Interaction of mechanical and thermal lesions induced by high intensity focused ultrasound (HIFU)

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Interaction of Mechanical and Thermal Lesions Induced by High Intensity Focused Ultrasound (HIFU)

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ABSTRACT

High-intensity focused ultrasound (HIFU) is emerging as an effective therapeutic modality in both thermal ablation for solid tumor/cancer therapy and soft-tissue fragmentation. Mechanical as well as thermal effects play an important role in the HIFU treatment simultaneously. Further understanding the underlying mechanisms of HIFU ablation is critical for its safety and efficiency in the clinical application as well as developing new technology.

In order to achieve our goals, nonlinear acoustic propagation of a HIFU burst was first simulated using KZK equation. Then bubble dynamics at the focal plane of the HIFU transducer, where a soft tissue is located, was simulated by the Gilmore model and its diffusion in the interval time by a static diffusion model with the progress of HIFU ablation. The simulation results show that the bubble dynamics is not consistent for each burst exposure and in general the maximum bubble size reached and the associated radiation acoustic pressure in the collapse increase with the number of delivered bursts although there are significant variations. This finding has a good agreement with the erosion on the soft tissue during histotripsy, starting at the focus, extending laterally and then reaching the threshold for the erosion area.

Secondly, a novel lesion production strategy was developed and tested in both gel phantom and *ex vivo* tissue with the HIFU pulse duration much longer than that of histotripsy but shorter than boiling histotripsy. The characteristics of bubble cavitation by passive cavitation detection (PCD) for the inertial and stable cavitation and temperature elevation by a thermocouple were measured during the therapeutic procedure. Lesion would vary from a mechanical erosion in the shape of a “squid” to a “dumbbell” lesion with both

mechanical and thermal lesion and then to a “tadpole” lesion with mechanical erosion at the center and thermal necrosis on the boundary in the transparent gel phantom by changing the pulse duration from 5 *ms* to 30 *ms* and pulse repetition frequency (PRF) from 0.2 *Hz* to 5 *Hz*. HIFU-induced small bubbles will be pushed forward by the acoustic radiation force while those large ones will scatter the subsequent bursts towards the source. Analysis of PCD signals, white noise as inertial cavitation and strong harmonics as wave scattering, is accords with the lesion formation. The temperature in the prefocus ($z = -2.5$ *mm*) is higher than those at the focus and postfocus ($z = 2.5$ *mm*) in this study using all sonication parameters. Great variation of the PCD signal and temperature elevation because of the generation and persistence of boiling bubble occurs with the increase of pulse duration and PRF. Similar lesion pattern was also observed in the *ex vivo* porcine kidneys. Hyperechoes in the B-mode ultrasound imaging are comparable to shape and size of lesions in the tissue. Altogether, characteristics of bubble cavitation and thermal field vary with the progress of HIFU treatment at different pulse duration and PRF. Appropriate synergy and monitoring of mechanical and thermal effects would broaden the HIFU application and enhance its efficiency.

Thirdly, bubble dynamics produced by HIFU bursts were observed by high speed camera imaging system. The images showed that the bubble expanded and traveled significantly towards HIFU transducer at millisecond scale immediately after the termination of HIFU exposure, which could be classified as hydrodynamic cavitation. Subsequently, the large bubble motion, expansion and collapse may further enhance tissue erosion. In contrast, the acoustic cavitation may be more efficient in the dynamics of small bubble, such as those produced by histotripsy or at the initial stage of HIFU ablation, and become less significant with the increase of bubble size. Therefore, hydrodynamic cavitation may be another

important mechanism in HIFU-induced tissue erosion with long pulse duration and making use of it may have the potential of enhancing the efficiency of tissue erosion.

Fourthly, since the thermal necrosis was found in the both gel phantom and *ex vivo* tissue experiments, its possibility of hemostasis was evaluated in order to avoid severe vessel rupture and blood bleeding when emulsifying the hypervascular organ (i.e. liver) at HIFU focus. A blood circulating *ex vivo* porcine kidney perfusion model was established in the lab. Catheters were connected to the artery, vein and ureter of fresh porcine kidney, and then porcine blood was circulated by a peristaltic pump. Acculysis and histotripsy bursts were delivered to the kidney, and the urine was collected during the ablation to count the concentration of red blood cells (RBCs) for the propensity of vessel rupture. It is found that the number of RBCs in acculysis-treated samples was significantly lower than those in histotripsy samples, which provided evidence of hemostasis ability. However, this *ex vivo* model may not be perfect in evaluating the hemostasis abilities because of the immune response between circulating blood and kidney. So future *in vivo* animal experiment is required.

Finally, the results and contributions of this study were summarized. Future work and development directions of this new HIFU technology and its application were discussed in the last chapter. Altogether, it is believed that with the further understanding of HIFU mechanisms and technological improvement of HIFU would have a wide acceptance and application in clinics to benefit all human beings.

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1 INTRODUCTION AND BACKGROUND

Ultrasound is a well-established imaging modality in the areas of medicine, biology and engineering. Its characteristics of easy application, low cost, mobility/portability and non-ionization make it a medical option in most soft tissue diagnosis, especially for gynaecology and obstetrics. The application of heat to aid in the healing of injuries is a remedy that has been handed down through the ages. As technology advanced, so did the method of inducing heat into the body. Ultrasound has been under investigation since 1927 by Wood *et al*¹ as a modality for heating or destroying deep-seated tissue. In 1944, focused ultrasound was first proposed by Lynn as a potential technique for neurosurgery.² Fry *et al.* have further developed an early system of his, used for the treatment of Parkinson's disease by destructing deep-seated brain tissue with multiple ultrasound probes in 1967.³

High-intensity focused ultrasound (HIFU) is a noninvasive tissue ablation technology which used focused acoustic energy to heat and damage specific zone. HIFU can be used in the treatment of tumor without going through traditional surgery using scalpels and tweezers; instead it has the ability to ablating tumor by causing tissue necrosis without side effect in the intervening tissue⁴. HIFU has been successfully applied as a minimally or non-invasive surgical tool in the treatment of cancer tumor. Most of HIFU treatments are predominated by the thermal coagulation mechanism of ultrasound ablation⁵. In recent years, the mechanical ablation, termed as histotripsy, has been developed for clinical applications to remove the diseased or impeding tissue for a cellular destruction by acoustic cavitation and energetic microbubble activities⁶. Compared to thermal ablation, the clinical significance of histotripsy is to physically remove unwanted tissue instead of

thermal coagulation followed by *in situ* resection, e.g. perforation of the arterial septum for congenital heart disease.

Non-invasive monitoring of thermally induced lesion is difficult due to the lack of reliable, accurate real time thermometry as well as the difficulties in controlling size, shape and extension of the thermal lesion. Compared to the thermal ablation, the mechanically induced lesion, histotripsy, can be produced in a very controlled and precise manner due to the micro-bubble activities which provide real-time imaging feedback and tissue removal with exceedingly sharp boundaries between the treated and healthy tissue. However, for hypervascular tissue, such as the liver, bleeding may be a big concern if treated by purely mechanical ablation, i.e. histotripsy. Therefore, combination of the mechanical and thermal ablation to achieve effective removal and hemostasis simultaneously is greatly desired in clinical applications.

1.1 Mechanism of Tissue Ablation in HIFU Treatment

HIFU is a non-invasive tissue ablation technology which uses focused acoustic energy to heat and damage tissue inside the body without side effects in the intervening tissue. Its principle is similar to focusing optical light into a point using a magnifying glass. The schematic diagram of HIFU treatment is shown in Figure1-1.

Figure 1-1 (a) Schematic diagram of HIFU treatment and (b) HIFU-beam passes through overlying skin and other tissues without harming them and is focused to necrose a localized tumor region, which may lie deep within the body. There is a very sharp boundary between dead and live cells at this contour. (Courtesy of Yu-Feng Zhou⁶⁰)

1.1.1 Thermal Ablation

Thermal ablation resulting from absorption of acoustic energy in the focal region can be used either to promote low, sustained temperature elevation for hyperthermia purpose or to induce nearly-instantaneous tissue coagulation through rapid heating. Temperature elevation depends on the absorption coefficient of the tissue as well as the size and shape of the thermal source and thermal properties of the target (i.e. conductivity and diffusion). The biological changes that are induced by heating depend on the temperature reached and the duration of the exposure (the thermal dose). Above a certain threshold, thermal doses induce irreversible tissue damage in the form of coagulative necrosis.

(a)

(b)

Figure 1-2 Photographs of (a) superficial HIFU coagulation lesions (shown by arrow) in rabbit liver *in vivo* and (b) the corresponding ultrasound image.

Figure 1-2 (a) shows a coagulation lesion generated by 5s HIFU exposure (intensity 1100 W/cm^2 total acoustic power 25 W) in an *in vivo* rabbit liver model (open procedure). Figure 1-2 (b) shows the enhanced echo of the lesion (arrow) in ultrasound imaging.

The mathematical model to explain this process is BioHeat Transfer Equation (BHTE) ⁷ which explains the temperature elevation by considering the effects of blood perfusion, heat diffusion, and heat deposition shown in the formula below:

$$\frac{\partial T}{\partial t} = \frac{k_t}{\rho C_t} \nabla^2 T - \frac{W_b C_b}{\rho C_t} (T - T_a) + \frac{Q}{\rho C_t} \quad (1.1)$$

where $T(r,t)$ is the temperature of tissue, T_a is the ambient (arterial) temperature (37 °C), k_t is the thermal conductivity of the tissue, C_t and C_b are the heat capacity of the tissue and the blood respectively, W_b is the perfusion rate (cooling by blood flow) and Q is the heat deposition source term due to the acoustic field.

The threshold for thermal necrosis can be calculated by using thermal dose formula which is required to create a thermal dose for an exposure at 43°C for 240 minutes (hyperthermia principle) or above 70°C in 1-4 seconds to necrose the tissue immediately ⁸.

$$TD_{43^{\circ}C}(t) = \int_0^t R^{43-T(t')} dt' \quad (1.2)$$

where R is equal to 0.25 if the $T(t)$ is less than 43°C or 0.5 otherwise.

1.1.2 Mechanical Ablation

In recent years, the mechanical ablation, called histotripsy, has been developed for clinical applications to remove the diseased or impeding tissue achieved by acoustic cavitation using a number of short duration, but high intensity ultrasound pulses (20 μs pulse duration, 1kHz pulse repetition frequency, 18MPa rarefactional pressure)⁶. Compared to non-invasive thermal therapy, histotripsy has some important advantages as follows: Energetic micro bubble activities are visible on imaging and are able to provide real-time feedback, so the operator knows what is happening; Microbubbles produced at the ultrasound focus, are shown as bright spots on ultrasound imaging, enabling the operator to see the targeted volume. After treatment, the lesion appears darker on ultrasound imaging (Figure1-3(c)), so that the operator can identify what has been done. Furthermore, histotripsy technique can produce lesions in a well-controlled and precise manner (Figure1-3(a)(b)). In the near future, it is hoped that histotripsy can be provided to clinicians as a non-invasive surgical tool to significantly improve the quality of the currently available therapeutic modalities. Current clinical targets are: breast cancer, prostate cancer, several cardiac applications, and various benign diseases including breast fibroadenoma.

Figure 1-3 Representative (a) image and (b) histology of tissue erosion after histotripsy, and (c) “M” shape lesion generated by histotripsy shown in ultrasound imaging. (Adapted from University of Michigan <http://www.bme.umich.edu/research/labs/histotripsy>)

1.1.3 Cavitation and Boiling

Cavitation, or bubble activities, may be resulted from the boiling of fluid in tissue or blood, or the growth of small cavitation nuclei within the body resulted from the negative pressure of the acoustic wave when the ultrasound wave transmits. The oscillation of the bubbles in the acoustic field can be stable/non-inertial, or inertial. Stable cavitation is the relatively small oscillations of the bubble’s radius about its equilibrium radius. These oscillations may generate micro-streaming in the vicinity of the bubble leading to improved sonoporation. The amplitude of oscillations during stable cavitation experiences a resonance that depends on the bubble size and frequency of the sound. When a bubble is driven at its resonant frequency, it reaches higher oscillation amplitudes. The resonance frequency of a particular bubble size also depends on the ambient pressure, surface tension, and the liquid density. When measuring the resonance response during stable cavitation, numerous acoustic cycles are often needed to allow the transient behavior of the dynamic system to fade. Inertial cavitation causes the bubble to rapidly grow due to the violent expansion followed by disruption and destruction of the bubble. Its collapse produces a

very high temperature (2,000-5,000 *K*) and pressure (20,000-30,000 *bars*) locally at the core, depending on the inertial properties of the surrounding liquid.⁹

1.2 HIFU Treatment Challenges

HIFU has shown considerable promise in recent years as a tool for successfully treating a range of tumors noninvasively, such as prostate tumor^{10,11}, breast tumor¹², uterine fibroids^{13,14}, renal tumor¹⁵, liver tumor^{16,17}, pancreatic cancer^{18, 19, 20}, bone tumor²¹ as well as hemostasis²². Compared with traditional open surgery, it offers advantages of reducing transfusion requirements and associated infection risks and results in a shorter recovery and in-hospital stay, a reduction in cost of health care and generally superior therapeutic outcome. However, several major challenges for HIFU therapy include focal gain and acoustic window, real-time imaging, treatment planning.

In term of focal gain and acoustic window, the challenge is to obtain large focal gain to produce high intensities at the focus only. The characteristics of the tissue within the focal region are also important factors in HIFU application, since HIFU is an acoustic energy; any factor affecting acoustic energy wave propagation would be considered in HIFU planning, such as acoustic shadowing, reverberation and refraction.⁶⁰ Treatment planning is related to the effective parameters and protocols used in the treatment, including the frequency, geometry, and amplitude specifically for different tissue with different acoustic properties.

Finally, in the absence of a reliable method of monitoring the outcome of HIFU ablation in real time is the most difficult challenge in view point of clinical safety and effectiveness²³. HIFU is currently integrated with MRI or ultrasound for targeting and monitoring. Although MRI has excellent anatomical resolution and high sensitivity for tumor detection,

it cannot exclude small amount of residual cancer and real-time organ motion is to be overcome for MRI guided HIFU treatment. Ultrasound guided HIFU has the problem of lacking of reliable thermometry and lesion generation monitoring.

1.2.1 HIFU monitoring with MRI

The development of magnetic resonance imaging (MRI) methods to monitor temperature changes in the targets region in real-time has marked a turning point in the history of HIFU.^{24,25}

Integration between MRI and HIFU is shown in Figure1-3. The system includes transducer array, detection of cavitation and ultrasound reflection, treatment planning, sonications, MR imaging, and temperature monitoring.

Figure 1-4 MRI-guided HIFU system (Adapted from S. Vaezy²⁶)

Using MRI for HIFU treatment is desirable since it provides great discrimination of tissue structure (tumor/cancer and vital tissues such as a nerves and vessels) and lesion formation, does not produce ionizing radiation as compared to fluoroscopy, and it is also good at characterizing the physiological and functional tissue parameters such as

temperature, flow, diffusion, and perfusion.²⁴ A real-time monitor of increasing focal temperature is important since temperature elevations varied over the procedure of treatments and patients. Therefore, MRI has great potential in targeting and evaluating tissue volume.

