Non-Invasive Treatment with Focused Ultrasound Enhanced with Nano- to Micro-Particles

Shin-ichiro Umemura* and Shin Yoshizawa

* Professor
Department of Electrical and Communication Engineering, Graduate School of Engineering
E-mail: sumemura@ecei.tohoku.ac.jp

Abstract

The bioeffect of ultrasound can be enhanced by orders of magnitude with microbubbles. Enhancement of ultrasonic tissue absorption with a microbubble agent is demonstrated both in theory and in a vivo experiment. This effect will be useful in therapeutic application of ultrasound if a microbubble agent is delivered selectively to the target tissue.

1. Introduction

Ultrasound has moderate tissue attenuation and absorption coefficients at an appropriate wavelength for penetrating intervening tissues to reach, vibrate, and heat non-superficial tissue objects while maintaining the ability to focus energy into small volumes. This is a unique advantage when compared to electromagnetic modalities such as laser beams in the application to non-invasive treatment of non-superficial tumors. Bioeffects of ultrasound, which can be potentially be used for such a kind of treatment, are shown in Fig. 1.

In the existence of microbubbles, the ultrasonic vibration amplitude is enhanced in their vicinity by orders of magnitude, and both mechanical and heating effects can be thereby accelerated. Furthermore, sonochemical effects can be induced when microbubbles collapse.

Recently, the heating effect was started being clinically used as the thermal coagulation treatment with high intensity focused ultrasound (HIFU). In this paper, the acceleration of heating [1] is chosen as an example of the ultrasonic bioeffects enhanced with microbubbles.

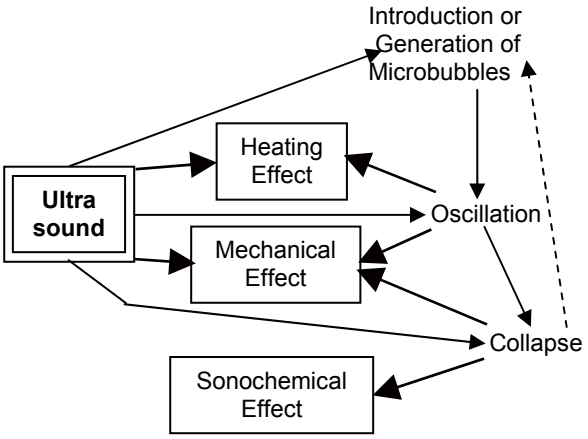


Fig. 1. Bioeffects of ultrasound and their enhancement with microbubbles.

2. Mechanism of Enhancement with Microbubbles

Microbubbles, subjected to ultrasonic pressure in the same frequency range with their resonant frequency, convert the acoustic energy to heat through their volume oscillation. The energy conversion has two mechanisms: (1) viscous heating and (2) a pressure-volume hysteresis loop. In the mechanism (1) heat is generated by the shear motion of liquid surrounding a volumetrically oscillating microbubble.

The mechanism (2) originates from the temperature change of the gas inside a microbubble: the temperature increase during its compression and the decrease during its expansion. The heat transfer taking place inside the bubble during the temperature change results in phase discrepancy between its pressure change and its volume change from 180°. A part of volumetric oscillation energy of the bubble is converted to heat in the heat cycle, which is opposite to that of a heat engine converting heat to mechanical energy.

