

Site-selective ultrasonic tissue-structure destruction for drug delivery control

超音波による局所的な組織構造破壊を用いる薬物送達技術

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1. Introduction

Ultrasound bio-effects can roughly be categorized into two; thermal[1] and mechanical[2] ones. The former and latter effects coagulate and fragment tissues, respectively.

We propose a new Drug Delivery System (DDS) utilizing the latter effects. In DDS, controlling spatial and temporal distribution of drugs are pursued and most cases, such control is performed by modifying properties of drugs. In our proposed approach, we aim to modify properties of target tissue, not drugs, for the control. More specifically, we destroy the biological structures of the target site selectively to cause 1) increased drug permeability in the tissue and 2) cell membrane damages which further cause increased drug uptake by the cells.

Such a DDS method would control spatial and temporal distribution of drugs without designing new drugs. However, it is known that extremely high acoustic intensity typically more than 10 kW/cm² is needed to reproducibly induce mechanical bioeffect of ultrasound, which prevents widely applicable therapeutic system.

In this paper, we focus on investigating the effect of chemical sensitizers to reduce acoustic intensity for inducing such mechanical effects, which is a key for our DDS. As a sensitizer, we selected a phase change nano-droplet (PCND)[1], a formulation of superheated perfluorocarbon droplets, which we have been investigated as sources of microbubbles (MBs) for diagnostic and therapeutic purposes. In ultrasound medicine, MB is the most commonly utilized for contrast agents and therapeutic sensitizers[4]. Unfortunately, MBs are generally fragile to ultrasound pressure. They will rupture even by diagnostic ultrasounds. For the purpose of this study, ultrasound pressure to be used will be higher than those used in diagnoses thus pressure-resistant MBs are needed. Increasing number density of MB would be a solution to make MB less sensitive to ultrasound pressure. To have high number density, using PCND is advantageous over using MB because PCND's diameter is about an order of magnitude smaller than MB.

The purpose of this study is investigate if PCND could be used as a sensitizer for destruction of tissue structures with ultrasound pulses whose intensity is in the same range as high intensity focused ultrasound (HIFU) therapy by using exteriorized tissues. Moreover, we investigated the dependency of the volume of destroyed regions to acoustic parameters such as pulse durations.

2. Materials and Methods

PCND and tissue sample

The procedure for preparing PCND has been described in detail elsewhere[3]. Freshly exteriorized chicken breast tissues cut into approximately 50 × 50 × 20 mm block were used as samples. 0.5 ml of PCND suspension was injected to samples before ultrasound exposure.

Experimental setup for ultrasound exposure

The samples were placed in a tank filled with continuously degassed water kept at 37±1°C. A 1.1-MHz focused transducer with a 58-mm-diameter aperture and 58 mm focal length was used as the ultrasound source. A convex probe (Hitachi Aloka Medical EUP-C532) connected to a medical ultrasound scanner (Hitachi Aloka Medical EUB-8500) hold at the center of the transducer aiming at the center of the tissue sample.

Exposure sequence

In this study, tissue-structure destruction, ultrasound pulses were repeatedly exposed to samples for 60 s. Parameters were changed and their effects on tissue-structure destruction were investigated. Parameters investigated were, pulse intensity, pulse duration, and pulse interval.

Measure of tissue-structure destruction

As a measure to quantify tissue-structure destruction, destructed volume was used. It was found that ultrasound exposure induces gross change in tissue sample. White-opaque region are created which can be scraped by spatula. The size of the white-opaque region was measured in the axial and azimuth direction, and the volume of the destroyed region was calculated by ellipsoidal assumption.

3. Results and Discussion

The pulse exposure to tissue samples in the presence of PCND induces hyper-echoic change at the focus of ultrasound. The hyper-echoic region is created in front of PCND-injected region. No apparent changes were observed when PCND was not injected. The hyper-echoic change remains at least an hour. White-opaque lesions are created in samples at corresponding sites of the hyper-echoic regions. It was found with thermometer measurements that the temperature rise at the focus by the ultrasound exposure was at most 40 °C which was too low to produce thermal lesion. Thus, the tissue changes were considered not induced by thermal coagulation but mechanical tissue destruction.

We further investigated on the parameters affecting the tissue structure destroying. It was found that destroyed volume increases as the concentration increases in the range of 0.001-0.1%. The result suggests that the volume is proportional to the logarithm of PCND concentration. Such a result should be tightly related to tissue destruction mechanism. Further investigations such as the destroyed volume dependence on the size distribution of PCND are needed.

Figure 1 shows the dependence of the destroyed volume on ultrasound pulse intensity. No significant tissue destruction was observed in the absence of PCND. In the presence of PCND, the destroyed volume increased as intensity increased. Intensity threshold needed to induce tissue destruction was about 2.2 kW/cm², and with intensity higher than the threshold, the volume increased almost linearly as intensity increased. Because intensity threshold for microbubble generation from PCND is estimated as 1.7 kW/cm², obtained result may indicate that the bubble activity generated from PCND plays an important role and the degree of volume change of bubbles may be an important factor.

The dependency of the destroyed volume on ultrasound pulse duration was also investigated. No significant tissue structure destruction was observed in the absence of PCND with duration in the range of 20-400 cycles. In the presence of PCND, the destroyed volume increased as intensity increased. Duration cycle needed to destroy tissues was found to be longer than 100. The volume reached a plateau at 300 cycles and increasing cycle number did not increase the volume. Not like pulse intensity, the effect of pulse duration does not seem to be input-energy based.

In this study, we could achieve the tissue structure destruction as a prerequisite for a novel DDS. It was found that acoustic intensity at about 2 kW/cm² is enough to destroy tissue structure, which

is within a range used in HIFU therapy. The value is very low considering previous studies on inducing mechanical effects inside body. Ever reported ultrasound intensity required for reproducible mechanical effects is higher than 10 kW/cm²[2]. The effect of the sensitizer, PCND is significant in this study. In our preliminary investigations, a microbubble agent Sonazoid® could not induce tissue-structure destruction with acoustic conditions used in this study. It is thus suggested that, as intended, PCND works as a source of pressure-resistant microbubble.

If we could induce tissue-structure destruction in

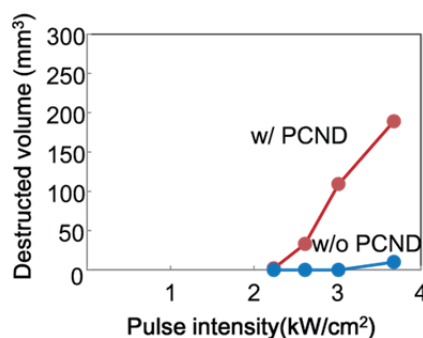


Figure 1 Dependency of pulse intensity on destroyed volume

PCND: 0.03%, pulse duration: 300 cycles
tumor tissues, several mechanisms for obtaining anti-tumor effects are expected to be utilized. 1) Direct lysis of tumor cells by mechanical stress, 2) Increased sensitivity of tumor cells to anti-tumor drugs, and 3) Immune responses. Results obtained suggest the involvement of direct mechanical effect on cells. When the mechanical stress is strong enough, that would cause lysis of cells, and if the stress is below a threshold, that would cause partially damages cells. In such a damaged state, cells are expected to be more sensitive to drugs because most of damages are considered to be induced at the cell membrane. Drugs have more possibilities to be incorporated into cells when membranes are damages.

Overall, we could demonstrate that tissue structure destruction is possible with ultrasound pulses with several kW/cm² intensity ranges by using a MB precursor, PCND. For developing a novel pancreatic tumor treatment, further investigation with anti-tumor drugs are planned.

References

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