Although MRI has advantages mentioned above, MRI has poor temporal resolution (1-3s). In addition, MRI is relatively expensive and is a magnetic device which needs a certain environment to minimize the possibility of side effects. For example, the use of metal parts in HIFU assembly is prohibited since it may cause the distortion of images.

1.2.2 HIFU monitoring with ultrasound-based method

Ultrasound has advantages in HIFU monitoring since it has improved temporal resolution and lower cost and accessibility. Since ultrasound image guidance uses the same form of energy as HIFU therapy, the ultrasound image transducer is usually incorporated into the treatment head as shown in Figure 1-5.

Figure 1-5 Different type of US-guided HIFU transducer of (a) an extracorporeal (FEP-BY02, Beijing Yuande Biomedical Engineering Inc, China) and (b) a transrectal (Ablatherm, Edap-Technomed, France) HIFU transducer. (Courtesy of Yu-Feng Zhou⁶⁰)

Several ultrasound imaging methods are being used and investigated²⁶:

1) Ultrasound monitoring based on change of mechanical properties

This method is to determine HIFU-induced lesion by comparing the stiffness of the region-of-interest and its surrounding tissue before and after the treatment (elastography)²⁷, because biological tissues become harder under the action of temperature. However, the required tissue compression may complicate positioning and reference during treatment planning and guidance. The contrast of an image of the displacement induced by global stress is generally poor for elasticity imaging and is influenced by factors that are unrelated to the distribution of tissue stiffness.

2) Measuring the tissue temperature at the treatment site using local variations in the sound speed and tissue thermal expansion.²⁸

This can be done by measuring the variation of sound speed in tissue as a function of temperature in order to obtain a spatial map for guiding or monitoring the HIFU treatment. However, many of those works are limited to *in vitro* conditions, and implementing such techniques *in vivo* requires further investigation.

3) Real time, synchronous, B-mode ultrasound imaging using HIFU bursts that are synchronized with imaging interrogation. This approach is to observe the hyperechogenicity at the HIFU treatment site in B-mode US image²⁹. However, coagulative tissue can only be detected when cavitation, or boiling occurs due to the high bubble concentrations that cause hyperechoic appearance in the treated area and the bubble occurrence is usually unpredictable, and therefore unreliable for HIFU treatment monitoring.

In conclusion, there is a lack of low-cost ultrasound based imaging approach for accurately and reliably guiding HIFU ablation in real-time. It is the main reason why the HIFU treatments haven't been accepted world-wide.

Histotripsy is a promising mechanical method for tissue erosion in a very controlled and precise manner. Compared to thermal therapy, histotripsy has some important advantages as follows: Energetic micro bubble activities are visible on imaging and are able to provide real-time feedback, so the operator knows what is happening; Microbubbles produced at the ultrasound focus, are shown as bright spots on ultrasound imaging, enabling the operator to see the targeted volume.³⁰ However, the erosion rate is relatively low at $8.33\text{ }\mu\text{m/s}$ compare to traditional surgery⁶ using scalpels and tweezers and hemorrhage may be a potential risk for hypervascular tissue, such as liver. Those limitations encourage the author to develop a novel method to combine thermal and mechanical ablation simultaneously for cancer treatment.

1.3 Significance of Proposed Research

The non-invasive HIFU is a promising thermal ablation method. However, the lack of real-time, intraprocedural imaging tools for quantitative assessment of the tumor boundaries and of the margins of the thermally ablated region is one major limitation of its wide acceptance in clinical application because of concerning about patient's safety, especially in USA, and this is the main reason why the HIFU treatment is still under review for market clearance. Mechanical ablation induces gas bubbles in the treatment area shown as hyperechoic spots clearly shown in B-mode ultrasound image. The echogenicity in B-mode ultrasound image will be changed significantly at mechanically induced lesion after treatment. However, hemostasis may be a major drawback of mechanical ablation and low

erosion rate may affect treatment efficiency. Therefore, combination of the mechanical and thermal ablation to achieve effective removal and hemostasis is greatly desired.

This study aims to develop a novel method which combines the mechanical and thermal ablation to optimize HIFU treatment under real-time ultrasound image guidance and monitoring the cavitation activities by measuring the PCD signals. The proposed novel treatment approach will generate mechanical and thermal lesion simultaneously to achieve significant tissue erosion and hemostasis. It would improve safety significantly since the HIFU induced mechanical lesion could be monitored by real-time B-mode US image basing ultrasound echogenicity. The relationship between both mechanical and thermal lesion and ultrasound parameters would be investigated in this thesis. With understanding of bubble dynamics and lesion formation, the results from this research could enhance the HIFU ablation applications in a controlled and safe way in varieties of human organs, such as liver.

1.4 Scope

Ultrasound parameters (i.e., frequency, pulse duration and pulse repetition frequency) have a great effect on the bubble cavitation and the consequent lesion formation. The goal of thesis work is to investigate and understand bubble cavitation mechanism and how ultrasound parameters affect the tissue lesion formation. The underlying hypothesis of this research is that optimized ultrasound parameters would achieve mechanical and thermal lesions simultaneously and as a result, a new type of lesion can generate tissue erosion as well as hemostasis.

In Chapter 2, the acoustic characterization of HIFU field was also simulated by KZK – a type of nonlinear parabolic equation in this chapter. The dynamics of a single bubble

according to the acoustic waveform was simulated by Gilmore model and its diffusion in the interval time by a static model. The progressive maximum bubble radii and radiating acoustic pressure along HIFU bursts was studied. The general trend of bubble dynamics within the focal plane was summarized.

In Chapter 3, a new tissue erosion method, whose pulse duration is much longer than histotripsy ($20\mu s$) but shorter than the boiling time ($\sim 103\ ms$), was proposed and tested in the gel phantom by utilizing both thermal and mechanical effects simultaneously, and the mechanism of tissue erosion by bubble cavitation was investigated with the progress of HIFU treatment. The bubble cavitation was measured throughout the HIFU exposure using the methods of PCD, and lesion formation in the gel phantom recorded by a camcorder. The temperature at the lesion area was measured by thermocouple simultaneously to distinguish between cavitation and boiling. Ultrasound parameters (i.e., frequency, pulse duration and pulse repetition frequency) were varied to generate different types of lesions.

In Chapter 4, new method was further tested in *ex vivo* porcine kidney. The bubble cavitation was measured throughout the HIFU exposure using the methods of PCD and active cavitation detection (ACD, ultrasound B-mode imaging). The lesion was reconstructed to form 3D space from 2D slice pictures and the volume of mechanical erosion and thermal coagulation was calculated by 3D DoctorTM software. The relationship between thermal and mechanical lesions was evaluated and the relationship between lesion shape/size and the echogenic spot in B-mode US images was also evaluated for safe control of future clinical application.

In Chapter 5, a high speed imaging system was applied to study the lesion formation and bubble dynamic with the progress of HIFU pulse with selected ultrasound parameters from

the previous chapter. The bubble dynamics after HIFU exposure termination was investigated and hydrodynamic cavitation was observed and discussed in this chapter. This finding could potentially enhance the efficiency of tissue erosion.

In Chapter 6, an *ex vivo* porcine kidney perfusion model was designed to test the feasibility of hemostasis of this novel treatment strategy. The perfusion model experiments showed that the new type of lesion (thermal lesion on the boundary and soft tissue emulsification in the core) has ability of stopping blood bleeding due to thermal coagulation.

In Chapter 7, the results and contributions of this work were summarized, and potential future directions were described.

2 NUMERICAL INVESTIGATION OF ACOUSTIC FIELD AND BUBBLE DYNAMICS IN HIFU FIELD

2.1 Introduction

The acoustic characteristics of HIFU field are high intensity in the range of 100-30,000 W/cm^2 at the focal point with peak positive pressure up to 100 MPa and peak negative pressure up to 20 MPa . The nonlinear acoustic propagation of HIFU pulse has been observed to have an effect on heat deposition^{31,32}. This nonlinearity appears because of the finite-amplitude of the acoustic wave; wave will travel faster if the amplitude is higher and vice versa. Nonlinear effects accumulate as wave travels through a distance and causes alteration of temporal waveform, leading to the formation of a shock front which increases the heat deposition significantly³³. HIFU field is often characterized using direct measurement techniques such as radiation force balance and hydrophone. Alternatively, numerical modeling has been applied to predict the HIFU field. The nonlinear parabolic Khokhlov-Zabolotskaya-Kuznetsov (KZK) equation was applied in this chapter to simulate the acoustic wave profile and distribution in the HIFU field.

Bubble cavitation plays an important role in the HIFU ablation as well as histotripsy. In therapeutic ultrasound, bubble cavitation causes the tissue breakdown into sub-cellular structure and, subsequently, mechanical ablation. In the acoustic field, microbubbles oscillate by cavitation and have a positive effect in enhancing heat deposition³⁴ through viscous damping, diffusion of heat from the inner of bubble, and more energy conversion by the wave scattering or radiation of these bubbles. Understanding and controlling the bubble cavitation activity is very important in HIFU application.

The first mathematical model of bubble dynamics was developed by Rayleigh in 1917³⁵.

$$\frac{\Delta P}{\rho} = \frac{P_i - P_\infty}{\rho_l} = \frac{3}{2}\dot{R}^2 + R\ddot{R} \quad (2.1)$$

where P_i is the pressure in the bubble, P_∞ is the pressure at infinity, ρ_l is the density of liquid, R is the bubble radius and dot denotes differentiation with respect to time. This equation has been solved for the collapse of a bubble in an ideal liquid which means significantly larger body of liquid and ignores the surface tension of the bubble and the viscosity changing with time. With those assumptions, Rayleigh has analyzed the collapse of an empty spherical cavity and found the time the cavity requires to collapse was:

$$\tau_{\text{collapse}} = 0.915 R_{\text{max}} \sqrt{\frac{\rho}{P_\infty}} \quad (2.2)$$

where R_{max} is the initial maximum radius of the cavity, and ρ and P_∞ are the density and pressure of the ambient fluid. Plesset introduced the effect of vapor pressure, non-condensable gas, surface tension and a time varying ambient pressure into the Rayleigh-Plesset equation in 1949.³⁶

$$R\ddot{R} + \frac{3}{2}\dot{R}^2 = \frac{1}{\rho_l} \left(P_i - P_\infty - \frac{2\sigma}{R} - 4\mu \frac{\dot{R}}{R} \right) \quad (2.3)$$

where μ is the viscosity of liquid and σ is the interfacial tension. The Rayleigh-Plesset equation explained the relationship between the oscillating bubble radius, its derivatives and the pressure function. However, the Rayleigh-Plesset equation ignored the compressibility of the medium surrounding the bubble. F. Gilmore has further improved the Rayleigh-Plesset equation for radial oscillations of a bubble in a compressible medium; the Gilmore equation has capability of calculating the response of a bubble at high acoustic pressure without requiring the complexity and computational cost of three dimensional model of bubble dynamics.⁵⁵

However, it is still quite difficult to model bubble dynamics with the progress of histotripsy, because the residual bubble (cavitation memory) will affect the following bubble growth. Up to date, no mathematical model has been applied to this area. In this chapter, the bubble dynamics with the progress of HIFU exposure would be modeled by the Gilmore equation using the simulated pressure waveform at the focal plane using the KZK equation to investigate inertial bubble cavitation at high acoustic pressure level at the interface of water and tissue.

2.2 Acoustic Characterization of HIFU Field

The acoustic field of an annular focused HIFU transducer (H-102, outer diameter = 69.94 mm, inner diameter = 22.0 mm, F = 62.64 mm, Sonic Concepts, Woodinville, WA, USA), which will be used in the following experiments, was predicted by a numerical modeling, KZK equation, a type of nonlinear parabolic equation.^{37,38}

$$\frac{\partial}{\partial \tau} \left[\frac{\partial p}{\partial z} - \frac{\beta}{\rho_0 c_0^3} p \frac{\partial p}{\partial \tau} - L_{abs}(p) \right] = \frac{c_0}{2} \Delta_{\perp} p \quad (2.4)$$

where p is the acoustic pressure, z is the propagation coordinate along the axis of the HIFU beam, $\tau = t - \frac{z}{c_0}$ is the retarded time, c_0 is the ambient sound speed, ρ_0 is the ambient density of medium, β is the coefficient of nonlinearity, $\Delta_{\perp} = \frac{\partial^2}{\partial r^2} + \frac{r^{-1} \partial}{\partial r}$ is the Laplacian operator with respect to the transverse coordinate τ , and L_{abs} is the linear operator that accounts for frequency-dependent absorption and dispersion in the medium. For simulation in water, thermoviscous absorption was included as

$$L_{abs} = \frac{b}{2\rho_0 c_0^3} \frac{\partial^2 p}{\partial \tau^2} \quad (2.5)$$

where b is the dissipative parameter of water. The values of the physical constants used for the modeling in water were $\rho_0 = 998 \text{ kg/m}^3$, $c_0 = 1500 \text{ m/s}$, $\beta = 3.5$, $b = 4.33 \times 10^{-3} \text{ kg s}^{-1} \text{ m}^{-1}$. Equation (2.4) was solved numerically in the frequency domain by applying a finite difference algorithm developed by Filonenko and Khokhlova in 2001³¹. The acoustic pressure waveform was modeled by the Fourier transform

$$p(z, r, \tau) = \sum_{-\infty}^{\infty} c_n(z, r) \cdot e^{-in2\pi f_0 \tau} \quad (2.6)$$

where c_n is the complex amplitude of the n -th harmonic. The initial pressure waveform at the source was a harmonic signal of the fundamental frequency f_0 and $c_n(z = 0, r) = 0$ for $n \neq \pm 1$.

The numerical solution of Equation (2.4) was calculated on a polar cylindrical grid. The source was modeled as a spherical cap (bowl) having azimuthal symmetry about the axis of the source. The acoustic and thermal fields need only computation using a two dimensional spatial grid, z , in the axial direction and r in the radial direction (R_{focal} at focus point plane). The finite-difference time-domain (FDTD) method was applied to model the propagation of finite-amplitude sound in a homogeneous thermoviscous field. The FDTD method approximates the spatial and temporal partial derivatives with discrete differences which are obtained from Taylor series expansions about each node on the computational grid³⁹. The grid consisted of two spatial dimensions with uniform spacing Δz and Δr , indexed by (i, j) and a temporal dimension with uniform spacing Δt , indexed by n . Absorbing boundary conditions were applied at the edge of the grid to prevent numerical reflections from affecting the results.