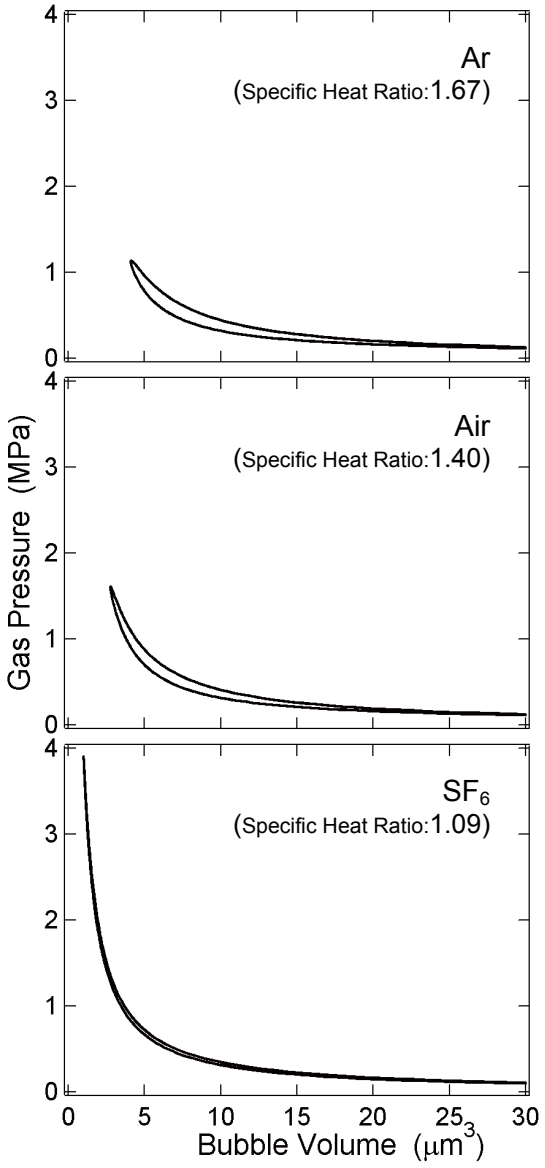


Fig. 2. Thermal cycle of resonant microbubbles containing different gas species subjected to ultrasonic pressure at 2 MHz and 0.5 MPa (peak).

The temperature changes due to compression and expansion of a microbubble increase as the specific heat of gas inside the bubble increases. The amount of acoustic energy converted to heat in a heat cycle also increases as the specific heat increases. A heat cycle of a microbubble containing air (with a specific heat ratio of 1.40) is shown in Fig. 2 compared with that of Ar with a large specific heat ratio of 1.67 and that of SF_6 with a small specific heat ratio of 1.09. The area inside the pressure-volume hysteresis loop equals to the heat converted from acoustic energy in a heat cycle, similarly as that of a heat engine. The loop area of Ar is approximately twice that of air. In contrast, that of SF_6 is negligibly small in comparison with air. The heat cycle of SF_6 is very close to isothermal change.

SF_6 is one of the gas species contained in stabilized microbubbles of the newest generation contrast agents developed for diagnostic ultrasound. Perfluorocarbon, which is also contained in the newest generation ultrasonic contrast agents, has an internal degree of freedom even larger and a specific heat ratio even closer to 1 than SF_6. Therefore, the heat cycle of a microbubble containing perfluorocarbon is very close to isothermal., and its heat generation of the mechanism (2) is negligible in comparison with that of the mechanism (1).

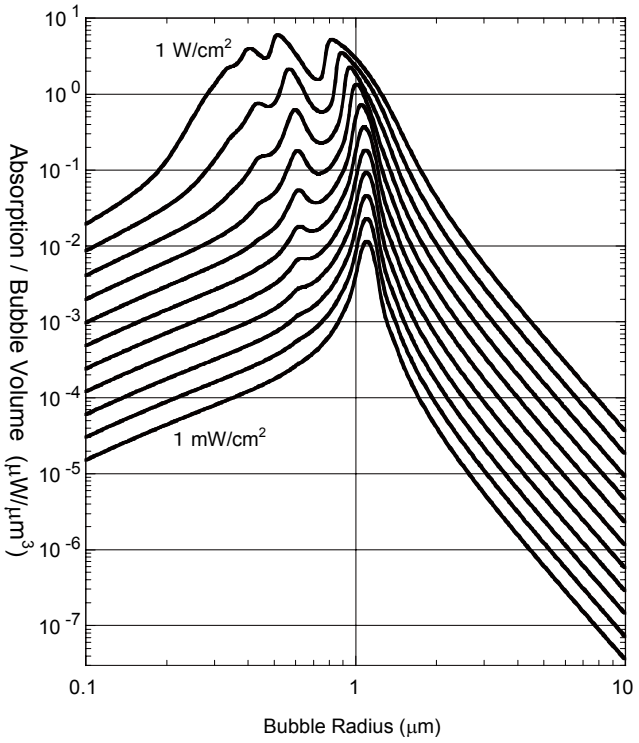


Fig. 3. Ultrasonic abosorption by microbubble at 3 MHz, theoretically predicted by numerically solving the Rayleigh-Plesset equation. Ultrasonic intensity is varied as a parameter from 1 mW/cm^2 to 1 W/cm^2 in a geometric series.

