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A Preliminary Study of Drug Perfusion Enhancement by Microstreaming Induced By an Oscillating Microbubble

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***Abstract*—**Microbubbbles incorporating ultrasound have been used to increase the efficacy of targeted drug delivery, because microstreaming induced by cavitating bubbles affects the drug perfusion into the target cells and tissues. In order to clarify the physical effects of microstreaming on drug perfusion into tissues, a preliminary experimental study of perfusion enhancement by a stably oscillating microbubble was performed. Microstreaming was induced by an oscillating bubble at 15 kHz, and perfusion of dye into an agar phantom was optically measured by histology on agar phantom. Surface color intensity and the penetration length of dye in the agar phantom were increased more than 70% and 30%, respectively, due to the microstreaming induced by an oscillating bubble. The mass of dye perfused into a tissue phantom for 30 s was increased about 80% in the phantom with an oscillating bubble. This preliminary experiment shows the physical effects of steady streaming by an oscillating bubble can enhance the drug perfusion into the tissues while minimizing the biological effects.

***Keywords*—**Bubble, Mass Transfer, Microstreaming, Drug Delivery, Acoustic Wave.

I. Introduction

MICROBUBBLES driven by ultrasound have been used to enhance drug delivery to target cells and tissues in clinical applications. Pressure and stress fields generated by cavitating bubbles may affect the drug perfusion into the tissues via two different pathways. They may affect not only the cell membrane permeability [1] and cellular junction integrity [2], but also affect the drug availability near the target cells and tissues by convective mixing motion of streaming flow fields. In contrast to the inertial cavitation which accompanies bubble collapsing and microjetting, a stable cavitation can enhance drug perfusion to the tissues without cellular damage by streaming which is induced by gentle oscillation of microbubbles. Therefore, it has been used to enhance the efficacy of thrombolysis [3]-[6], targeted drug delivery [7]-[9], and drug delivery across the blood brain barrier (BBB) [10], [11]. Many theoretical [12], [13] and experimental [14], [15] analyses on the steady streaming flow fields have been performed, but studies on mass transfer enhancement by stable cavitation are limited. A preliminary experimental study of perfusion enhancement induced by a stably oscillating microbubble was performed in order to clarify the physical effects of microstreaming on drug perfusion into tissues.

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II. Methods

Agarphantoms have been used to mimic neurological tissues [16] and thrombus [17]. A tissue phantom was prepared by mixing a solution of 0.6 wt% agarose powder (Samchun Chemical, Korea) and distilled water. The mixture was heated until all the agarose powder was dissolved, and then it was boiled for 10 minutes. After the mixture solution was cooled to the room temperature, approximately 40 g was poured into a rectangular container made of transparent plastic mold (50 x 50 x 20 mm). Samples for the experiment were prepared by cutting the agar phantom into the hexahedron block (15 x 15 x 12 mm). Aqueous solution of safranine (Samchun Chemical, Korea) was used to mimic water soluble drug, and drug perfusion was optically measured by histology on agar phantom [18].

In order to quantify the color intensity in the sample, the correlation between safranine concentration and image color intensity was obtained as follows. The 0.15 ml of safranine solution with various concentrations (0.001, 0.003, 0.005, 0.0075, 0.01, 0.03, 0.05, 0.075, 0.1 wt %) was infused into the chambers made of microscope slides (76 x 26 x 1 mm) using a pipette. A safranine solution of the known concentration was placed on the light illumination panel (Dae Jin Trading Co., Ltd, Korea) and then the color image was captured by a camera (AM 313, Dino-Lite, ANMO Co., Taiwan). The digitally image was imported into ImageJ (National Institutes of Health, USA). The color intensity was decomposed into red-green-blue color intensities, and they were correlated with the safranine concentration.