Temporal derivatives were calculated to second order accuracy, $O(\Delta t^2)$ as follows⁴⁰

$$\frac{\partial^2 p}{\partial t^2} \approx \frac{1}{(\Delta t)^2} (p_{i,j}^{n+1} - 2p_{i,j}^n + p_{i,j}^{n-1}) \quad (2.7)$$

$$\frac{\partial^3 p}{\partial t^3} \approx \frac{1}{(2\Delta t)^3} (6p_{i,j}^n - 23p_{i,j}^{n-1} + 34p_{i,j}^{n-2} - 24p_{i,j}^{n-3} + 8p_{i,j}^{n-4} - p_{i,j}^{n-5}) \quad (2.8)$$

The absorption and nonlinear terms used backward-time differences. The spatial differences were computed using a fourth order accurate, $O(\Delta z^4, \Delta r^4)$, centered differencing,

$$\frac{\partial p}{\partial r} \approx \frac{1}{12\Delta r} (-p_{i,j+2}^n + 8p_{i,j+1}^n - 8p_{i,j-1}^n + p_{i,j-2}^n) \quad (2.9)$$

$$\frac{\partial^2 p}{\partial r^2} \approx \frac{1}{12(\Delta r)^2} (-p_{i,j+2}^n + 16p_{i,j+1}^n - 30p_{i,j}^n + 16p_{i,j-1}^n - p_{i,j-2}^n) \quad (2.10)$$

$$\frac{\partial^2 p}{\partial z^2} \approx \frac{1}{12(\Delta z)^2} (-p_{i,j+2}^n + 16p_{i,j+1}^n - 30p_{i,j}^n + 16p_{i,j-1}^n - p_{i,j-2}^n) \quad (2.11)$$

The numerical simulation of KZK model was performed with a computational tool in Matlab code developed by US Food and Drug Administration (FDA).⁴¹

The simulated pressure waveform at the focal plane and the distribution of the peak positive pressure p_{max} and peak negative pressure p_{min} are shown in Figure 2-1(g) and representative pressure profiles at the focal plane.

(a)

(b)

(c)

(d)

(e)

(f)

Figure 2-1 Representative pressure profile at point (a) $R_{focal}=0mm$, (b) $R_{focal}=0.264mm$, (c) $R_{focal}=0.528mm$, (d) $R_{focal}=1.386mm$, (e) $R_{focal}=1.782mm$, (f) $R_{focal}=2.508mm$ and (g) transverse distribution of the p_{max} and p_{min} simulated by KZK model.

2.3 Gilmore Bubble Dynamics Equation

The collapse of a spherical bubble in an infinite volume of water is analyzed in the situation where the liquid flow is irrotational, that is, gravity effects and surface oscillations are left aside thus assuming sphericity at all times⁴². Considering the compressibility of the liquid, the radial oscillation of a single spherical bubble can be described by Gilmore formation⁴³

$$R \left(1 - \frac{U}{C}\right) \frac{dU}{dt} + \frac{3}{2} \left(1 - \frac{U}{3C}\right) U^2 = \left(1 + \frac{U}{C}\right) H + \frac{1}{C} \left(1 - \frac{U}{C}\right) R \frac{dH}{dt} \quad (2.12)$$

where R is the bubble radius, $U(=dR/dt)$ is the velocity of the bubble wall, and C and H are the speed of sound in the liquid at the bubble wall and enthalpy difference between the liquid at pressure $P(R)$ and pressure P_∞ respectively, which are determined by

$$H = \int_{P_\infty}^{P(R)} \frac{dP}{\rho} \quad (2.13)$$

$$C = [C_l^2 + (m - 1)H]^{1/2} \quad (2.14)$$

where P and ρ are the time-varying pressure and density of liquid, P_∞ is the pressure at infinity, C_l is the infinitesimal speed of sound in the liquid, and m is a constant normally set to 7. To determine H and C , the equation of state for a compressible fluid and the pressure at the bubble wall, $P(R)$, in relation to the gas pressure inside the bubble (P_g), liquid viscosity (μ), and surface tension (σ) in the liquid are considered and given in the following,

$$P = A(\rho/\rho_0)^m - B \quad (2.15)$$

$$P(R) = P_g - \frac{2\sigma}{R} - \frac{4\mu}{R} U \quad (2.16)$$

where ρ_0 is the equilibrium liquid density, and A and B are constants describing the fluid, $A = C_l^2 \rho / P_0 m$ and $B=A-l$. The instantaneous number of moles of gas n in a bubble was

calculated from the zeroth-order solution to the diffusion equation given by Eller and Flynn⁴⁴

$$n = n_0 - 4(\pi D)^{1/2} \int_0^\tau F(\tau') (\tau - \tau')^{-\frac{1}{2}} d\tau' \quad (2.17)$$

where D is the diffusion constant of the gas in the liquid, n_0 is the number of moles of gas initially present in the bubble,

$$\tau = \int_0^t R^4(t') dt' \quad (2.18)$$

$$F(\tau) = C_0 \left(\frac{P_g}{P_o} \right) - C_i \quad (2.19)$$

where

$$P_g = \left(P_0 + \frac{2\sigma}{R_0} \right) \frac{n}{n_0} \left(\frac{R_0}{R} \right)^{3\eta} \left(\frac{R_{0n}}{R_0} \right)^{3(\eta-1)} \quad (2.20)$$

where R_0 is the initial equilibrium radius of the bubble, $R_{0n}(t)$ is the time-varying equilibrium bubble radius, η is the polytropic exponent of the gas, C_o is the saturation of the gas in the liquid and C_i is the initial concentration of the gas in the liquid far from the bubble. The pressure distribution in the liquid at a distance r from the bubble center is described by Akulichev as following⁴⁵

$$P_r(t) = A \left[\frac{2}{m+1} + \frac{m-1}{m+1} \left(1 + \frac{m+1}{rC_l^2} G \right)^{1/2} \right]^{2m/(m-1)} \quad (2.21)$$

where G is an invariant of bubble motion that is specified at bubble surface by

$$G = R \left(H + \frac{U^2}{2} \right) \quad (2.22)$$

The constants used for simulation are listed in the Table 2-1

Table 2-1 Constants and initial values used in the Gilmore model simulation

Description	Value	Description	Value
Ambient pressure P_0 :	$1.01 \times 10^5 Pa$	Initial bubble radius R_0 :	$3 \times 10^{-6} m$
Liquid density ρ_0 :	$998 kg/m^3$	Surface tension σ :	$72.583 \times 10^{-3} N/m$
Sound speed C_l :	$1500 m/s$	Liquid viscosity μ :	$1.046 \times 10^{-2} kg/m \cdot s$
Diffusion constant D :	$2.42 \times 10^{-9} m^2/s$	number of moles of gas n_0 :	$6.9 \times 10^{-15} moles$
Polytropic exponent η :	1.4	Saturation of the gas C_0 :	$0.7899 mol/m^3$
		Initial concentration of the gas C_i :	$0.9 C_0$

The finite-difference time-domain (FDTD) method as mentioned in Section 2.3 is used to calculate the acoustic pressure waveform and distribution.

2.4 Numerical simulation results

Figure 2-1 shows the pressure profile, one can see that the profile shape is characterized by a considerable asymmetry of the positive and negative phases. The positive peak value of the profile at the focus is almost four times as large as the peak negative value. The peak negative pressure in the acoustic waveform is primarily responsible for bubble growth and subsequent collapse.

To explore how HIFU pulse affects the bubble dynamics (radial expansion and collapse), the Gilmore model was used to simulate the response of a single $3\mu m$ bubble nuclei to a HIFU exposure at the center frequency of $3.3 MHz$, $1 Hz$ pulse repetition rate at the transverse positions at the focal plane.

Figure 2-2 shows the bubble growth in the first HIFU pulse at varied locations across the transverse focal plane and the bubble nuclei absorbs the acoustic energy and expands about

ten times from the original size of $3\ \mu\text{m}$ to $22.80\ \mu\text{m}$ at $R_{focal}=0\ \text{mm}$, $21.3\ \mu\text{m}$ at $R_{focal}=0.066\ \text{mm}$ and $19.4\ \mu\text{m}$ at $R_{focal}=0.462\ \text{mm}$, etc. The bubbles size decreased with the distance away from the focal point along transverse direction, e.g. $5.98\ \mu\text{m}$ at $R_{focal}=1.254\ \text{mm}$ and $1.14\ \mu\text{m}$ at $R_{focal}=2.244\ \text{mm}$.

Representative bubble dynamic changes simulated by the Gilmore model at the focal point are shown in Figures 2-3&4 and, the equivalent spherical bubble radii vs. time is shown in Figure 2-5. Figure 2-3 shows the selected sequence of bubble radius change at $R_{focal}=0\ \text{mm}$ from the 1st to the 8th pulse, and Figure 2-4 shows the selected sequence of bubble radius change at $R_{focal}=0\ \text{mm}$ from the 15th to the 22nd pulse. The bubble oscillates within HIFU pulse duration ($7\ \mu\text{s}$ in this simulation) and the bubble grows rapidly to the maximum radius when the acoustic pressure termination. Finally, the bubble collapses, and radius drops exponentially.

The bubble radius also oscillated with the progressive HIFU exposure, increased from the 15th to the 17th (from $20.84\ \mu\text{m}$ to $32.86\ \mu\text{m}$) and decreased at the 20th pulse ($20.90\ \mu\text{m}$) and increased again to even higher value at the 21st and 22nd pulse ($33.60\ \mu\text{m}$ and $39.60\ \mu\text{m}$, respectively). Compare to the 15th to the 22nd pulse, the bubble oscillation from the 1st to the 8th pulse is weak with variation from $21.60\ \mu\text{m}$ to $32.30\ \mu\text{m}$ in the maximum bubble size.

To investigate the bubble dynamic during HIFU exposure, the maximum bubble radius as well as the radiation acoustic pressure vs. the number of HIFU pulses was plotted in Figure 2-5. Clear trends can be summarized. The oscillation in bubble cavitation is in the same phase of the oscillation of the maximum acoustic radiation pressure which is generated by bubble collapse, so the bigger and accumulative bubble generates higher radiation pressure. The maximum bubble radius, variation of the bubble oscillation and pressure

decrease with the distance away from the focus from $39.9\ \mu\text{m}$, $27.1\pm 5.36\ \mu\text{m}$ and 1.52MPa at $R=0\ \text{mm}$ to $34.2\ \mu\text{m}$, $24.8\pm 5.19\ \mu\text{m}$ and $1.12\ \text{MPa}$ at $R_{\text{focal}}=0.396\ \text{mm}$, and then further to $7.1\ \mu\text{m}$, $5.98\pm 0.187\ \mu\text{m}$ and 0.11MPa at $R_{\text{focal}}=1.254\ \text{mm}$. Although the oscillation pattern is not same along the transverse focal plane, it can be observed that the great oscillation in bubble cavitation correlates well with high radiation acoustic pressure in the collapse, e.g. from the 60th to the 67th pulse at $R_{\text{focal}}=0\ \text{mm}$ the maximum bubble radius varies from $22.1\ \mu\text{m}$ to $39.9\ \mu\text{m}$ and acoustic pressure varies from $1.14\ \text{MPa}$ to $1.52\ \text{MPa}$; from the 39th to the 46th pulse at $R_{\text{focal}}=0.396\ \text{mm}$ the maximum bubble radius varies from $19.1\ \mu\text{m}$ to $34.2\ \mu\text{m}$ and acoustic pressure varies from $0.879\ \text{MPa}$ to $1.12\ \text{MPa}$; from the 55th to the 60th pulse at $R_{\text{focal}}=0.594\ \text{mm}$, the maximum bubble radius varies from $17.7\ \mu\text{m}$ to $28.5\ \mu\text{m}$ and acoustic pressure varies from $0.638\ \text{MPa}$ to $0.772\ \text{MPa}$, etc. Another trend is that the bubble size would gradually become large with the progress of HIFU exposure if the number of bursts is sufficient (the number of burst is only 100 in this study due to the limited PC memory). Because of the gas diffusion into the bubble may not be in the same phase of bubble oscillation, especially at higher number of molecules, the oscillation pattern is not exactly identical. For example, at $R_{\text{focal}}=0.198\ \text{mm}$, bubble radius of $37.8\ \mu\text{m}$ at the 59th pulse is larger than that of $35.3\ \mu\text{m}$ at the 31st pulse; At $R_{\text{focal}}=0.264\ \text{mm}$, bubble radius of $37.2\ \mu\text{m}$ at the 51st pulse is larger than that of $35.9\ \mu\text{m}$ at the 26th pulse, etc.

Figure 2-2 Bubble dynamic change by a HIFU burst ($f_0= 3.3$ MHz, $p_+ = 78.96$ MPa, $p_- = -15.18$ MPa, $RPF=1$ Hz, pulse duration= $7\mu s$) at the focal plane for the first burst exposure

N=1

N=2

N=3

N=4

N=5

N=6

N=7

N=8

Figure 2-3 Gilmore simulation of bubble dynamic change by HIFU burst ($f_0 = 3.3$ MHz, $p_+ = 78.96$ MPa, $p_- = -15.18$ MPa, RPF=1Hz, Pulse duration= 7 μ s) at focal point $R_{focal}=0$ mm

Figure 2-4 Progressive bubble dynamic change by a HIFU burst ($f_0 = 3.3$ MHz, $p_+ = 78.96$ MPa, $p_- = -15.18$ MPa, $RPF = 1$ Hz, Pulse duration = $7\mu s$) at the center of focal plane ($R_{focal} = 0$ mm)

Figure 2-5 Representative maximum radiated acoustic pressure and maximum bubble radius respect to HIFU exposure pulse at transverse plane at focal point (a) $R_{focal}=0$ mm, (b) $R_{focal}=0.198$ mm, (c) $R_{focal}=0.264$ mm, (d) $R_{focal}=0.396$ mm, (e) $R_{focal}=0.528$ mm, (f) $R_{focal}=0.594$ mm, (g) $R_{focal}=0.66$ mm, (h) $R_{focal}=1.254$ mm

2.5 Discussion

Non-linear acoustic propagation effects are amplitude dependent. Higher amplitude portions of the wave travel faster than lower ones. In medical ultrasound diagnosis, the changes in the sound speed with amplitude are small (typically only a few percent of the small-signal sound speed). However, nonlinear effects accumulate on the propagation pathway towards the focus and can result in significant distortion of the temporal acoustic waveform. In the focused fields, nonlinear effects are the strongest within the focal zone, where the amplitudes are the highest. When distortion of the waveform occurs, higher harmonics of the fundamental are generated. These additional frequencies are more effectively absorbed by tissue, and converted to heat. Therefore, the acoustic nonlinearity could enhance heating and lead to quick temperature rise over 100 °C in boiling histotripsy. As a result, thermal ablation is more efficient. Swindell and Hynynen have demonstrated that the acoustic nonlinear propagation increased the energy absorption in the focal region in both theory⁴⁶ and *ex vivo* tissue experiment⁴⁷. Khokhlova *et al.* had observed that nonlinear ultrasound propagation accelerates lesion inception and growth in the gel phantom experiments.⁴⁸ In addition, high acoustic pressure in the nonlinear propagation induced stronger oscillation of microbubbles grown from gaseous nuclei in the tissue. The presence of cavitation also enhances the subsequent heating in the focal region, especially the pre-focal one^{49,50}.