The acoustic power converted to heat by a microbubble in isothermal heat cycles is numerically calculated and the power per bubble volume is plotted against the bubble radius in Fig. 3. The ultrasonic

intensity is geometrically varied from 1 mW/cm^2 to 1 W/cm^2.

A microbubble has a single resonant radius and shows an approximately linear response at an ultrasonic intensity of 1 mW/cm^2. In contrast, it starts showing its nonlinear response and having plural of resonant radii such as a half and a third of the fundamental, as the ultrasonic intensity increases as high as 1 W/cm^2. A stabilized microbubble agent normally has a size distribution in the order of twice. Owing to this nonlinear response, most of the microbubbles varying in size can contribute to thenultrasonic energy conversion to heat. It is estimated that the ultrasonic absorption by tissue is doubled in the existence of approximately 10 microbubbles in 1 mm^3 of tissue.

3. Methods

A prototype split-focus power transducer with two elements, originally constructed for transrectal treatment of a prostate, was used in exposure experiments. The dual element PZT transducer (Fuji Ceramics, Shizuoka, Japan) had a resonant frequency of 3.2 MHz, a spherical curvature radius of 35 mm, and an aperture of 40 mm x 20 mm. The area of each element was 40 mm x 10 mm. It was contained in an aluminum housing as shown in Fig. 4, in combination with a small imaging probe (EUP-F331, Hitachi Medical, Tokyo, Japan) at 6.5 MHz having a convex array curvature radius of 10 mm. The position and angle of the imaging probe, relative to the power transducer, was calibrated and adjusted in prior to the in vivo exposure experiment.

When the two elements of the power transducer were driven at opposite phases, the focused beam is split into two. This dual split-focus beam was used instead of the conventional single-spot focal beam for suppressing the spatial peak intensity to minimize the possibility of excessively destroying the microbubble agent and inducing irreversible changes in the tissue. The schlieren images of the focal field from the prototype transducer are shown in Fig. 5. The side image and the front images of both single-spot and split-focus fields are shown. In the split-focus mode, the two focal beams were separated about a millimeter from each other.

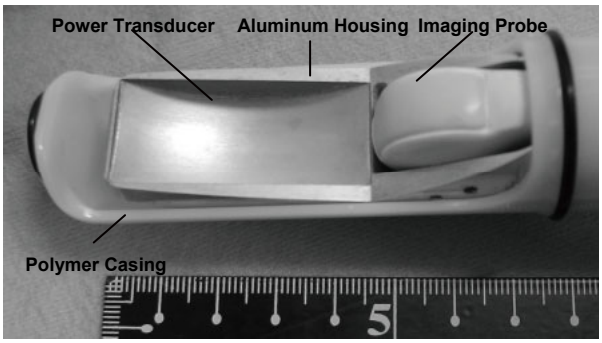


Fig. 4. HIFU transducer at 3.2 MHz used in *in vivo* experiment.

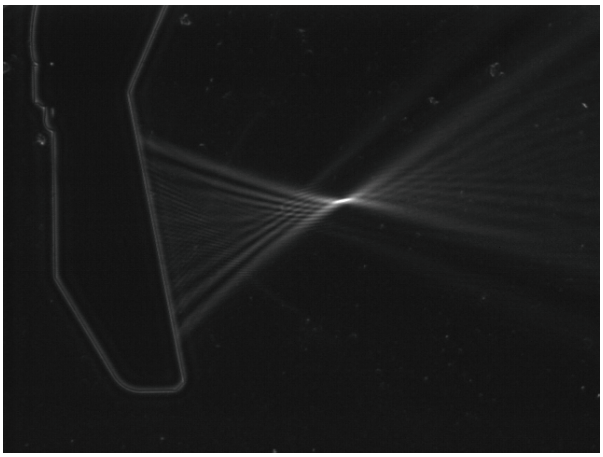


Fig. 5. Focal ultrasonic field of the HIFU transducer at 3.2 MHz.

The total acoustic power output from the transducer was calibrated against the drive voltage by measuring the radiation force on a hollow aluminum plate in degassed water. The acoustic intensity distribution pattern on the focal plane was measured in degassed water with a 0.5-mm-diameter needle-type hydrophone (Imotec, Coesfeld, Germany) at a low drive voltage. From these results the spatial peak acoustic intensity was obtained as a function of drive voltage assuming that the intensity distribution pattern does not significantly change as the drive voltage increases. This peak acoustic intensity in water was used to describe the exposure condition in the in vivo experiments, ignoring the ultrasonic attenuation while traveling a short distance in tissue.