The experimental set up is shown in Fig. 1. The phantom was immersed in safranine solution (0.01 wt%, 50 ml), and fixed at the bottom of a chamber (40 × 40 × 30 mm). An air bubble of 500 μm diameter was generated using a microsyringe (600 Series MICROLITER™ Syringes model 62, Hamilton Company, USA), and placed on the tip of a Teflon (Dupont, USA) coated rod. A bubble is placed 0.5 mm away from a phantom surface using a three dimensional traverse system. For acoustic excitation, a sine wave voltage was generated by a function generator (33210A, Agilent Co., USA), and amplified up to a few hundred volts by a voltage amplifier (PZD700, Trek

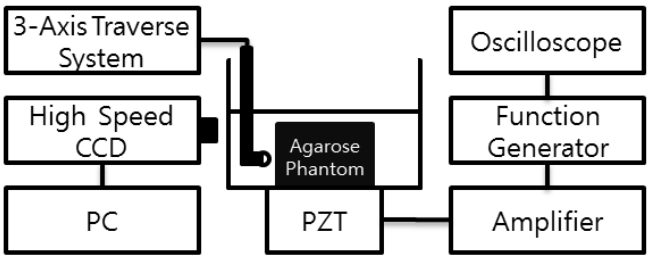

Fig. 1 Schematic diagram of experimental set-up

Co., USA). The amplified voltage signal was transmitted to a cylinder-type piezoactuator (disk type PRYY-1133, PI Ceramics, Germany) attached to the bottom of the chamber. The bubble was excited by the acoustic wave generated by a piezoactuator excited by 12 kHz voltage signal with the amplitude of 300 volt, and the oscillation of a bubble was observed using a charge coupled device (CCD, EO-1312C, Edmund Optics, USA) integrated with a zoom lens (VZM™ 450i, Edmund Optics, USA).

The phantom was exposed to the streaming flow induced by oscillating bubbles for 30 s. A 0.7 mm slice of the phantom was taken at the location closest to the bubble center, and it was imaged by a camera. The digitally captured image (Fig. 3) was imported into ImageJ, and the color intensity was measured across the cross section the phantom sample in order to determine the dye (safranine) perfusion into a phantom. Red, blue and green color mapping was used to demonstrate the optical color intensity of the safranine solution. Experiments were performed for more than 11 phantom slices, and at least 4 color intensity profiles were extracted for each sliced sample.

III. Results

In order to estimate safranine concentration perfused into a phantom tissue, the correlation between safranine concentration and color intensity was obtained. The digital image of a perfused sample was color mapped to red, green and blue color intensity. The intensity of each color component was corrected for background by subtracting background intensity. The differences of color intensity of red, green and blue are well correlated with the safranine concentrations. The dye color intensities (R-G-B) were calculated by subtracting the background corrected intensities of green and blue from that of red. The color intensity R-G-B shows a linear correlation with concentration for low concentrations, and it is saturated as the concentration increases (Fig. 2).

Fig. 3 shows the color images of sliced samples with and without bubble oscillation for 30 s. The increases of color intensity and perfusion length are noticeable for the sample exposed to microstreaming induced by bubble oscillation. The color intensity of the digital image was mapped to the R-G-B intensity and the distance from the phantom surface was determined by converting digital pixels into distance. The color intensity of R-G-B is higher along the perfusion length in a phantom with oscillating bubble as shown in Fig. 4.

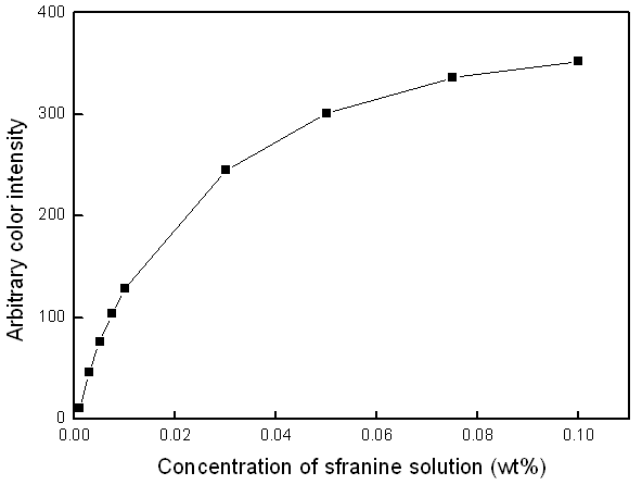

Fig. 2 The R-G-B color intensities for different concentrations of aqueous safranine solution

