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Ultrasound-enhanced chemiluminescence tomography in biological tissue

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ABSTRACT

This paper reports ultrasound-assisted optical imaging of chemiluminescent probes in biological tissue. A focused low power ultrasound sonochemically enhances a peroxyoxalate chemiluminescence (CL) that involves indocyanine green (ICG) as luminescent pigments. By scanning the focus, it produces tomographic images of CL in scattering media. The authors demonstrate imaging using a slab of porcine muscle measuring 50 × 50 × 75 mm, in which a capsuled CL reagent is embedded at 25 mm depth. Spatial resolution of imaging and concentration characteristics of CL reagents to enhanced CL intensity are also studied to evaluate the potential for use in bio-imaging applications with exploring the CL enhancement mechanisms. CL enhancement ratio, defined as the ratio of ultrasonically enhanced CL intensity to the base intensity without ultrasound irradiation, was found to be constant even in varying ICG and oxidizer concentrations, implying to be applicable for quantitative determination of these molecules.

1. Introduction

The combined modalities of optical measurement with ultrasound to assist in revealing spatial information of optical characteristics in light-scattering media are promising methods for biomedical imaging. In particular, various photoacoustic imaging techniques are developed and applied for practical in vivo imaging [1]. These techniques achieve tomographic imaging beyond the optical diffusion limit in biological tissue by detecting ultrasonic pressure waves that are converted thermoelastically from pulsed photons bearing local absorption properties in light-scattering media. By contrast, a variety of multimodal methods by which focused ultrasound serves to assist determination of spatial information of optical properties (absorption, fluorescence, or luminescence) in light-scattering media are proposed and studied [2–8]. Most of them are based on ultrasound-modulated optical phenomena, however these techniques have a challenging issue of low modulation depth. Recently, we proposed a novel tomographic imaging technique based on the sonochemical effect of ultrasonic enhancement of chemiluminescence (CL), with demonstration of imaging capabilities using a peroxyoxalate chemiluminescence (POCL) system [9]. The POCL reaction is a well-known highly efficient CL system containing fluorophore (in our case, indocyanine green; ICG) as finally excited molecules. The fluorophore molecules are excited through energy transfer from a high-energy intermediate (HEI) generated in oxidation of oxalate with hydrogen peroxide. Thereby, this system is generally used for the quantitative detection of a small amount of fluorophore or H_2O_2 in analytic chemistry. Results showed that POCL system is sensitive to low-power ultrasonic waves that can be applicable for in vivo bio-imaging. By combination with focused ultrasound, which has power of less than 0.14 W/cm^2, the spatial distribution of CL substances in optically turbid media can be resolved, leading to functional or structural CL probe imaging. The effect of ultrasonic CL enhancement is considered to originate in ultrasonic promotion of chemical reactions, known to be used in the process of sonochemistry, which is derived in the emergence of microscopic high-pressure and high-temperature regions through cavitation to accelerate oxidation, generally accompanied by free radical generation. These facts imply that this phenomenon is not specific to POCL systems, but is instead extensible for other CL systems leading to the development of ultrasound-sensitive CL probes. In a previous paper [9], the CL enhancement ratio of POCL, defined as the ratio of increased CL intensity to the base intensity, is constant even in varying conditions of the base CL intensity, suggesting that it is useful for the quantitative determination of fluorophore or H_2O_2.

This paper demonstrates the capability of the CL enhancement technique to apply it for in vivo imaging in biological materials. We report the imaging performance using an agar base phantom and a practical tissue slab of porcine muscle in which CL targets

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are embedded at depths of 25–30 mm from the detecting surface. We also discuss the mechanism of the CL enhancement through characterization of the enhancement effect under various concentrations of the oxidizer and fluorophore.

2. Materials and methods

Experimental arrangement is described in a previous paper [9]. Configuration between the direction of optical detection (X axis) and that of the ultrasound beam (Y axis) was perpendicular, as shown in the schematic diagram displayed in Fig. 1. An ultrasonic transducer (V302-SU; Olympus Corp., Japan), which has a 25-mm-diameter element with 46.5 mm focal length, equipped on the side wall of a water tank was placed on an X–Y translational stage for mechanical scanning of the ultrasound focus, probing the inside of phantoms set in a sample holder. The sample holder was fixed from the outside of the water tank. All of this equipment was installed in a dark box. A photomultiplier tube (PMT, R669; Hamamatsu Photonics K.K, Japan), which has a 46-mm-diameter photocathode, was attached on the side wall of the dark box to detect light through the transparent water tank. Distance between the center of the sample holder and the PMT photocathode was constant (200 mm) even during X–Y scanning. We used 1 MHz continuous-wave (CW) driven focused-ultrasound that has focal area measuring 2.9 mm on the transverse axis (X) (beam diameter) and 35 mm on the longitudinal (Y) axis (focal zone), which determines the spatial resolution of the acquired image. The transducer was driven with sinusoidal wave of 180 V amplitude through a power amplifier, which was the maximum limit for CW driving of the transducer. Sound pressure at the focus, which was measured using a calibrated hydrophone (NH8144 needle-type hydrophone; Toray Engineering Co. Ltd., Japan), was 61 kPa, being equivalent to 0.14 W/cm^2 ultrasound power. In this condition, the temperature rise at the focus measured with a thermocouple was 0.7 °C/hr. Samples that we have used to demonstrate imaging performance were an agar base phantom molded with an agarose gel mixed with Intralipid solution and a practical tissue made of a slab of porcine muscle prepared from pork meat. The agar base phantom was made with mixing Intralipid solution, which is fat emulsion widely used as a standard optical scattering medium. 20 mL/L concentration of Intralipid (Intralipid-10%; Fresenius Kabi AG, Germany) in water and glycerol (final concentration 20%) was mixed to adjust the optical scattering coefficient (estimated reduced scattering coefficient was ca. 1.5 mm^{-1}), and molded with agarose (2 wt% Yamato agar; Ina Food Industry Co. Ltd.) with dimensions of 60 mm (X) × 60 mm (Y) × 75 mm (Z). The tissue sample of porcine muscle was prepared with cuts of lean pork purchased from a supermarket to fit in the sample holder having dimensions of 50 mm (X) × 50 mm (Y) × 75 mm (Z). A CL target of POCL solution filled in a small capsule was embedded in the phantom or tissue sample to simulate a localized CL probe in a deep site of light-scattering media. The embedded position of the target was the center on the X–Y plane with 45 mm depth on the Z-axis. For an experiment to evaluate the spatial resolution, the second target was additionally embedded in the agar phantom, 15 mm distant from the center target on the X-axis.