Optison^{TM}, a suspension of microspheres of human serum albumin with perflutren, C_3F_8, (Amersham Health, Princeton, NJ) was used as the microbubble agent in the experiment. The concentration, size

distribution, and total volume of microbubbles in the suspension were measured using a Coulter Multisizer III instrument (Beckman Coulter, Fullerton, CA), fitted with an orifice tube of 30 μm aperture after diluted with phosphate buffer saline, pH 7.4, by 200 times.

After surgical anesthesia with sodium pentobarbital was given to a female Sprague-Dawley rat (approximately 250 g), the left kidney was mobilized and exteriorized through an incision. A polyethylene tube cannula, 0.5 and 0.8 mm in inner and outer diameter, respectively, was inserted into a jugular vein for systemic administration of the microbubble agent. The rat was held vertically in degassed saline at 33°C as shown in Fig. 6.

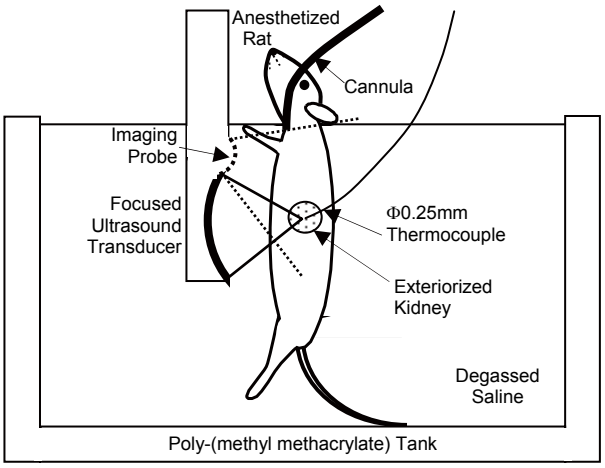


Fig. 6. Experimental setup of HIFU exposure.

A very-thin sheathed thermocouple (Sukegawa Electric, Ibaraki, Japan) was chosen to minimize the potential artifacts in tissue temperature measurement. The 0.25-mm diameter thermocouple consisted of a pair of 0.05-mm diameter chromel and alumel wires running through a 0.025-mm thick stainless-steal sheath filled with packed, ultra fine magnesium oxide powder. Its longitudinal heat conductance is estimated to be 0.36 μWm/°C, which is equivalent to that of a 0.9-mm diameter cylindrical rod of tissue. It was inserted into the renal cortex tissue. The position of the rat in saline, relative to the transducer, was adjusted so as to locate the thermocouple right in the middle of the focal heating zone, first by using the B-mode images taken with the 6.5-MHz probe, and then by using the thermocouple output at low ultrasonic exposure intensity.

A murine kidney for histological examination was excised immediately after a series of ultrasonic exposure, fixed in 10% formaline, and later stained with hematoxylin and eosin (H & E). No thermocouple was inserted into this group of renal tissues to avoid the effects of a mechanical wound on the histology. The position of the rat, relative to the transducer, was adjusted only by using the B-mode images.

Three animals were used in each experiment to check the repeatability. The experimental animals were treated following the guideline proposed by the Science Council of Japan.

4. Result

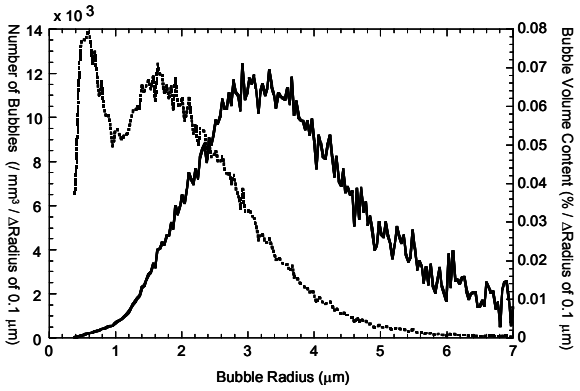


Fig. 7. Size distribution of Optison (dotted line) and distribution in volume content (solid line). It was measured using a Coulter Multisizer III instrument (Beckman Coulter, Fullerton, CA), fitted with an orifice tube of 30 μm aperture after diluted with phosphate buffer saline, pH 7.4, by 200 times.