In the theoretical studies of bubble dynamics, such as the Rayleigh, Rayleigh-Plesset, and Gilmore model as mentioned before, most interest is focused on the variation of the radius and wall velocity of a single bubble after a single ultrasound burst exposure (a few cycles). Although strong collapse of a single and large bubble attached on the surface of a cell, such as by laser tweezer, may generate a pore on the cell membrane, which is termed as

sonoporation. In the macroscopic view, usually a single burst exposure may not be able to produce detectable or measurable bioeffects. The significant ultrasound-induced bioeffects on the cell, such as apoptosis, drug penetration through the membrane pores, calcium pathway regulation, need a certain time for a complete response. For example, cells are exposed to low-intensity pulsed ultrasound (LIPUS) for at least 1 minute and then fixed for the observation of the morphology by surface electronic microscope (SEM). The main reason may be much smaller bubble produced by LIPUS and the cavitation effects on cell should be accumulated with the time in order to reach a threshold for observable response. Strong collapse pressure produced by the bubble cavitation surrounding the kidney calculus surface would weaken the structure of stone and make it more vulnerable for comminution for the incoming bursts. There are similarities in the erosion on the tissue surface using histotripsy. The erosion originates from the focal point after a few thousand bursts and then expands laterally to a certain size. Afterwards, further exposure will only enhance the erosion depth but not its width. Overall, it is desirable to investigate the progressive effect of bubble cavitation in a long ultrasound exposure, such as shock wave lithotripsy and histotripsy. In this chapter, we simulated the dynamics of a single bubble by the Gilmore model and its diffusion in the interval time by the static model. Although the pressure waveform is assumed identical for each burst exposure, the bubble nuclei size and concentration of gas inside it, which are used as initial conditions for the Gilmore model, are not consistent for each burst exposure. Therefore, the corresponding bubble dynamics is different. It is noted that such a change is not monotonical as shown in Figures 2-4 and 2-5, which may be due to the gradual coalescence of the last bubble oscillation with the last second one. Although the number of bursts is only 100 in this study due to the memory limitation of our PC, the general trend can be figured out, increase with great variation in

the maximum bubble size reached and the radiation acoustic pressure in the collapse. These findings may explain the phenomenon of erosion extension in histotripsy. And it suggests that the investigation of such progressive bubble cavitation may illustrate more underlying mechanism in the ultrasound-induced bioeffects.

However, there are some limitations of this preliminary study. First, we assume a uniform bubble distribution (initial size and gas concentration) at the focal plane. It is well known that bubble cavitation is the major mechanism of ultrasound-induced bioeffects. Because of the small size of those bubble nuclei (1-3 μm), it is hard to measure their distribution even in the free field (i.e. water). The determination is more challenging for *in vivo* situation. Therefore, uniform distribution is used in this study for simplicity. Second, the boundary condition, rigid or soft wall, close to the bubbles has a significant impact on their oscillations⁵⁵. Symmetric and spherical expansion and collapse of bubbles will be changed to asymmetric one. The formation of high-speed and high pressure jet towards the boundary has been considered as one of the mechanisms of erosion of propeller⁵¹, fragmentation of kidney calculi in shock wave lithotripsy^{52,53}, vessel rupture induced by intraluminal bubble dynamics^{54,55,56}. But the computation is complicated and time consuming. Thus, consideration of the boundary effect would increase the simulation burden tremendously, which will be studied in the next step. Third, oscillating bubbles nearby may merge into a big one, especially with the presence of a boundary, such as the huge one in the proximal surface of a kidney stone in the shock wave lithotripsy^{57,58}. As a result, the subsequent bubble oscillation and collapse would be enhanced. Usually the bigger merged bubble the strong cavitation it is. In this preliminary study, the bubble coalescence is not considered for simplicity. Finally, this model intentionally does not include energy dissipation in the study of bubble cavitation occurred at free-field condition

(large enough water) under short HIFU pulse duration (pulse duration= $7\mu\text{s}$, RPF= 1Hz), therefore no energy loss or heat conversion is considered.

3 EFFECT OF PULSE DURATION AND REPETITION

FREQUENCY ON THE LESIONS FORMATION BY HIFU IN GEL PHANTOMS

Ultrasound parameters (i.e., frequency, pulse duration and pulse repetition frequency) have a great effect on the bubble cavitation and the consequent lesion formation. In this chapter, different types of lesions could be produced in the gel phantom at varying pulse duration and PRF of HIFU burst. Bubble cavitation and temperature in the focal region were measured throughout the HIFU exposure using the methods of PCD and thermocouples, respectively. Lesion formation in the gel phantom was captured by a digital camcorder, simultaneously.

3.1 Introduction

High-intensity focused ultrasound (HIFU) is an emerging therapeutic modality for both benign and malignant tumors/cancers.^{59, 60} In Asia and Europe, more than 100,000 patients were involved in the HIFU clinical treatment with promising results. Application, investigation, and development of HIFU are becoming more and more attractive to physician, patients, researchers, and medical device manufacturers since its first clinical trial in the 1990s. The major mechanism of HIFU is the thermal coagulation in the targeted volume due to the temperature elevation over 65 °C and consequent protein denature caused by the absorption of acoustic energy. Alternatively, because the acoustic intensity implemented in the HIFU is usually on the order of 1000 W/cm², the influence of bubble activities is also of importance in the focal region. Bubbles may form from boiling of fluid or by the growth of tiny cavitation nuclei in blood or tissue under the negative

pressure of the acoustic wave. Bubbles are strong scatters of acoustic wave because of much lower acoustic impedance in comparison to that of water and soft tissue. Therefore, they will shield the propagation of HIFU bursts and cause more energy to be absorbed proximally, resulting in lesion distortion from a symmetric cigar shape to an asymmetric tadpole one with its head moving towards the source.⁶¹

In comparison, the mechanical effects of high-intensity ultrasound waves (i.e., bubble cavitation, acoustic streaming, and radiation force) dominate in the burst model. Interaction of acoustic cavitation bubbles and ultrasound burst (20 μ s pulse duration with pulse repetition frequency (PRF) of 1 *kHz* and rarefactional pressure of 18 *MPa*) would lead to mechanical fractionation of tissue structure, which is named as histotripsy.^{6, 62, 63}

The transducer used has similar geometry and acoustic specification as the HIFU type, but at a little low frequency because of the proportionality between the cavitation threshold and ultrasonic frequency.⁶⁴ Both at the fluid-tissue interface and in bulk tissue, histotripsy mechanically fragments tissue to a liquefied substance at the subcellular level with sharply demarcated boundaries. Histotripsy provides an option in clinical tissue ablation and removal, such as perforation of the atrial septum in the treatment of congenital heart disease, due to its technical advantages, such as well-controlled and precisely noninvasive lesion production, real-time monitoring energetic microbubble activities and lesion appearance as bright and dark spots on the B-mode ultrasound imaging, respectively.

Furthermore, nonlinear propagation of HIFU waves not only becomes distorted along the propagation path but also forms shock waves in tissue. As a result, the heating rate is significantly higher than that predicted for undistorted and weakly distorted waves. Boiling in tissue (e.g., temperature around 100 °C) occurs in milliseconds. Emulsified lesions without thermal denaturation occurred only when both initiation of boiling in less

than 20ms and shock waves were present, could also cause tissue erosion, but in a larger size (a few mm).^{65, 66} This effective and reliable method resolves problems of inherent performance in the other tissue emulsification technologies stemming from the stochastic nature of cavitation. The lesion size and degree of thermal effects can be predicted and controlled through the proper operation parameters (i.e., HIFU frequency and pulsing scheme). Higher duty factors (> 2%) or longer pulses (> 30 ms) produced varying degrees of thermal denaturation combined with mechanical emulsification.

Altogether, bubble cavitation plays an important role in the HIFU ablation of soft tissue, either thermally or mechanically. Because of heat accumulation between HIFU pulses, the time-to-boil decreases with successive pulses. Although bubble cavitation or liquid boiling could be detected as a fluctuation in the HIFU driving voltage and passive cavitation detection (PCD) signals, little study was carried out on the bubble dynamics with the progress of the treatment. Furthermore, the dominance of mechanical and thermal effects during the HIFU ablation could be varied by simply adjusting the HIFU parameters. In this study, different types of lesions could be produced in the gel phantom at varying pulse duration and PRF of HIFU burst. Bubble cavitation and temperature in the focal region were measured throughout the HIFU exposure using the methods of PCD and thermocouples, respectively. Lesion formation in the gel phantom was captured by a digital camcorder, simultaneously. It is found that the characteristics of bubble cavitation and temperature elevation vary with the progress of HIFU ablation and depend on the operation parameters. Small bubbles will be pushed forward by the acoustic radiation force while those large ones will scatter the subsequent acoustic burst. It is suggested that appropriately utilizing the mechanical and thermal effect of HIFU exposure would broaden

its application and monitoring cavitation activities would provide a feedback in control the effectiveness as well as safety of HIFU therapy.

3.2 Materials and Methods

3.2.1 Experiment setup

Figure 3-1 Gel Phantom experiment setup

The gel phantom experiment set up is shown in Figure 3-1 and the details of devices used are described in following sections.

3.2.2 HIFU Transducer

An annular focused HIFU transducer (H-102, outer diameter = 69.94mm, inner diameter = 22.0 mm, $F = 62.64$ mm, Sonic Concepts, Woodinville, WA, USA) was used in this study (Fig. 1). The HIFU transducer, immersed in the degassed and deionized water ($O_2 < 4$ mg/L, $T = 25$ °C, measured by DO700, Extech Instrument, Waltham, MA, USA) of a Lucite tank ($L \times W \times H = 70 \times 50 \times 30$ cm), was driven by sinusoidal bursts produced by a

function generator (AF3021B, Tektronics, Beaverton, OR, USA) together with a 55dB power amplifier (150W, A150, ENI, Rochester, NY, USA). The HIFU transducer was running at its third harmonic frequency, 3.3 MHz. An acoustic absorber was put in the opposite wall of the testing tank to prevent the ultrasound reflection. The HIFU transducer was attached to a three-axis positioning system (BiSlide, Velmex, Bloomfield, NY, USA) to align the focus with the desired position within the exposure sample. A LabView program (National Instruments, Austin, TX, USA) was written to trigger the function generator for various pulsing protocols, motion of HIFU transducer, and data collection.

3.2.3 Gel Phantom Preparation

Polyacrylamide with 7% of bovine serum albumin (BSA) was used to prepare optically transparent gel phantoms, which have the acoustic impedance of 1.6 MRayls, the speed of sound of 1544 m/s, and the acoustic attenuation of 0.15 dB/cm/MHz⁶⁷. Temperature above 60-65 °C would cause the BSA to denature and become opaque for thermal lesion observation. The liquid mixture of gel constituents was degassed for about 1 h in a descant chamber (420100000, Scienceware, Pequannock, NJ, USA) by a vacuum pump (VTE8, Thomas, Sheboygan, Wisconsin, USA) at pressure of 150mbars, then poured into a mold for polymerization at room temperature within 1 minute of adding N,N,N',N'-tetramethylethylene/diamine (TEMED, Sigma, Singapore).

3.2.4 Cavitation detection and lesion observation

A focused ultrasound probe (A319S, $f = 15$ MHz, $D = 12.7$ mm, $F = 65$ mm, Olympus-IMS, Waltham, MA, USA) was aligned confocally and coaxially with HIFU transducer and

worked as a PCD sensor.⁶⁸ PCD signals of each burst were recorded by a digital oscilloscope (Wavesurfer MXs-B, LeCroy, Chestnut Ridge, NY, USA) at a sampling frequency of 100 MHz and then transferred to a personal computer (PC) for data analysis. Variations of the peak-to-peak PCD values present the amplitudes of bubble cavitation during HIFU ablation.⁶⁹ Small-time Fourier transforms (STFT) were then performed on the collected PCD data in Matlab (The Mathworks, N. Nortick, MA, USA) for the corresponding spectrogram, $F(t, f)$. A specific frequency window, 7.5-9 MHz, whose central value is the mean value of the 2nd and 3rd harmonic frequencies, was chosen to evaluate the amount of inertial cavitation-induced broadband noise during the HIFU exposure by calculating the temporal-average frequency-average amplitude

$$\bar{F} = \frac{\int_0^T \int_{f_1}^{f_2} F(t, f) df dt}{T \cdot (f_2 - f_1)} \quad (3.1)$$

where T is the pulse duration time, f_2 and f_1 are the upper and lower bounds of integral, respectively^{70,71}. Meanwhile, the amplitude of the 4th harmonic frequency ($f_1 = 13.1$ MHz and $f_2 = 13.3$ MHz) was used to represent the stable cavitation⁷² as shown in Figure 3-2. Figure 3-2(a) shows a representative PCD signal produced by the 50th HIFU burst with the pulse duration of 30 ms and a pulse a pulse repetition of 1 Hz. Figure 3-2(b) shows the spectral content of the PCD signal at Figure 3-2(a). The values at the top of the frames are the number of bursts delivered. Production of more and stronger harmonics is due to the stable cavitation meanwhile white noise in the spectrogram is associated with inertial cavitation. Temporal-average frequency-average spectrogram within the dashed (7.5-9 MHz) and dotted (13.1-13.3 MHz) regions are used to present the inertial and stable cavitation dose, respectively.

(a)

(b)

Figure 3-2 (a) Representative PCD signals and (b) the corresponding spectrogram produced by the 50th HIFU burst with the pulse duration of 30 ms and a pulse repetition frequency of 1Hz.

A 100W white-light bulb under the water tank provided the illumination source through the gel phantom for the photography. The progress of bubble formation and lesion growth in the BSA-embedded transparent gel phantom was recorded by a digital camcorder (Vixia HF M500, Canon, Tokyo, Japan) at a frame rate of 50 *Hz* and shutter speed of 1/2000 *s*. The photos were extracted from the videos (Movie Maker, Microsoft, Redmond, WA, USA) and further processed in Photoshop (Adobe, San Jose, CA, USA) for auto color and contrast correction.

3.2.5 Temperature measurement

To measure the thermal field in gel phantom during HIFU ablation, three thermocouples (TJ36-ICSS-020G-6, Omega Engineering Inc., Stamford, CT, USA) with a diameter of 0.5 mm were inserted into the focal region ($z = -2.5, 0, \text{ and } 2.5 \text{ mm}$) through guided holes in a 2-cm thick Lucite plate under the guidance of the digital camcorder and connected to a data acquisition (DAQ) unit (NI-9214, National Instruments, Austin, TX, USA). Sampling rate was 1000*Hz*, and the data were then transferred to PC and processed in Matlab. The viscous heating produced by the insonation would lead to a rapid temperature increase close to the thermocouple⁷³. The thermal couple measurement in the original a few pulses was compared to the theoretical simulation using the BioHeat equation⁷⁴, and the discrepancy was due to the thermoviscous artifacts at the tip of thermocouple, which can be modelled as a Gaussian heat source with a $1/e$ -radius governed by the junction size. So, we estimate the total temperature rise to be

$$T(0, t) = \frac{\mu_a I \alpha^2}{4k} \ln \left(1 + \frac{4kt}{\alpha^2} \right) + B \alpha_v^2 \ln \left(1 + \frac{4kt}{\alpha_v^2} \right) \quad (3.2)$$

where α_v is the effective radius of the viscous heat source and B is an empirically determined factor which may be considered to be strength of the viscous interaction.

Then the artifact was minimized by removing the contribution of the viscous heat source throughout the whole measurement ⁷⁵ as shown in Figure 3-3.