The CL target capsule was a 0.5-mm-thick silicone tube with 2 mm inner diameter and 22 mm length, with total volume of 70 μL. The reagent for POCL reaction was a mixture of equal volumes of oxalic acid (bis [3,4,6-trichloro-2-(pentyloxycarbonyl) phenyl] oxalate, Tokyo Chemical Industry Co. Ltd., Japan; CPPO: concentration 20 mg/mL), 1% hydrogen peroxide (Wako Pure Chemical Industries Ltd., Japan), sodium salicylate (concentration 0.2 mg/mL; Wako Pure Chemical Industries Ltd.), and ICG (concentration 1 mg/mL; Tokyo Chemical Industry Co. Ltd.). Solvents for these chemicals are dimethyl phthalate for CPPO, a 4:1 mixture of dimethyl phthalate and t-butyl alcohol for H_2O_2 and sodium salicylate, and ethanol for ICG.

To investigate the mechanism of CL enhancement with exploration of the dependence of CL reagent concentrations, the final concentration of ICG was varied from 3.2 to 320 μM and H_2O_2 was varied from 0.27 to 81.6 mM in final concentration. For these experiments, 2 mL/L concentration of Intralipid solution without agar molding (liquid phantom) was used as a light-scattering medium to assess the changes of CL intensity during ultrasound irradiation at the fixed position of the focus corresponding to the CL target.

For tomographic imaging, we operated the X–Y translational stage to scan the ultrasound focus with step movement of 0.5 mm (X) or 2 mm (Y). The PMT was operated under cooling for optimum condition in photon-counting mode to detect weak CL at 830 nm from ICG. The gate-time for photon-counting was 500 ms, synchronized the step movement of the water tank. For data processing after the measurement, scanned data were corrected with normalization, considering the temporal decrease of the base CL intensity and the spatial distribution of background obtained without ultrasound irradiation.

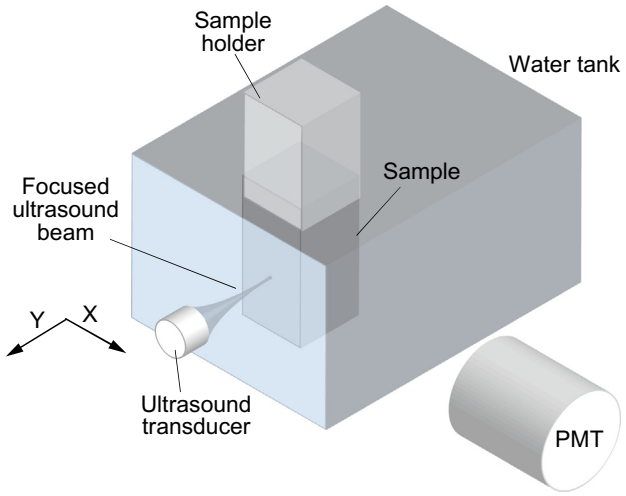

Fig. 1. Schematic diagram showing experimental configuration. All of this equipment except a photomultiplier tube (PMT) is in a dark box. The PMT housing is attached on the side wall of the dark box.

3. Results and discussion

3.1. Tomographic imaging using agar phantoms

A tomographic image observed with an agar phantom (a photograph of the phantom put in a sample holder is displayed as Fig. 2(b)) is depicted in Fig. 2(a). The X-coordinate origin is the phantom surface of the opposite side of the PMT. The Y-coordinate origin is the opposite side of the surface that fronts onto the ultrasound transducer. The image of an embedded CL target observed as an ellipsoidal shape is portrayed. A line profile obtained with transverse scanning on the X-axis at the focal distance of ultrasound on the Y-axis is presented in Fig. 2(c). A Y-profile at the center on the X-axis of the phantom is shown in Fig. 2(d). The observed width (full-width-at-half-maximum; FWHM) on the transversal (X) axis of ultrasonic wave is approximately 6 mm, which is consistent with the beam diameter at ultrasound focus with consideration of the sample capsule diameter. On the longitudinal (Y) axis, because of the limitation of Y-scanning range, FWHM is not exactly determined, but it can be estimated more than 30 mm. Fig. 2(c) shows the asymmetric shape of the