In prior to the series of HIFU exposure experiments, the size distribution of the microbubble agent, Optison , which was used in the experiments, was measured and plotted in Fig. 7. Regarding the number of microbubbles, there are two peaks in radius around 0.6 μm and 1.6 μm, but there is only one gradual peak in radius around 3.2 μm regarding their volumes. The total bubble volume content of Optison^{TM} was calculated to be 2.2%.

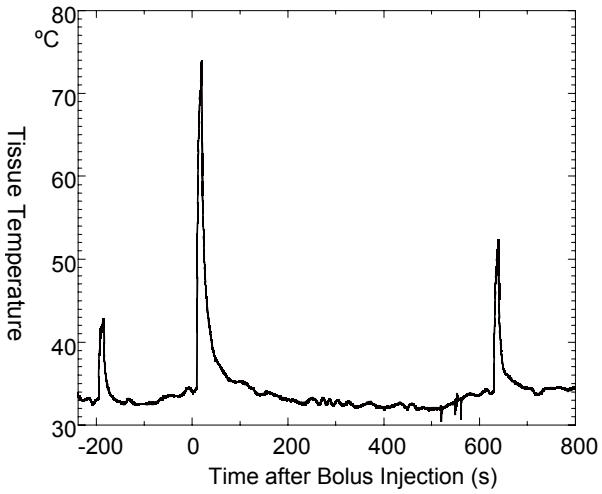


Fig. 8. Murine kidney tissue temperature with HIFU exposure at 290 W/cm^2 before and after intravenous bolus injection of Optison. 0.2 ml/kg Optison was injected at time 0.

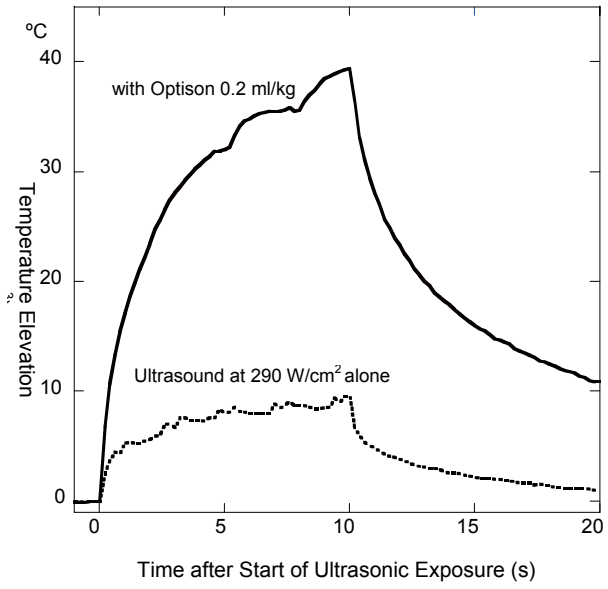


Fig. 9. Tissue temperature elevation with HIFU in the presence and absence of Optison.

In Fig. 8, the temperature change in the kidney due to three-time ultrasonic exposure at an ultrasonic intesnsity of 290 W/cm^2 for 10 s is plotted. Optison with a dose of 0.2 ml/kg was injected at time 0 in the horizontal axis. In Fig. 9, the temperature curves right before and after the injection are compared by expanding the horizontal time axis. Optison multiplied the tissue temperature rise due to HIFU by 4-5 times.

In Fig. 10, the temperature change in the kidney due to seven-time ultrasonic exposure at an ultrasonic intensity of 150 W/cm^2 for 10 s is plotted. The first and second exposures were done right before and after the injection of 0.2 ml/kg Optison at time 0, respectively. The second to seventh exposures were done with a constant period of 5 min. Even lower ultrasonic intensity was chosen to prevent irreversible change of the tissue due to the repetitive exposure.