Figure 3-3 Comparison of temperature measured by a thermocouple (black solid line), simulated by a theoretical model (red middle dashed line), viscous heating artifact fitted with an effective Gaussian radius (blue solid line), and simulation result including the viscous heating artifact (short dashed line) in the focal region of a HIFU transducer with the pulse duration of 20ms and a pulse repetition frequency of 1 Hz. A good agreement is found between the measurement and simulation results. The viscous heating artifact decays quickly after the termination of HIFU exposure and then its influence is minimized from the thermocouple measurement.

3.2.6 Statistical analysis

At each testing condition, at least five data were collected. ANOVA¹ was performed in SPSS[®] Statistics (IBM Software, Somers, NY, USA) to determine the statistical difference

¹ Analysis of variance (ANOVA) is a statistical analysis tool that separates the total variability found within a data set into two components: random and systematic factors. The p-value is the mean difference and the variance and n-value is the sample size.

between the groups that was fixed at $p < 0.05$.

3.3 Results

The acoustic pressures of the HIFU transducer with the peak-to-peak voltage of 1 V from the function generator were $p^+ = 46.8 \pm 1.3 \text{ MPa}$ and $p^- = -15.7 \pm 0.8 \text{ MPa}$ at the focus with -6 dB beam size of $0.5 \times 4 \text{ mm}$ measured by a fiber-optic probe hydrophone (FOPH-500, RP Acoustics, Leutenbach, Germany)⁷⁶, and the acoustic power was $105.6 \pm 7.2 \text{ W}$ measured by a radiation force balance (RFB-2000, Onda Corp., Sunnyvale, CA, USA). The boiling time of a single HIFU burst in the gel phantom was $81.4 \pm 17.2 \text{ ms}$ indicated as a significant increase in the measured PCD signals⁶⁵, which is close to the theoretical simulation result of temperature over 100°C at 103 ms . Sonication parameters in the gel phantom are listed in Table 3-1.

Table 3-1 Summary of sonication parameters in gel phantom experiments

PRF (Hz)	Pulse (ms)	PRF (Hz)	Pulse (ms)	PRF (Hz)
1	5	0.2	20	1
1	10	0.5	20	1
1	15	1	20	1
1	20	2	20	1
1	25	5	20	1
1	30			

3.3.1 Effect of the pulse duration

The progressive growth of a lesion produced in the transparent gel phantom was captured with a digital camcorder (Figure 3-4). In the first HIFU pulse, the bubble nuclei will absorb the acoustical energy and then expand. Because of the acoustic reflection at the

interface of phantom and air, dense bubble clouds were observed to form proximally towards the transducer⁷⁷. It is interesting to note that some bubbles appear distally (i.e., ~ 1 mm away from the lesion tip). They may be produced in the focal region initially and then pushed forward with the response to the acoustic radiation force⁶⁶, and aggregation of these bubbles will occur in the succeeding exposure with a long lifetime. Details of this motion will be investigated in the future by using high-speed imaging. Interaction between 5-*ms* HIFU burst and these bubbles led to the formation of a “squid” lesion, which was predominantly mechanical erosion and quite flat (the thickness is less than 0.3 mm, much smaller than the projected lesion size). With the increase of the pulse duration time, bubbles produced in the focal region became large, and the acoustic scattering effects from bubbles were more significant because the acoustic intensity of the scattered wave is proportional to r^6 , where r is the radius of a spherical source⁷⁸. A dumbbell lesion was induced by 10-*ms* bursts (Figure 3-4c). Longer pulse duration led to the domination of thermal effect in the pre-focal region by the backscattering waves, the introduction of large boiling bubble (~3.5*mm* in diameter at $N = 10$ in Figure 3-4d), and then formation of tadpole lesion moving towards the transducer although the forward motion of bubbles and mechanical erosion in the post-focal region still existed. Meanwhile, the high-speed jet emitting from the bubble along the ultrasound propagation direction was clearly illustrated, which was considered the mechanism of tissue fractionation^{66, 79}.

Figure 3-4 Progressive lesion production by a HIFU burst ($f_0 = 3.3$ MHz, $p_+ = 46.8$ MPa, $p_- = -15.7$ MPa) in a transparent 7% BSA gel phantom with the pulse duration of (a) 5 ms, (b) 10 ms, (c) 20 ms, and (d) 30 ms and a pulse repetition frequency of 1 Hz. The values at the top of the frames are the number of bursts delivered. Production of large boiling bubbles with a jet in the HIFU propagation direction is illustrated at $N = 10$ in **Figure 3-4(d)**. Dark area in the pre-focal region presents the opaque thermal lesion due to BSA denaturation while more transparent one is generated by the mechanical erosion. Arrows show bubbles in the distal end of the lesion, which has a long lifetime after the bubble coalescence and may be emitted by the jet from the focal region and pushed forward by the acoustic radiation force of HIFU burst. Many small bubbles are also found surrounding the lesion shown as the arrowhead. With the increase of pulse duration, the lesion shape will change from “squid” to “dumbbell” and then to “tadpole” with thermal effect dominating in the prefocal region. Ultrasound propagates from the bottom to the top. The scale is 2mm.

Meanwhile, PCD signals from cavitation bubbles produced in the focal region throughout the HIFU exposure were recorded using the confocal and coaxial ultrasound transducer. It is found that with the increase of the HIFU pulse duration from 10 *ms* to 30 *ms* there are significant differences between the peak-to-peak PCD signals (Figure 3-5a). When the pulse duration is smaller than 20 *ms*, PCD signal increased with the progress of the sonication despite higher variation in longer bursts. At the pulse duration of 30 *ms*, PCD signals reached the maximum after about 60 bursts. Afterwards, a reduction in the bubble activity was found, even less than those produced by 20-*ms* burst. Thus, there is no statistical difference between the maximum or average PCD signals for 20-, 25-, and 30-*ms* bursts (0.44 ± 0.11 vs. 0.46 ± 0.08 vs. 0.49 ± 0.07 V and 0.24 ± 0.05 vs. 0.23 ± 0.03 vs. 0.23 ± 0.03 V, respectively, $p > 0.05$, Figure 3-5b).

Figure 3-5(a) Representative peak-to-peak PCD signals with the progress of HIFU exposure in the gel phantom, and (b) comparison of the maximum and average peak-to-peak PCD signals in gel phantom (solid line) during the 200-burst exposure with varied pulse duration up to 30 ms and a pulse repetition frequency of 1 Hz. ANOVA is used to determine the statistical difference ($p < 0.05$, $n > 5$).

Because of the large amount of data, PCD signals of every 25 pulses were transferred from the digital oscilloscope to the PC for STFT analysis to quantify both stable cavitation (SC) and inertial cavitation (IC) in Figure 3-6. SC, nonlinear bubble oscillation and acoustic scattering signals from bubbles, are represented by the appearance of the fundamental and harmonics (up to the 7th one due to the bandwidth of PCD transducer) of the driving burst⁸⁰. Appearance of higher-order harmonics and increase of spectrum amplitude may indicate the larger bubble. Meanwhile, IC is shown as the white noise between harmonics⁸¹. SC and IC doses were quantized for easy comparison in Figure 3-7. For the 1st burst, the cavitation doses increased with the pulse duration (i.e., SC from -18.0 ± 0.5 dB to -14.6 ± 1.9 dB and IC from -24.8 ± 0.1 dB to -22.5 ± 0.5 dB with the pulse duration increasing from 5 *ms* to 30 *ms*). SC increased with the progress of HIFU exposure for the pulse duration no more than 20 *ms* (i.e., from -16.6 ± 1.2 dB to -4.3 ± 1.1 dB for 20-*ms* burst). In comparison, SC dose reached its saturation quickly only after 50 pulses (i.e., 0.18 ± 2.4 dB) and began to decrease at the end of exposure for the pulse duration of 30 *ms* (Figure 3-7 (a)). Such a peak distribution is more noteworthy in the detected IC dose (Figure 3-7 (b)), whose value reached the maximum (i.e., -18.5 ± 1.0 dB) around the 50th pulse and then decayed quickly. Therefore, although SC signals were still strong (i.e., -1.4 ± 2.7 dB) at the end of the sonication, IC became considerably less (i.e., -24.2 ± 0.7 dB). SC strength is usually higher than that of IC because IC has a much broader bandwidth⁸¹.

Figure 3-6 Representative PCD signals(upper row) and the corresponding spectrogram(lower row) produced by the HIFU burst with the pulse duration of (a)10 ms,(b)20 ms, (c)30 ms and a pulse repetition frequency of 1 Hz. The values at the top of the frames are the number of bursts delivered. Production of more and stronger harmonics is due to the stable cavitation meanwhile white noise in the spectrogram is associated with inertial cavitation. Temporal-average frequency-average spectrogram within the dashed (7.5-9 MHz) and dotted (13.1-13.3 MHz) regions are used to present the inertial and stable cavitation dose, respectively.

Figure 3-7 (a) Stable and (b) inertial bubble cavitation dose with the progress of HIFU exposure in the gel phantom with varied pulse duration from 5ms to 30ms and a pulse repetition frequency of 1 Hz. Stable cavitation dose is usually higher than that of inertial cavitation.

The sum of simulated temperature elevation by HIFU burst and viscous heating artifact in an effective Gaussian diameter matched with the thermocouple measurement of the first 4 pulses quite closely (Figure 3-8), so the artifact could be minimized assuming its consistency throughout the exposure. The temperature rose rapidly for about 15°C in the 20-*ms* HIFU exposure and then decayed exponentially. Because of the long thermal diffusion compared to the pulse interval time, the ambient temperature increased exponentially with the progress of HIFU exposure. The temperature in the pre-focal region ($z = -2.5 \text{ mm}$) is always higher than that at the focus ($z = 0 \text{ mm}$) and in the post-focal region ($z = 2.5 \text{ mm}$) from the first pulse (63.2°C vs. 52.4°C and 40.5°C, respectively, Figure 3-8) due to the thermal enhancement by the acoustic scattering from the bubbles^{82,83}. Although temperature rise was quite consistent throughout the whole exposure (i.e., $\Delta T = 33\text{-}40^\circ\text{C}$ at $z = -2.5 \text{ mm}$), large variation occurred at the end of sonication (i.e., the smallest ΔT of 23.2°C around the 175th burst at $z = -2.5 \text{ mm}$), which correlates well with the significant changes in the peak-to-peak PCD signals (Figure 3-5a).

Figure 3-8 Envelopes of the temperature profile at $z = -2.5$, 0 , and 2.5 mm, respectively, during 200-burst HIFU exposure after artifact correction. Upper and lower lines of each plot are the peak and ambient temperature for each HIFU pulse exposure, respectively.

Although three thermocouples are close to each other ($\Delta z = 2.5$ mm), the measured temperatures are significantly different with varied pulse duration from 5 ms to 30 ms ($p < 0.05$ in Figure 3-9a). Over 100 °C could be reached with the appearance of the thermal necrosis in tadpole shape (Figure 3-4) if the pulse duration is longer than 15ms. Temperatures generally increased with the pulse duration despite a slight decrease at $z = 0$ and 2.5 mm for 30-ms HIFU burst. When the pulse duration is short, the temperature elevation of each HIFU burst exposure was quite consistent (i.e., $\Delta T = 14.7 - 29.1$ °C at $z = -2.5$ mm for 5-ms burst), shown as the small discrepancy between the 1st and 3rd quartile in the box plot (Figure 3-9b). With the increase of the pulse duration, the variation became significant (i.e., $\Delta T = 9.1 - 82.3$ °C at $z = -2.5$ mm for 30-ms burst) with the average moving to the 5th percentile, which may be due to the great attenuation of large bubble(s)

on the HIFU propagation for much lower temperature rise. These suggest that bubble cavitation have a great impact on the thermal field in HIFU ablation.

Figure 3-9(a) The maximum temperatures and (b) the box plot of temperature elevations of each burst at $z = -2.5$, 0, and 2.5 mm, respectively, by 200-burst HIFU exposure with varied pulse duration from 5 ms to 30 ms and a pulse repetition frequency of 1 Hz in the gel phantom ($n > 5$). Whiskers are 1.5 interquartil ranges. The lower and upper outlier shows the 5th and 95th percentile of the data, respectively. Great variations are indicated as the significant difference between high and low measurements.

3.3.2 Effect of pulse repetition frequency

Varying PRF could also adjust the role of mechanical and thermal effect on HIFU ablation (Figure 3-10). At low PRF of 0.2 *Hz*, bubbles may have sufficient time for dissolution because no persisting bubbles were found between bursts. Bubbles distal to the lesion were also found after 5 pulses. The lesion pattern (“squid” type) is similar to that produced using the pulse duration of 5 *ms* and PRF of 1*Hz* (Figure 3-4a), but larger in size (3.1×8.9 vs. 1.6×5.6 *mm*). As expected, with the increase of PRF, thermal effect induced by bubble scattering became significant and led to the growth of a “tadpole” lesion (i.e., 4.4×10 *mm* at PRF of 5 *Hz*) towards the transducer source and decrease of the mechanical erosion in the distal (Figure 3-10).

Figure 3-10 Progressive lesion production by a HIFU burst in a transparent 7% BSA gel phantom with the pulse duration of 20ms and a pulse repetition frequency of (a) 0.2 Hz, (b) 0.5 Hz, (c) 2 Hz, and (d) 5 Hz. The values at the top of the frames are the number of bursts delivered. Characteristics of lesions are similar to those produced by changing the pulse duration in **Figure 3-4** that with the increase of PRF thermal effect will play an important role in the prefocal region and change the lesion to “tadpole” shape. Arrow and arrowhead present the bubbles in the distal end of the lesion and scattered bubbles around the lesion, respectively. Ultrasound propagates from the bottom to the top. The scale is 2 mm.

Measured PCD signals at varied PRF (Figure 3-11a) illustrate similar characteristics as those with a varied pulse duration (Figure 3-5a). When PRF is 0.2 *Hz*, the peak-to-peak PCD signals increased slowly with the sonication progress. If PRF is higher than 1 *Hz*, the maximum PCD signal could be reached in the first 50-60 bursts and then followed by a decrease with notable variations. Both the maximum and average PCD signals increased with varied PRF from 0.2 *Hz* to 1 *Hz*, but decreased slightly at higher PRF with no statistical difference ($p > 0.05$, Figure 3-11b). SC dose increased with the sonication progress and PRF (i.e., from -16.9 ± 0.7 dB to -3.3 ± 1.9 dB in the first 25 pulses at PRF of 5 *Hz* in Figure 3-12). In contrast, IC dose increased almost monotonically with the number of HIFU pulses delivered at low PRF (i.e., 0.2 *Hz*). With the increase of PRF, the variation had a peak distribution with the summit moving towards the beginning and narrow width. At PRF of 5 *Hz*, IC dose increased slightly from -23.5 ± 0.4 dB at $N = 1$ to -22.5 ± 0.7 dB at $N = 25$ and then decreased greatly (i.e., -24.7 ± 0.4 dB at $N = 200$), which suggests that HIFU-induced bubbles at high PRF may coalesce into a large one for strong acoustic scattering and be resistant to collapse.

Figure 3-11(a) Representative peak-to-peak PCD signals with the progress of HIFU exposure in gel phantom, and (b) comparison of the maximum and average peak-to-peak PCD signals in the gel phantom (solid line) during the 200-burst exposure with the pulse duration of 20 ms and pulse repetition frequency from 0.2 Hz to 5 Hz.