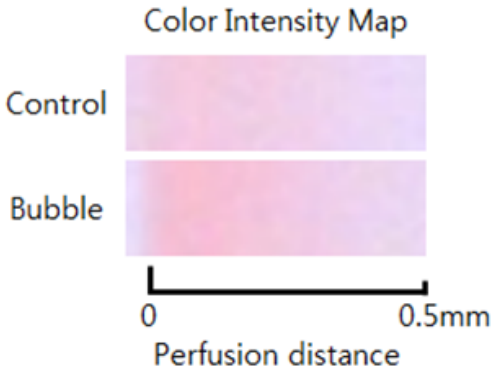

Fig. 3 The color images of the sliced agar phantom sample with and without bubble oscillation. Bubbles are activated by 12 kHz acoustic wave for 30 seconds

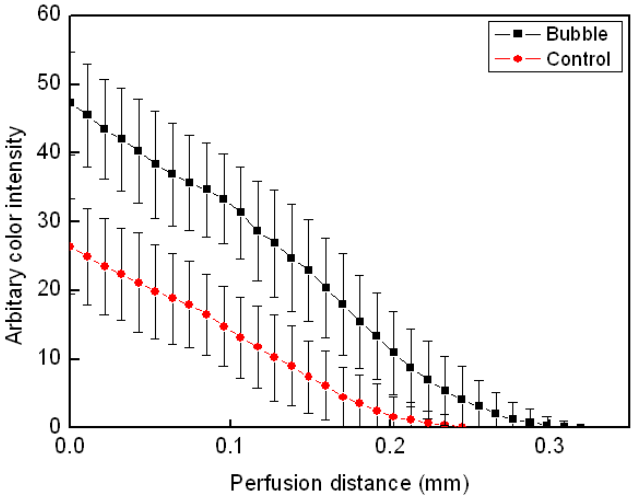

Fig. 4 The R-G-B color intensity distribution along the perfusion distance. Sold squares are the samples with bubble oscillation and solid circles are the samples without bubble oscillation. Vertical bars show standard deviations

Surface color intensity at the surface and the penetration length is increased more than 70% and 30%, respectively, for the phantom with bubble oscillation. The increase of dye concentration at the surface may owe to the reduced mass transfer resistance by pressure and shear as well as the

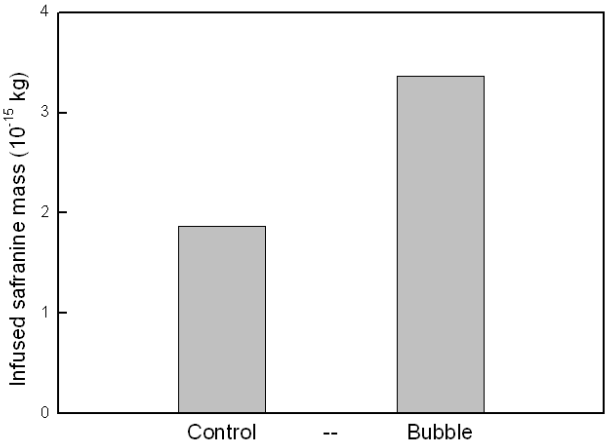

Fig. 5 Mass of safranine dye perfused into the phantom per 1 mm width with and without bubble oscillation

IV. Conclusion

Dye perfusion experiment was performed using an agar phantom in order to elucidate the effects of microstreaming induced by an oscillating bubble on drug delivery. We showed that perfused dye concentration in the agar phantom could be estimated by the color intensity of the cross section of a sliced sample. Noticeable increase of color intensity of dye in the phantom with an oscillating bubble was observed. Surface color intensity and the penetration length of dye in a phantom were increased more than 70% and 30%, respectively, due to the microstreaming induced by an oscillating bubble. The mass of dye perfused into a tissue phantom was increased about 80% in the phantom with an oscillating bubble. This preliminary experiment shows the physical effects of steady streaming, such as pressure, stress, and flow fields, can enhance the drug perfusion into the tissues while minimizing the biological effects. Further studies for micron size bubbles activated by ultrasound waves should be performed to validate the effectiveness of drug delivery enhancement by microstreaming of ultrasound contrast agents for clinical application.

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