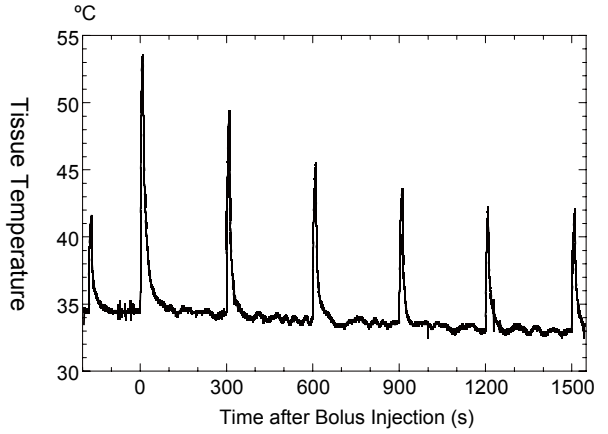


Fig. 10. Murine kidney tissue temperature with HIFU exposure at 150 W/cm^2 before and after intravenous bolus injection of Optison. 0.2 ml/kg Optison was injected at time 0.

The peak temperature rise at each time of exposure in Fig. 10 was also plotted in Fig. 11. The dotted curve is the result of a least squares fit to the second to seventh peaks. The fit curve exponentially approaches to the level of the first peak, which is the peak before the injection of Optison, with a time constant of approximately 9 min.

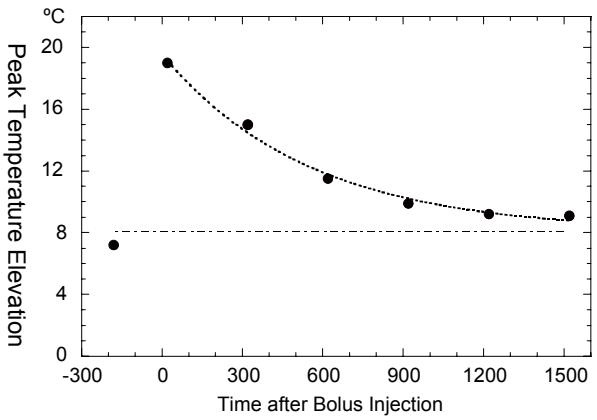
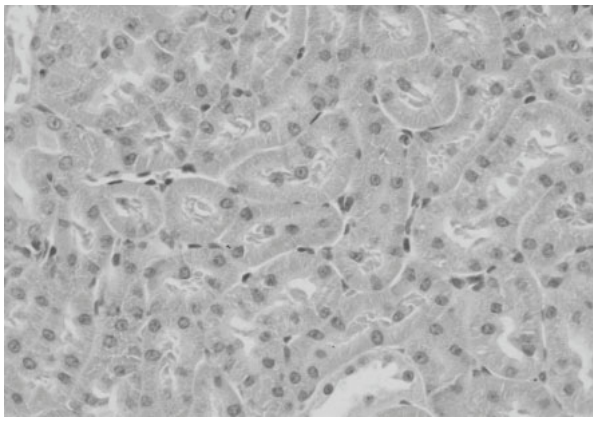


Fig. 11. Peak tissue temperature elevation during each ultrasonic exposure plotted against time after bolus injection of 0.2 ml/kg Optison. A least-squares fit monoexponential curve (dotted line) and its final value (dotted broken line) are also shown.

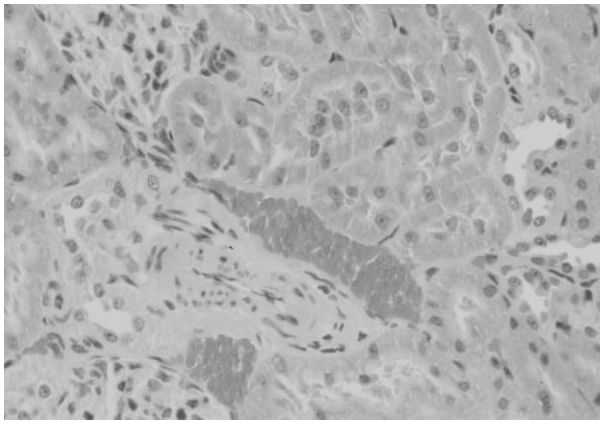
The photographs in Fig. 12 show typical histology of the renal tissue after each series of ultrasonic exposure. Extravascular accumulation of erythrocytes was sporadically found in the histology after the same series of ultrasonic exposure with Optison™ as in the second *in vivo* experiment at 150 W/cm^2 described above (b). Extensive coagulation necrosis of renal tubules was commonly seen in the histology after only one time exposure with Optison™ as in the first *in vivo* experiment at 290 W/cm^2 described above (c). No tissue damage was observed in the histology after ultrasonic exposure alone, even after a series of seven exposures at 290 W/cm^2 (a), the duration and repetition period of which were the same as in the second *in vivo* experiment.