Figure 3-12(a) Stable and (b) inertial bubble cavitation dose with the progress of HIFU exposure in the gel phantom with the pulse duration of 20 ms and varied pulse repetition frequency from 0.2 Hz to 5 Hz.

Figure 3-13(a) The maximum temperature and (b) the box plot of temperature elevation during each burst at $z = -2.5$, 0, and 2.5 mm, respectively, by 200-burst HIFU exposure with the pulse duration of 20 ms and varied pulse repetition frequency from 0.2 Hz to 5 Hz in the gel phantom ($n > 5$). Whiskers are 1.5 interquartil ranges. The lower and upper outlier shows the 5th and 95th percentile of the data, respectively.

The thermal field in HIFU ablation at the varied PRF showed distinct characteristics as that with the varied pulse duration (Figure 3-13). The maximum temperature increased with PRF in the focal and post-focal region (i.e., from 66.1 ± 3.9 °C at PRF of 0.2 Hz to 96.9 ± 3.0 °C at PRF of 5 Hz at $z = 0$ mm), which is due to rapid increase of the ambient temperature in the short interval time (high PRF). In contrast, the corresponding value at $z = -2.5$ mm decreased slightly from 111.5 ± 4.3 °C to 102.7 ± 4.8 °C. In addition, there is large variation in the temperature elevations during the sonication (i.e., high temperature rise initially but more low ones at the end) with the increase of PRF in the whole focal region (Figure 3-13b). At PRF of 5 Hz, although the temperature elevation at $z = -2.5$ mm had similar average value as that of the focus (14.0 ± 9.9 vs. 12.4 ± 5.4 °C), the variation range was much larger (6.6 – 91.2 vs. 4.8 – 37.3 °C).

3.4 Discussion

High-intensity short-duration focused ultrasound bursts were used in the tissue emulsification and ablation. In this study, bubble cavitation, thermal field, and the consequent lesion formation were monitored during the HIFU exposure at the frequency of 3.3 MHz and electrical output power of 150 W using PCD, thermocouple, and the digital camcorder, respectively. It is found that lesion would vary from a mechanical erosion in the shape of a “squid” to a “dumbbell” lesion with both mechanical and thermal lesions, and then to a “tadpole” lesion with mechanical erosion at the center and thermal necrosis on the boundary in the phantom by changing the pulse duration from 5 ms to 30 ms and PRF from 0.2 Hz to 5 Hz. Bubble cavitation activities and temperature profiles were found to vary significantly with the progress of HIFU ablation and sonication parameters. Spectrogram of PCD signals may provide information of the lesion formation, and the

correlation between quantitative SC and IC doses with the lesion volume will be investigated in the next study, which could evaluate the therapeutic progress passively (i.e., determining the transition from mechanical erosion to thermal coagulation) in a low-cost and real-time means⁸⁴. In general, the temperature in the pre-focal ($z = -2.5 \text{ mm}$) was always higher than that at the focus ($z = 0 \text{ mm}$) and post-focal ($z = 2.5 \text{ mm}$) region at all sonication parameters used in this study. The variation in the temperature elevation becomes significant with the increase of pulse duration and PRF because of the generation and persistence of boiling or large bubble(s). Monitoring the cavitation activities and temperature elevation and then dynamically adjusting the sonication parameters would be an effective and safe control scheme for HIFU ablation.

The occurring time of tissue boiling depends on the acoustic pressure in the focal region of the HIFU transducer and the tissue absorption. At the low acoustic pressure exposure, it varies from a few hundreds of milliseconds to several seconds. As a result, a vaporized core will appear in the center of a pre-existing thermally necrotic volume⁸⁵. Although similar lesions were produced with the pulse duration of 30ms and PRF of 1 Hz in our study, the mechanism is different. The lesion produced in the original 10 bursts is mechanical erosion. Afterwards, the increase of ambient temperature due to the thermal diffusion⁷⁴ and significant acoustic scattering from bubbles lead to the gradual dominance of the thermal effect, production of boiling bubbles, and formation of opaque BSA necrosis. In the previous studies, high power output (i.e., 300 W) could produce a boiling bubble within milliseconds using a single-element air-backed transducer at a frequency of 2.158 Hz ⁶⁶. When the interval time between bursts was sufficiently long for thermal diffusion, no thermal denaturation but purely mechanical erosion could be observed.

Increasing duty cycles or pulse duration would lead to the production of a thick white paste with different thermal denaturation, which was not found in this study because of the different time-to-boil (3 *ms* vs. 103 *ms*) and limited operation parameters. Altogether, varying the sonication parameters would produce different types of lesions.

Several phenomena are involved in the HIFU ablation, such as energy absorption, temperature elevation for necrosis formation, bubble cavitation, liquid boiling, acoustic scattering from bubbles, acoustic microstreaming, and atomization^{86,87}, which are usually categorized as mechanical and thermal effects and work synergistically instead of independently in the sonication. Introduction of commercially available ultrasound contrast agent would enhance the thermal accumulation due to the intense acoustic backscattering at the interface of microbubbles. However, the lesion will shift in the proximal direction and change the shape. For example, 0.001% of Definity® by volume in gel phantom produced a lesion 12 times larger with an acceptable shift towards the source in the HIFU ablation⁸⁸. 2 ml intravenous injects of SonoVue into a rabbit would result in the 3-fold increase of coagulated volumes in the liver with more severe cell ultrastructure disorder and more interrupted cell nuclear membranes after 2 seconds of HIFU exposure at the acoustic power of 600 *W*⁸⁹. Furthermore, low pulse duration and high PRF of HIFU burst (histotripsy) could cause noninvasive tissue erosion because of the interaction of bubbles and ultrasound burst, which is a purely mechanical effect. Making use of the significant heat increase rate by a shock wave in nonlinear acoustics could produce tissue boiling in milliseconds for large tissue erosion without thermal coagulation⁶⁶. Therefore, it suggests that appropriately utilizing both thermal and mechanical effects would enhance the efficiency of HIFU therapy in diverse applications. As indicated in this study, the

dominant mechanism varies with the advance of ablation using the same pulse duration and PRF. An adaptive method is in need to dynamically adjust the sonication parameters based on the real-time feedback in order to achieve the desired outcome.

In summary, HIFU bursts with pulse duration longer than that of histotripsy but shorter than the time-to-boil could produce various types of lesions, from a mechanical erosion in the shape of a “squid” to a “dumbbell” lesion with both mechanical and thermal lesion and then to a “tadpole” lesion with mechanical erosion at the center and thermal necrosis on the boundary, at different sonication parameters (i.e., pulse duration and PRF). Bubble activities and thermal elevation change with the progress of HIFU exposure and sonication parameters because of the varied dominant mechanisms (mechanical and thermal effects). Monitoring bubble activities in real-time not only enhances our understanding of bubble cavitation in the acoustical field but also provides feedback information to control the efficiency and efficacy of HIFU therapy.

4 EFFECT OF PULSE DURATION AND REPETITION

FREQUENCY ON THE LESIONS FORMATION BY HIFU IN *EX VIVO* TISSUE

In the previous chapter, we have already studied the effect of HIFU duration and repetition frequency to the lesion performance in homogeneous tissue-mimicking gel phantoms, which are less acoustically absorptive than a real tissue. Tissue contains heterogeneities and has significantly higher acoustic absorption than the BSA-embedded gel phantom described in the previous chapter (the absorption coefficient is about 3 times higher in tissue than in a BSA gel phantom). In this chapter, we evaluated the performance of mechanical and thermal lesion production by HIFU in *ex vivo* tissue samples, which would provide evidence for the future translation to *in vivo* animal studies and clinical trial.

4.1 Introduction

In the previous chapter studies, it is found that bubble cavitation plays an important role in the HIFU ablation of soft tissue, either thermally or mechanically. In this study, different types of lesions could be produced in *ex vivo* porcine kidney at varying pulse duration and PRF of HIFU burst. Not like in gel phantom, it is lack of reliable thermometry and lesion production monitoring method in *ex vivo* experiments. MRI is one of methods, however, it is a very expensive method and it has problem of MR compatible. There are several ultrasound methods under investigation, including sonographic thermometry⁹⁰ and elastographic techniques⁹¹. However, none of them have significant impacts on clinical application at this moment yet. From the results in Chapter 3, the temperature elevation in the gel phantom may relate to the generation and persistence of boiling or large bubble(s).

This finding encouraged the research to measure the bubble cavitation using the methods of active cavitation detection (ACD, ultrasound B-mode imaging) together with PCD in the *ex vivo* porcine kidney to investigate the relationship between lesion pattern, lesion size, temperature and the echogenic spot in the B-mode ultrasound image. The research result may provide a method to monitor HIFU treatment in real time and dynamically adjusting ultrasound parameters to achieve an effective and safe control treatment strategy.

4.2 Materials and Methods

4.2.1 Experiment setup

Figure 4-1 Schematic of the experiment set up

Figure 4-1 shows the configuration of experimental apparatus. The details of apparatus are described as below.

4.2.2 Device Development

A US device incorporated HIFU transducer (Same as Section 3.3.2), US image transducer (G5-2/60 GPS, Ultrasonix, Vancouver, BC, Canada), a passive cavitation detector (Same as Section 3.3.4) into a single device shown in figure 4-2, that was submerged into a custom build water tank. The three transducers shared a common focus point.

Figure 4-2 Photograph of US system, integrating a HIFU transducer with a PCD and an US imaging transducer

An annular focused HIFU transducer (H-102, Sonic Concepts) was used this study and the HIFU transducer was running at its third harmonic (3.3 MHz) frequency. The HIFU transducer has a 2.2 cm diameter hole at its center for placing a passive cavitation detector transducer coaxial to the HIFU path. A US diagnostic imaging transducer (G5-2/60 GPS, Vancouver, BC, Canada) was placed perpendicular to HIFU path so that its imaging plane intersected the HIFU axis along its length, such that the HIFU focus was at the center of US B-mode image. The real-time B-mode images (i.e. bubble cavitation and HIFU-induced lesions) on a diagnosis system (SonicTouch, Ultrasonix) during the sonication

were recorded.

4.2.3 HIFU focal point positioning

The US system was mounted on a XYZ table, which controlled by a servo motion controller (Galil DMC-2183, Gilil Motion Inc.) The HIFU focal point was determined by a mechanical pointer, which was manufactured according to HIFU transducer specification shown in Figure 4-3 and the mechanical pointer can be defined clearly in US image when the US system immersed in degassing water.

Figure 4-3 *Photography of HIFU pointer to identify the location of focal point physically and the focal point can be identified in US image*

The US system with HIFU focal point pointer was connected with XYZ translational stage and moved till it touched the surface of tissue. Afterwards, the US system was moved back 5mm by motion controller, and the mechanical pointer was removed. Then the US system was moved forward to the desired position.

4.2.4 Tissue Preparation

In the *ex vivo* experiment, fresh porcine kidney purchased from local slaughterhouse (Primary Industries Pte Ltd, Singapore) was used within hours for optimization of the HIFU parameters(exposure duration, duty cycle, pulse length and PRF) to produce mechanical and thermal effects simultaneously. The kidney tissue was immersed in 0.9% phosphate-buffered saline (PBS) solution at 4°C and degassed for at least 30 minutes before HIFU treatment. A tissue holder was designed and manufactured to mount tissue in exposure water tank. After HIFU exposures, the tissue samples were frozen at -20 °C overnight, dissected in a thickness of 0.6 mm by a food slicer (Toledo001, Jian Machinery Ltd, China), and recorded photographically by a digital camera (PowerShot SX230 HS, Canon). Mechanical erosion and thermal coagulation in each slice were automatically contoured and realigned, and then their volumes were reconstructed in three-dimensional space and calculated (3D Doctor², Able Software Corp., Lexington, MA, USA).

4.3 Results

HIFU parameters used in this *ex vivo* experiments were same as in gel phantom experiments shown in Table 3-1.

² 3D doctor is an advanced 3D modeling, image processing and measurement software for MRI, CT, PET, microscopy, scientific, and industrial imaging applications. <http://www.ablesw.com/3d-doctor/>

Figure 4-4 comparison of the maximum and average peak-to-peak PCD signals in ex vivo porcine kidney during the 200-burst exposure with varied pulse duration up to 30 ms and a pulse repetition frequency of 1 Hz. ANOVA is used to determine the statistical difference ($p < 0.05$, $n > 5$)

Figure 4-5 Comparison of the maximum and average peak-to-peak PCD signals in ex vivo porcine kidney during the 200-burst exposure with the pulse duration of 20 ms and pulse repetition frequency from 0.2 Hz to 5 Hz.

Figure 4-6 Representative B-mode ultrasound images during the HIFU treatment in ex vivo porcine kidney with the pulse duration of 20ms and a pulse repetition frequency of 1 Hz. The values at the top of the frames are the number of bursts delivered. White arrow shows the HIFU focus. Beam propagates from the left to the right. A significant hypoecho shown as arrow head may be the mechanical erosion while the surrounding region with lower gray levels shown as right arrow may be the thermal lesion produced by the HIFU pulses. Ultrasound propagates from the left to the right. The scale is 1cm.

Step 1: Delineate lesion boundaries in 2D image stacks

Step 2: 3D reconstruction

Step 3: Volume calculation

10ms Hz

20ms 0.5Hz

20ms 5Hz

15ms 1Hz

20ms 1Hz

25ms 1Hz

20ms 0.2Hz

20ms 2Hz

30ms 1Hz

Figure 4-7 3D reconstruction of mechanical (inner mesh) and thermal (outside mesh) lesion (the scale is 5 mm)

Figure 4-8 Representative lesions produced by HIFU burst with pulse duration of 20ms and PRF of 1 Hz

Figure 4-9 Comparison of the volumes of HIFU-induced mechanical and thermal lesions in ex vivo porcine kidney with varied pulse duration from 10 ms to 30 ms and pulse repetition frequency from 0.2 Hz to 5 Hz.

Characteristics of peak-to-peak PCD signals measured in the porcine kidney with varied pulse duration (Figure 4-4) and PRF (0.2 to 2 Hz of Figure 4-5) are similar to those in the

gel phantom although the amplitude is a little lower due to the higher acoustic attenuation in tissue. PCD signal remains flat in the gel phantom, but decreases in *ex vivo* tissue during the HIFU exposure with 5 Hz pulse repetition frequency. It is highly expected that PCD in the gel will further decrease at even higher PRF. The reason is the bubbles induced by HIFU block the acoustic energy of following pulses if they are not completely dissolved in the interval time (higher PRF). Density of bubble nuclei in the *ex vivo* may be lower than that of in the gel because the cell structure makes degassing more difficult than in the solution. Hyper-echoes were observed immediately on the sonography after the delivery of the first pulse with pulse duration of 20 ms and PRF of 1 Hz and then grew into a tadpole shape with very high echogeneity at the center, which may be mechanical erosion and thermal necrosis induced by HIFU pulses, respectively ($N = 200$, Figure 4-6). Although the active cavitation detection (ACD) may be less sensitive to the presence of bubbles than PCD⁹², the shape and size of hyper-echo (7.1×10.1 mm) measured by the sonography caliper were close to the lesion found in the tissue (6.5×9.3 mm in Figure 4-8). 3D lesion was reconstructed from the tissue slices, and mechanical erosion and thermal necrosis were presented by the inner and outside meshes, respectively, and characteristics of lesion production in the tissue are similar to those in the gel phantom (Figure 4-7 and Figure 4-9). When PRF is 1 Hz, the thermal lesion volume increased from $2.0 \pm 0.8 \text{ mm}^3$ at the pulse duration of 10ms to $139.1 \pm 17.6 \text{ mm}^3$ for 30-ms burst. However, the largest mechanical lesion occurred at the pulse duration of 20 ms (i.e., $11.3 \pm 3.8 \text{ mm}^3$). Similarities in the trend of lesion production were found by varying PRF. Thermal lesion increased from $1.1 \pm 0.9 \text{ mm}^3$ at PRF of 0.2Hz to $409.5 \pm 84.9 \text{ mm}^3$ at PRF of 5 Hz. Although

the projected area of a “squid” lesion is big, its volume is usually small because of the thin thickness.

