the temperature-responsive polymer PDA is appropriate for use as a sensing material for an irreversible thermochromic tissue-mimicking phantom.

Here, we describe a thermochromic phantom that measures the temperature changes of the target site and histotripsy lesions simultaneously during continuous wave (CW) HIFU exposure. To achieve similarity with biological tissue, we determined the optimum mixing ratio of agar, water, PDA nanocomposites, sucrose and calcium chloride while precisely measuring the acoustical parameters such as density, sound speed and attenuation coefficient. Because this transparent PDA hydrogel phantom (PHP) has these color-changing characteristics, the change in temperature inside the PHP can be analyzed by the color ratio during HIFU treatment.

METHODS

Materials

Agar (agar ash ≤2.0%), L-α-phosphatidylcholine (purity 99%), calcium chloride ($CaCl_2$, purity 97%, anhydrous) and 10,12-pentacosadiynoic acid (purity 97%) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Sucrose was obtained from Kanto Chemical Company (Tokyo, Japan). Ultrapure de-ionized water was used for preparation of PHPs.

Synthesis and characterization of PDA liposomes

Liposomes were prepared using the thin-film rehydration method. Briefly, L-α-phosphatidylcholine (PC, 10 mM stock solution in chloroform, 250 μL) and 10,12-pentacosadiynoic acid (PCDA, 10 mM stock solution in chloroform, and 750 μL) were mixed in a 20-mL glass vial. The chloroform was evaporated by a stream of N_2 gas to yield a thin uniform translucent film, and 10 mL of de-ionized water was added. This solution was sonicated at 80°C using probe sonication (Sonic & Materials, Inc., Newtown, CT, USA, VC505 model, 20% amplitude) for 30 min, and the resulting solution was filtered through a cellulose acetate filter (0.8 μm) while hot. The filtrate was cooled and stored at 4°C overnight. The resulting solution was polymerized at room temperature by irradiating the solution with 254 nm ultraviolet light (4-W, 254-nm tube, Vilber Lourmat, Marne La Vallee Cedex, France) for 3 min. After irradiation, the colorless liposome solution turned blue and the final concentration of PDA liposome was 1 mM. Liposome size was determined with the dynamic laser light scattering technique. The average size of the PDA liposome in water was determined to be 84.4 nm at 25°C, as measured with a Malvern Zetasizer Nano ZEN5600 dynamic light scattering analyzer (ZEN5600, Malvern Instruments Ltd., Worcestershire, UK).

Fabrication of agar-based PDA hydrogel phantoms

The ultrasound phantoms were made of 5% (w/v) by weight dried agar powder. Two grams of agar (per 4, 8, 12, 16 and 20 g of sucrose for 10, 20, 30, 40 and 50% w/v, respectively) or 2 g agar/8 g sucrose (20% w/v)/$CaCl_2$ (2 M stock solution in water, 4, 8, 12 and 16 mL for 0.2, 0.4, 0.6 and 0.8 M, mol/L, respectively) was added to a 100-mL glass beaker. De-gassed, distilled water was then added to make a total volume of 40 mL. The mixture was heated until it turned from cloudy to clear using 700-W microwaves. Once the agar was completely in solution, hot de-ionized water was added to compensate for the volume loss during heating. The hot solution was then poured into a plastic cylinder container and mixed using a Thinky (Tokyo, Japan) super mixer (non-vacuum type, mixing mode: 2000 rpm, 1 min, defoaming/degassing mode: 2200 rpm, 1 min).

To prepare phantoms with different concentrations of PDA, 2 g agar/8 g sucrose (20% w/v)/16 mL $CaCl_2$ (0.8 M mol/L) was added to a 100-mL glass beaker. De-gassed, distilled water was then added to make a total volume of 40 mL. The solution was dissolved in water, and the resulting solution was mixed, using the same procedure as mentioned above, with the Thinky super mixer. After the mixing step, the hydrogel was cooled to 45°C and PDA liposomes were added to the hydrogel solution in a plastic cylinder container, along with the desired concentration of PDA liposome (1 M stock solution in water; 0.4, 0.8, 1.2, 2.0 and 2.8 mL for 10, 20, 30, 50 and 70 μM, 10^{-6} mol/L, respectively). The resulting hydrogel solution was mixed using the Thinky super mixer (non-vacuum type, mixing mode: 2000 rpm, 30 s, defoaming/degassing mode: 2200 rpm, 30 s). After the mixing step, the hydrogel was poured into an acrylic phantom mold (diameter = 5.4 cm, height = 1.0 cm) and allowed to solidify into a gel.