5. Discussion

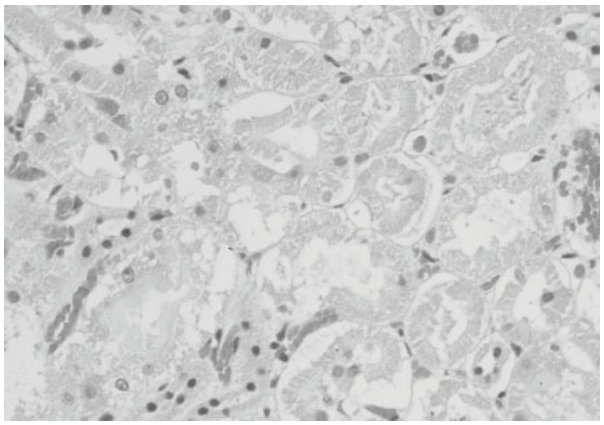
The dose of Optison used in the experiments, 0.2 ml/kg, is larger than the clinical dose normally used in ultrasonic diagnosis by an order of magnitude, but still within the clinically approved safe dose, with which the biological safety was confirmed. The significant enhancement of ultrasonic tissue heating 4-5 times with a microbubble agent at a safe dose demonstrated the high potential of this kind of approaches.



(a) After seven-time ultrasonic exposure alone at 290 W/cm^2.



(b) After seven-time ultrasonic exposure with Optison™ at 150 W/cm^2.



(c) After one-time ultrasonic exposure with Optison™ at 290 W/cm^2.

Fig. 12. Typical histology of the renal tissue after each series of ultrasonic exposure.

The gas contained in a stabilized microbubble agent such as Optison is excluded from the body by the gas exchange in circulating through lungs. The exclusion time constant is estimated to be approximately 5 min for a human form gas-chromatography experiments.

Considering the difference between the species, it is reasonable to interpret that the time constant of approximately 9 min, seen in Fig. 10, corresponds to that of excluding Optison from the body. This result strongly supports the hypothesis that the observed enhancement of ultrasonic tissue heating is proportional to the concentration of Optison in blood.

The magnitude of the observed enhancement of ultrasonic tissue heating with microbubbles agreed well with the theoretical prediction, in which the heat converted by a microbubble was numerically calculated.

In the theoretical prediction, the nonlinear effect was seen even at ultrasonic intensity of 1 W/cm^2, which is much lower than the intensity normally used as HIFU. Because of this nonlinear effect, many peaks of ultrasonic absorption other than the fundamental resonance peak are also seen as a function of bubble radius in Fig. 3. Microbubbles in a stabilized microbubble agent normally have a relatively broad distribution in their radius as shown in Fig. 6. If each bubble had to be excited at its resonant frequency, the exposure would have to be done at many different ultrasonic frequencies. However, the real microbubbles have a broad frequency response due to their nonlinear character as seen in Fig. 3. This should be the reason why the sufficiently large enhancement of ultrasonic heating was observed even at a single ultrasonic frequency.

Discussions on a point of view a little different from above, to interpret the results, are also written below.

A microbubble in liquid automatically collapses in a short period of time because its high inner pressure to compete against the surface tension results in fast gas diffusion outward. Therefore, surfactants reducing the surface tension or a shell resisting to gas diffusion are used in a stabilized microbubble agent. In Option, used in the experiments, an albumin shell is used to stabilize a perfluorocarbon microbubble. This albumin shell cannot stand the high acoustic pressure of HIFU, and it may have been destroyed when the microbubble started converting acoustic energy into heat. Assuming this situation, the theoretical results shown in Fig. 3 were calculated for a microbubble without a shell.

After the shell was destroyed, the shell fragments may start working as cavitation nucli. The cavitating bubbles may contain not only perfluorocarbon, carried over from Optison, but also air, originally contained in blood. For air as the inner gas, the mechanism (2) has to be taken into account, and the theoretical prediction may produce a little different results.

In the series of events on this hypothesis, the shell fragments rather than the perfluorocarbon gas enhance the ultrasonic heating. Therefore, the time constant of approximately 9 min, seen in Fig. 9, should correspond to time to exclude the albumin shell fragments rather than the perfluorocarbon gas from the body.