4.4 Discussion

The results from this study confirm the new type of lesion (thermal lesion on the boundary and soft tissue emulsification in the core) was also formed in *ex vivo* tissue using our new HIFU strategy, which may be developed due to both thermal and mechanical effects of HIFU. In the clinical application of tissue removal, the blood bleeding is a big concern. The new type of lesion may have an advantage over histotripsy technology developed at University of Michigan^{62,63}, in term of ability of hemostasis, because of outer thermal lesion. Another advantage over histotripsy is the new type of lesion has quite large erosion area, a few millimeters after 100 exposures. The interaction of the HIFU-induced cavitation bubbles with the subsequent ultrasound burst would lead to the atomization effect at the interface of gas and soft tissue and result in emulsification of soft tissue in the core. At the same time, the bubble produced by HIFU also scatters acoustic waves with the consequently enhanced temperature elevation for hemostasis of potential blood bleeding. Thermal lesion and mechanical erosion can be generated simultaneously, and bubbles are present at the site of HIFU treatment around the time of appearance of a hyperechoic region in a diagnostic B-mode US image.

In the tissue removal by either conventional scalpel or recent radio-frequency (RF) or ultrasound method, the hemorrhage caused by the rupture of vessels or capillaries is the most important safety issue in the clinics, which may be fatal in case of hemostatic defects. For example, dissection of the liver parenchyma using the standard techniques may cause

death rate ranging from 4% to 20%, considerable blood loss, and other serious complications, such as liver failure, hematoma, infections, and bile leakage.^{93,94} Although histotripsy has already proven effective in the noninvasive removal of canine prostate, the degree of hemorrhage in the treatment was not reported.⁹⁵ Meanwhile, HIFU has also been applied in hemostasis in a variety of organs (i.e., liver and spleen) by activating the platelet using bubble cavitation and temperature elevation.²⁶ A new type of lesion with tissue fragmentation at the center and thermal coagulation on the boundary was produced in this study. The ratio of inner and outer radius of the lesion could be adjusted simply by changing the ultrasound parameters. Therefore, combination of tissue erosion and thermal necrosis simultaneously may be a solution in resecting the hypervascular tissue. This hypothesis will be evaluated in *ex vivo* fresh porcine kidney perfusion model in Chapter 6 and in future animal experiment.

5 EFFECT OF HYDRODYNAMIC CAVITATION IN THE TISSUE EROSION

Currently, the mechanism of tissue erosion by using HIFU still has not been fully understood. There are two factors believed to affect the formation of HIFU lesion⁹⁶, i.e. bubbles⁴⁹ and nonlinear acoustic propagation⁹⁷. Hydrodynamic cavitation as illustrated in this study may be another possible mechanism for the bubble dynamic and subsequent tissue erosion immediately after the termination of HIFU exposure, which is different from acoustic cavitation in terms of pressure and time scale of bubble cavitation.⁹⁸ From previous chapter results, the erosion mainly occurred in the pre-focal region towards HIFU transducer. In this chapter, a high speed image system was applied to study the bubble dynamic and consequent lesion formation in more details

5.1 Introduction

Hydrodynamic cavitation is generated by the pressure gradients in a stream of variable speed in which nucleuses of bubble migrate.⁹⁹ The hydrodynamic cavitation causes the bubble collapse which produces pressure, thermal shock waves and microjets or microstreamings. As the results of these intense local forces, the material in proximity to the collapsing bubble will be eroded. The damage starts as pitting and may gradually become large holes.¹⁰⁰ Jets were defined as a bubble collapse near a wall, and the concept of microjets responsible for cavitation damage was first brought out by M. Kornfeld and L. Suvorov in 1944¹⁰¹ and Knapp, Daily and Hammitt suggested three possible jet models¹⁰² as shown in Figure 5-1.

Figure 5-1 Jet collapse models (Knapp, Daily and Hammitt)¹⁰²

In the role of erosion due to hydrodynamic effect, the pioneer study was carried out by Frenkle who found that purely hydrodynamic force could break covalent bond of long polymers and in yeast cell disintegration experiments, Doulah and Hamord, proposed a disintegration mechanism for yeast breakage in hydrodynamic field when the dynamic pressure difference across the drop exceeds its surface tension^{103,106}. Later, Save et al investigated the possibility of hydrodynamic cavitation for large scale cells disruption and concluded that hydrodynamic cavitation was an efficient way for cell breakage¹⁰⁴. Those studies based on single cavity were on chemistry and food applications. The hydrodynamic cavitation concept was applied here to discuss lesion formation under HIFU exposure.

5.2 Material and methods

5.2.1 High Speed Image System

Figure 5-2 Experimental setup

Bubble dynamics observations were carried out in the tissue mimicking gel phantom as described in Chapter 3 which was chosen because of its optical transparency for high speed imaging and its close acoustic properties to soft tissue. The experimental setup is similar as Figure 3-1 in Chapter 3 except the camcorder was replaced by a High Speed Camera (AOS Technologies AG, S-PRiplus), working at 1000 frames per second with a resolution of 640 by 480 pixels. The camera was mounted with a Nikon lens (12.5 to 53mm, $f/1.8D$) and a set of close-up lens to achieve a magnified view of bubble cavitation with 0.04mm/pixel resolution. The camera was triggered by a digital pulse delay generator (DG535, Stanford Research Systems, Inc) to synchronize with HIFU pulse delivery. To capture bubble dynamics in the whole progress of HIFU exposure, the camera starts and ends 10ms before and after HIFU pulse duration. For example, for 10ms pulse duration, images were

recorded from -10 to 20ms; for 15ms pulse duration, images were recorded from -10 to 25ms. A 1000W white-light bubble (Model H10, Hi-Tech Electronics) was used as back-illumination in the imaging. Using it, the gel phantom and bubble appeared as white background and dark region, respectively. The individual frame was extracted from the captured video using the software companied with the high speed camera (AOS imaging Studio V3, AOS Technologies), and then the quantitative data of bubble dynamics is calculated and summarized).

5.2.2 Bubble dynamic parameter definition and measurement method

(a) Measurement

(b) Calibration 0.04mm/pix

(c) Bubble displacement and expansion

Figure 5-3 Bubble expansion calculation schematic diagram

In order to describe the bubble dynamics after HIFU exposure, two parameters were introduced here. One is bubble displacement to describe how far the bubble travels under

the pressure gradients, and the other is the bubble expansion to describe bubble growth according to the hydrodynamic cavitation as shown in Figure 5-3.

The camera software was used to take measurements in the still images selected to calculate the bubble motion with respect to the time frame as well as the diameter change. Global crosshair was used as a reference point for each image as shown in Figure 5-3(a). Then, distance from the crosshair to the targeted bubble was measured. Similar work was repeated for the subsequent frames. The time between two frames is 1ms determined by the frame rate of a high speed camera.

5.3 Results

High speed camera recorded the videos for HIFU exposure duration plus 10 ms more for all pulse duration .i.e. 10-, 15-, 20-, 25- and 30- ms bursts. 200 HIFU pulses were exposure for all pulse duration to study the bubble dynamics and selected image to present the bubble dynamic as shown in Figure 5-4 and 5-5.

Figure 5-4 Representative high speed camera images, $f_0 = 3.3\text{MHz}$, $\text{PRF} = 1\text{Hz}$, Pulse duration = 20ms , $N = 1, 50, 150, 200$

Figure 5-4 shows the representative high speed camera images for HIFU pulses with 20 *ms* pulse duration and 1 *Hz* PRF at 1st, 50th, 100th, 150th and 200th pulse. The first column frame 20 shows the bubble at the exact termination of HIFU. Column 2 to 5 (frame 21 to 24) show continues 4 frames starting from 21 *ms* until 24 *ms* with 1 *ms* interval.

Figure 5-5 presents two still image sequences showing the bubbles bounced back towards the transducer after the exact termination of HIFU exposure and the bubble displacement, which may be caused by the sudden change of pressure or applied acoustic radiation force to them. Meanwhile, it also shows the corresponding bubble expansion due to the hydrodynamic cavitation.

	10ms		20ms		30ms	
N	frame 0	frame -1	frame 0	frame -1	frame 0	frame -1
1
5
10
20
30
40

	10ms		20ms		30ms	
N	frame 0	frame -1	frame 0	frame -1	frame 0	frame -1
50
60
70
100
120
150

Figure 5-5Representative photography from high speed camera images of bubble growth under HIFU pulse exposure and bubble expansion 1ms time after HIFU exposure

Quantitative analysis of the bubble dynamic change is shown in Figure 5-6 and Figure 5-7. Figure 5-6 illustrates the bubble displacement along the HIFU pulse axis, and Figure 5-7 presents the bubble diameter change assuming the bubble keeps its spherical shape. It is found that the bubble displacement increase sharply from the 1st to the 50th pulse for all HIFU burst with pulse duration 10-, 15-, 20-, 25- and 30-*ms*. After about 50 pulses, the bubble displacement of 10-, 15 and 20-*ms* burst becomes saturated until about 120 pulses. In comparison, the backward bubble motion induced by 25-*ms* and 30-*ms* bursts reaches the maximum (0.760 ± 0.181 *mm* and 0.752 ± 0.125 *mm*, respectively) and then decreases, which is due to the large size and mass of bubble motion. This finding has a similar trend as peak-to-peak PCD signals shown in Figure 3-5(a) and Figure 3-6. Figure 5-6 also shows that the displacement range from 5 to 25 pixels in 1*ms*, corresponding to the velocity of 200-1000 *mm/s* (high speed camera image calibration factor= 0.04 *mm/pixel*)

Figure 5-6 Bubble displacement 1*ms* after HIFU exposure with varied pulse duration from 10*ms* to 30*ms* and pulse repetition frequency of 1*Hz*.

Figure 5-7 (a) absolute diameter expansion, (b) relative diameter change R_1/R_0 1ms after HIFU exposure with varied pulse duration from 10ms to 30ms and pulse repetition frequency of 1Hz.

Figure 5-7 shows the bubble expansion increased with the progress of HIFU exposure

starting from the 10th pulse and reached saturation after 100 pulses and then began to decrease. The peak bubble expansion had been detected for all pulse duration from 10- to 30-*ms* within 200 bursts. Apparently, 20-*ms* pulse duration had the highest value for both displacement and diameter expansion (i.e., $0.856 \pm 0.0456 \text{ mm}$ at the 120th pulse and $0.52 \pm 0.0748 \text{ mm}$ at the 100th pulse, respectively). This finding is similar to the volume of HIFU-induced mechanical lesion in *ex vivo* porcine kidney shown in Figure 4-9, in which, 20-*ms* pulse duration produced the largest mechanical lesion. Contrarily, 30-*ms* pulse duration had the lowest value for both displacement and diameter expansion at the end of 200 pulses (i.e., $0.272 \pm 0.0868 \text{ mm}$ and $0.176 \pm 0.0328 \text{ mm}$ respectively), which is opposite to the volume of HIFU-induced thermal lesion in *ex vivo* porcine kidney in Figure 4-9 which shows that 30-*ms* HIFU bursts produced the largest thermal lesion at the same pulse repetition frequency of 1*Hz* compare to other pulse duration. This finding provides suggestion that thermal lesion may restrict mechanical expansion and the ratio of thermal lesion volume to that of mechanical one could be adjusted by changing the HIFU parameters.

5.4 Discussion

Different from hydrodynamic cavitation produced by pressure gradients in a stream of variable speed in which nucleuses if bubble migrate, acoustic cavitation is produced by sound waves in a liquid due to pressure variation.⁹⁸ The minimum pressure acts as driving force during bubble growth for both hydrodynamic cavitation and acoustic cavitation and can be calculated basing on the following equations⁹⁸:

$$P_{min}(\text{Acoustic cavitation}) = P_{ext} - \sqrt{I2c\rho} \quad (5.1)$$

$$P_{min}(Hydrodynamic\ cavitation) = P_{in} - \frac{1}{2}\rho(v_{max}^2 - v_{in}^2) - k \quad (5.2)$$

In Equation (5.1), P_{ext} is the external pressure over the liquid, I is the intensity of the sonication, ρ the liquid density and c the sound speed in the liquid. In Equation (5.2), P_{in} is the inlet pressure v_{max} is the maximum liquid velocity and v_{in} is the inlet velocity and k is the pressure losses. The pressure to cause acoustic cavitation is estimated much higher, e.g. 100 *atm* to cause acoustic cavitation in water. In practice, the value is lower (less than 20 *atm*) due to the presence of weak spots in the water.¹⁰⁵ This pressure depends on the acoustic properties, such as intensity and frequency, while the pressure related to hydrodynamic cavitation depends on the liquid velocity and local static pressure reduction under vapor pressure of liquid.

Another parameter that would be used to differentiate those two types of cavitation is the time scale of the processes. Hydrodynamic cavitation process works from 1 to 50 *ms* which is three orders of magnitude longer than 1 to 50 μs for acoustic cavitation⁹⁸. The results from this study provide evidence that the bubble dynamics after HIFU exposure could be classified as hydrodynamic cavitation because the bubble growth is in *millisecond* order.

Bubble cavitation plays an important role in the tissue erosion, in both histotripsy and boiling histotripsy. However, how the bubble cavitation generates the mechanical erosion still remains unclear. Moreover, current studies focused on the cavitation during HIFU exposure and ignore the bubble dynamics between HIFU pulses. This study has observed the hydrodynamic bubble cavitation at the termination of HIFU exposure. Hydrodynamic cavitation was considered as power transformers by which acoustic energy is converted

into hydrodynamic energy due to the release of elastic waves¹⁰⁶ and is believed to be a stable cavitation¹⁰⁷. This steady oscillation causes the fluid nearby to move rapidly, which is known as microstreaming effect or microjet effect. The microstreaming causes large localized forces to shear the cell membrane surface resulting in physical damage to the cells, which have been shown in both theoretical and experimental studies.^{99, 108, 110}. The shear stress caused by microstreaming can be modeled as

$$S = \frac{2\pi\eta f \varepsilon_0^2}{A\delta} \quad (5.3)$$

where δ is the boundary layer thickness, A is the initial bubble radius, η is the shear viscosity of the medium, f is the frequency, and ε_0 is dependent on peak rarefactional pressure¹⁰⁹. The shear stress due to microstreaming increases with the applied peak rarefactional pressure, and could be as high as $600\text{-}800\text{dyn/cm}^2$ ¹¹⁰ which are strong enough to be one of important mechanisms in tissue erosion.