This hypothesis can be tested by using Optison after destroying its shells and sucking perfluorocarbon away under low pressure.

There may be another hypothesis that the perfluorocarbon gas itself is working as cavitation nucli. The cavitated microbubbles may contain air also in this hypothesis. Perfluorocarbon gas can work as cavitation nucli because it is extremely hydrophobic. The mechanism (2) should also be taken into account in the theoretical prediction on this hypothesis.

6. Conclusion and Future Work

It was demonstrated that a microbubble agent, in its clinically approved dose range, multiplies the heating effect of ultrasound in both theory and experiment.

However, if it is distributed in the whole body at a similar tissue concentration, the ultrasonic attenuation in the intervening tissue will be multiplied at the same time. Ultrasound will be mostly attenuated before it reaches to the target tissue in the focal zone. Therefore, a microbubble agent should be delivered selectively to the target tissue, as shown in Fig. 13, to fully utilize the observed effect.

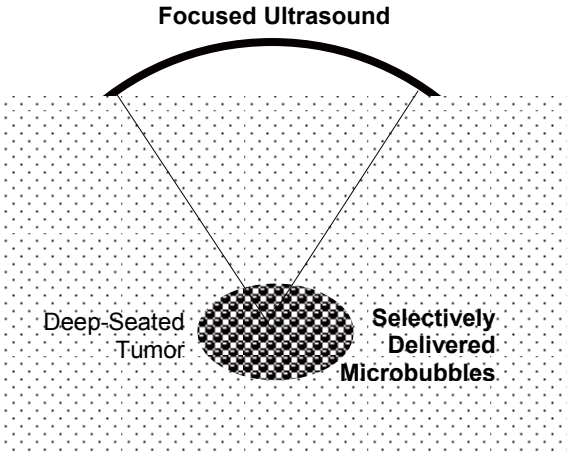


Fig. 13. Selective delivery of microbubbles. Microbubbles should be delivered selectively to the target tissue to fully utilize the observed effect.

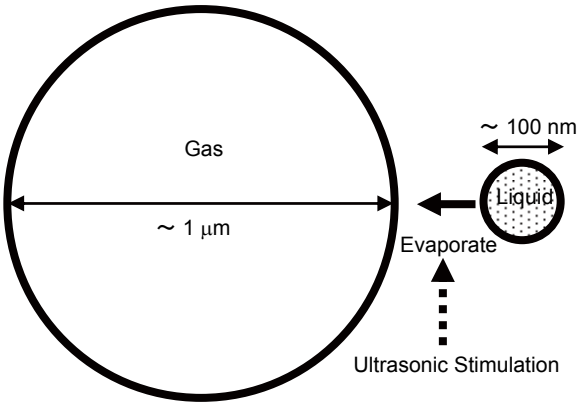


Fig. 14. Midrobubble and its precursing nano-particle. A liquid particle approximately 100 nm in radius can be converted with ultrasonic stimulation to a gas bubble approximately 1 μm in radius, which can efficiently convert ultrasonic energy at a frequency in a MHz range into heat.

The delivery of microbubbles to target tissue as phase conversion nano-particles may have the potential to satisfy this requirement. A liquid particle approximately 100 nm in radius, which may have the EPR effect to accumulate in tumor tissues, can be converted with ultrasonic stimulation to a gas bubble approximately 1 μm in radius, which can efficiently convert ultrasonic energy at a frequency in a MHz range into heat and significantly enhance ultrasonic tissue heating [2], as schematically shown in Fig. 14.

Acknowledgements

The author acknowledges Kazuaki Sasaki and Ken-ichi Kawabata of Hitachi Central Research Laboratory for collaboration in producing the experimental result.

References

[1] Umemura S, Kawabata K, and Sasaki K. Acceleration of ultrasonic tissue heating by microbubble agent. *IEEE Trans Ultrasonics, Ferroelectrics, and Frequency Control* **52**, 1690-1698, 2005.

[2] Kawabata K, Sugita N, Yoshikawa H, Azuma T, and Umemura S. Nanoparticles with Multiple Perfluorocarbons for Controllable Ultrasonically Induced Phase Shifting. *Jpn J Appl Phys* **44**, 4448-4852, 2005.