The hydrodynamic cavitation occurred towards HIFU transducer because of the pressure difference after HIFU exposure. This finding had a good agreement with the finding of gel phantom and *ex vivo* tissue experiment in which the larger mechanical erosion is located in the pre-focal region.

The results of high speed imaging system showed that 20 *ms* ultrasound burst had the highest value of bubble displacement and expansion, which is the similar trend of mechanical lesion generated in the gel phantom and *ex vivo* tissue that, 20 *ms* HIFU burst generated the largest mechanical lesion. This finding provided evidence that a large bubble had strong cavitation with subsequently large tissue erosion. Same finding was

observed from studies of kidney stone comminution by shock wave lithotripsy (SWL)^{53,111}. In order to enhance the tissue erosion efficiency, a new treatment strategy should induce bubble growth as large as possible. As a result, the subsequent bubble oscillation and collapse would create large tissue erosion. Progressive increment of bubble or boiling bubble size may be a solution to achieve large erosion. Another alternative solution is to introduce one secondary, low amplitude ultrasound pulse between two primary HIFU bursts to induce large bubble. This concept has been applied to enhance the efficiency of kidney stone comminution in the SWL⁷⁰ and will be investigated in the future study. The hydrodynamic cavitation scenario was observed from high speed camera imaging and its limitation is that the observation was doable in transparent gel phantom only and the scenario will observe in *ex vivo* tissue from high speed active cavitation detector (B-mode ultrasound imaging system) in the future. With planar wave excitation, the frame rate of B-mode ultrasound image could be larger than 100 fps.

6 HIFU HEMOSTASIS EFFECT IN *EX VIVO* EXPERIMENT

The results from the gel phantom and *ex vivo* tissue experiments show that HIFU exposure with appropriate pulse duration and pulse repetition frequency is able to produce both mechanical and thermal lesion simultaneously. Optimized parameters can generate the maximum mechanical tissue erosion and appropriate thermal lesion for the purpose of hemostasis. This chapter will try to prove the feasibility of hemostasis using a fresh *ex vivo* porcine kidney perfusion model.

6.1 Introduction

The thermal ablation is emerging as an effective and non-invasive HIFU treatment method and histotripsy is proved to physically remove tissue successfully without thermal coagulation⁶. However, bleeding may be a major drawback of histotripsy in the treatment of hypervascular tissues e.g. liver. Although, some investigations of stopping blood bleeding with thermal HIFU ablation had been conducted successfully in animal experiments²², to date, no result has been reported for hemostasis effects together with mechanical tissue erosion. In *ex vivo* tissue experiments in Chapter 4, we have successfully created a new type of lesion with tissue erosion at the center and thermal coagulation on the boundary by using certain ultrasound parameters i.e. pulse duration and pulse repetition rate. Although thermal coagulation, which is one of main reasons for hemostasis, was found, further evaluation is needed for the feasibility of hemostasis in order for the clinical translation. An *ex vivo* porcine kidney perfusion model was designed to prove the hemostasis effects of the novel treatment strategy before moving to the animal studies.

Porcine kidney has a capability of living a long time and still performing its function (around 10 hours) after the pig is harvested. The function of the kidney is to excrete the waste from blood to the ureter as urine (7 in Figure 6) when the blood flows through the glomerulus. The kidneys receive blood from the renal arteries (3 in Figure 6) and returns to veins (4 in Figure 6) after filtration. In a healthy condition, the red blood cells (RBCs) should not pass the glomerulus into the urine so that the RBCs number difference in urine before and after HIFU treatment could be an indicator of vessel rupture.

Figure 6-1 Porcine kidney perfusion model (from Wikipedia) 3-renal artery 4- renal vein 7-Ureter. HIFU lesion shows a thermal boundary in yellow and mechanical erosion in blue dot

6.2 Materials and methods

6.2.1 Experimental setup

The experimental setup and devices used here were similar as those described in Section 4.2 (see Figure 4-1). In this experiment, PCD transducer was omitted and only ultrasound

imaging transducer was used to monitor lesion location and lesion generation. Same focal point positioning device as Section 4.2.3 was used here to locate the focal point at the B-model US image.

Figure 6-2 Schematic diagram of experimental setup

6.2.2 Fresh Porcine Kidney and Blood Preparation

Figure 6-3 Porcine kidney Tube stitched to Ureter Tube stitched to Arterial 1- Kidney 2- Tube attached to ureter 3- Tube attached to renal artery 4- Tube attached to vein

The researchers have entered the local slaughterhouse (Primary Industrial Pte Ltd, Singapore) to collect fresh porcine kidneys after obtaining the approval from Agri-Food Veterinary Authority of Singapore (AVA). Porcine kidney samples were flushed by injecting 0.1% Phosphate Buffer Solution (PBS) solution through the renal artery until no blood coming out of the vein. Flushed kidneys were then put into an ice box to prevent intrarenal thrombosis. At the same time, 4500 *ml* porcine blood was stored into a container, mixed with 500 *ml* anti-blood clotting agent (ACD-A solution; Dextrose monohydrate-12.25 g, Anhydrous citric acid- 3.65 g, Sodium Citrate-11 g in 500 *ml* water), and put into the ice box to prevent the blood clotting. Once transferred to the lab, the kidney samples were flushed with 0.1% PBS solution again to make sure no blood clot inside them. Three silicone tubes were connected to the kidney's artery, vein and ureter, respectively, by a surgical suture method as shown in Figure 6-3. After fixing all tube connection, 0.9% Sodium Chloride Solution (AHB7127, Baxter) was first circulated to confirm that there is no leakage. Then porcine blood circulated from a blood bag to the kidney's artery through a peristaltic pump (BT300-2J, LongerPump) and exited from the vein.

The kidney with tubes was placed on a customized sample holder and immersed into the water tank filled with degassed water as described in Section 4.2.

Figure 6-4 Experiment arrangement, 1- Kidney 2-HIFU transducer, 3-Ultrasound B-mode transducer

6.2.3 Sample collection and RBCs Count

(a)

(b)

Figure 6-5 Sample collection and RBC count (a) Urine sample collected in 5min interval (b) representative photo from hemacytometer

After the kidney was placed inside the water tank, the peristaltic pump (BT300-2J, LongerPump) was turned on at a flow rate of 20 *ml/min* to simulate the normal heart pressure at 120 *mmHg* to circulate the blood through the kidney. The ureteric effluence

flow out was collected at 6 minutes as the control sample. After that, 200 HIFU pulses with 20-*ms* duration at 1Hz pulse repetition rate were applied to the kidney to generate tissue erosion guided by B-mode US image. Two samples labeled as *HIFU* were collected at 3 minutes and 6 minutes time after HIFU exposure respectively. The other kidney from the same pig was treated by 200k histotripsy bursts with 20 μ s duration, 1*kHz* pulse repetition. Same as HIFU samples, two samples labeled as *Histotripsy* were collected at 3 and 6 minutes shown in Figure 6-5(a). All samples were sent to Biologic Lab for the RBCs counting by a hemacytometer (Neubauer Improved Brightline Hemacytometer) (Figure 6-5b).

6.3 Results

Figure 6-6 RBCs analysis

RBCs number counting results are shown in Figure 6-6 in which the control samples had lowest value of $6.56 \pm 1.31 \times 10^6$ cells/ μ l at 6 minutes. The RBCs number in both HIFU

and histotripsy samples increased from 3 minutes (HIFU: $13.7 \pm 1.50 \times 10^6$, Histotripsy: $16.3 \pm 2.26 \times 10^6$) to 6 minutes (HIFU: $20.7 \pm 2.60 \times 10^6$, Histotripsy: $37.7 \pm 1.74 \times 10^6$). Moreover, the RBCs number in histotripsy samples increased by 131.3% which was much higher than that of 51.1% in HIFU samples. The results also show there is no significant difference between the RBCs number in HIFU and histotripsy samples at 3 minutes, but the RBCs number in histotripsy samples is 82.1% higher than that using HIFU at 6 minutes. In addition, the RBCs number in both HIFU and histotripsy samples is much higher than in the control samples ($6.56 \pm 1.31 \times 10^6$).

6.4 Discussion

The fresh porcine kidney perfusion model was used to test the ability of hemostasis of novel HIFU treatment strategy to create mechanical erosion at the center and thermal coagulation on the boundary. Histotripsy was applied to remove tissue mechanically only, and our preliminary results showed that the RBCs number in histotripsy samples increased because of the vessel rupture by the acoustic energy. Although HIFU pulses also caused the tissue erosion, the thermal coagulation layer on the lesion boundary produced by HIFU pulse had an ability of hemostasis because the RBCs number is much less than that in histotripsy samples. However, the hemostasis effect is not perfect since the RBCs number is about three times as the control. Thus, HIFU parameters such as pulse duration and pulse repetition frequency need to be optimized to achieve complete hemostasis effect.

The fresh porcine kidney perfusion model is a preliminary model to evaluate the ability of hemostasis of our novel HIFU technology. However, there are some limitations of this

experiment. First, plenty of blood clots were found when slicing the kidney after the experiment, which may be the immune response between kidney and the circulating blood that is not collected from the same pig. Second, the blood used in this experiment was circulated without complete degassing. The air bubble in the blood shielded acoustic energy delivered to the desired location. In live human or animal body, gas (i.e. oxygen) in the circulating blood exists in the molecular scale and will not cause the shielding problem. Therefore, the ability of hemostasis will be further tested in the animal experiment, and the thermal lesion at the boundary of lesion will be collected and fixed immediately after ablation for biological and pathological analysis to investigate whether the blood vessel is closed up to prevent blood flow.

Figure 6-7 Representative photo of dissected porcine kidney after perfusion experiment.
Blood clots are visualized as dark area

7 SUMMARY AND CONCLUSIONS

7.1 Contribution

HIFU treatment is a promising non-invasive treatment method in the applications of thermal ablation and mechanical erosion. In this thesis, understanding of the lesion growth with the progress of HIFU and the effect of ultrasound parameters on the lesion formation were studied. A novel treatment strategy was developed to generate mechanical erosion at the center and thermal coagulation on the boundary simultaneously. Furthermore, its outcome was proved in both gel phantom and *ex vivo* porcine kidney samples.

The opening chapter briefly introduced the history, mechanism and clinical application of HIFU technology. The challenges and limitations of HIFU in clinic were also discussed here. The main limitation of HIFU thermal ablation is lack of real-time monitoring of lesion formation and temperature evaluation; meanwhile, no proven hemostasis may limit the wide acceptance and application of mechanical erosion. These challenges and limitations encouraged the researcher to develop a novel method of combining the mechanical and thermal ablation to optimize HIFU treatment under the guidance of real-time ultrasound image and the monitoring of cavitation activities in order to improve safety and efficiency in clinics.

The dynamics of a single bubble was simulated by the Gilmore model and its diffusion in the interval time by the static model. Although the pressure waveform is assumed identical to each burst exposure, the bubble nuclei size and concentration of gas inside it, which are

used as initial conditions for the Gilmore model, are not consistent with the progress of HIFU exposure. Therefore, the corresponding bubble dynamics is different. It is noted that such a change is not monotonic, which may be due to the gradual coalescence of the last bubble oscillation with the last second one. The general trend is increase of the maximum bubble size reached and the radiation acoustic pressure in the collapse with great variation. These findings may explain the phenomenon of erosion extension in histotripsy and illustrate more underlying mechanism in ultrasound-induced bioeffects.

Because of the complexity of HIFU field, the theoretical results can't be applied to predict all HIFU treatment results directly. In Chapter 3, a series of experiments in the BSA-embedded gel phantom were carried out to investigate how the ultrasound parameters such as pulse duration and pulse repetition frequency affect the bubble cavitation, temperature evaluation and the consequent lesion formation. Bubble cavitation and temperature in the focal region were measured throughout the HIFU exposure using the methods of PCD and thermocouples, respectively. Lesion formation in the gel phantom was captured by a digital camcorder, simultaneously. The study showed that lesion would vary from a mechanical erosion in the shape of a "squid" to a "dumbbell" lesion with both mechanical and thermal lesions, and then to a "tadpole" lesion with mechanical erosion at the center and thermal necrosis on the boundary in the phantom by changing the pulse duration from 5 ms to 30 ms and PRF from 0.2 Hz to 5 Hz at the driving frequency of 3.3 MHz. Bubble cavitation activities and temperature profiles were found to vary significantly with the progress of HIFU ablation and sonication parameters.

Tissue has significantly higher heterogeneities and acoustic absorption than the BSA-embedded gel phantom; therefore, similar parameters done in Chapter 3 were repeated in

ex vivo porcine kidney tissue to further evaluate our novel strategy. The bubble cavitation was measured by passive cavitation detector and active cavitation detector (sonography). The lesion was reconstructed in 3D space from pathological slices, and then the volumes of mechanical erosion and thermal coagulation were calculated by the software. The results from this study confirmed that a new type of lesion (thermal lesion on the boundary and soft tissue emulsification in the core) could be formed using appropriate HIFU pulses in tissue, which may be due to interaction of both thermal and mechanical effects of HIFU. The volume ratio of mechanical lesion to thermal necrosis could be varied simply by changing the ultrasound parameters. This new type of lesion may be a solution in dissecting the hypervascular tissue. It showed that the echogenic spot in the B-mode ultrasound image was in a good agreement with the lesion shape and size in the dissected tissue, which may provide a reliable monitoring approach in the future clinical application.

The mechanism of tissue erosion with HIFU progress has not been fully understood. In Chapter 5, the high speed imaging system was applied to study the lesion formation and bubble dynamic with the progress of HIFU pulse with selected ultrasound parameters from the previous chapter. The experimental observations showed a remarked bubble expansion and motion towards HIFU transducer after the termination of HIFU exposure. Such phenomenon was classified as hydrodynamic cavitation because of the time scale of the process was in the range of 1-50 ms, three orders magnitude higher than that of the acoustic cavitation. The bubble displacement and expansion with varied pulse duration had a similar trend as peak-to-peak PCD signals measured in Chapter 3, which may suggest that the mechanical erosion in the pre-focal region is mainly created by the hydrodynamic cavitation.

Hemostasis ability of this novel treatment strategy developed in Chapter 3 and 4 needs more confirmation for future clinical translation. In Chapter 6, an *ex vivo* perfusion model was designed to circulate the blood through a fresh porcine kidney and to count the RBCs numbers in the urine samples after either HIFU or histotripsy exposure to the renal tissue on the site. The results showed that the lesion produced by HIFU pulse (200 pulses with pulse 20 *ms* duration and 1 *Hz* PRF) has much lower RBCs number may than histotripsy (200K pulses with 20 μs and 1 *kHz* PRF), but still higher than control samples, which may be due to immune response to the fixed porcine blood in the experiment. Therefore, *in vivo* animal trial is greatly desired to evaluate the ability of hemostasis.

The main contributions of this thesis are summarized as below:

- 1) Applied the Gilmore model to simulate the bubble dynamic with respect to the nonlinear acoustic pressure waveform at the focal plane with the progress of HIFU pulse,
- 2) Investigation of lesion formation with varied ultrasound parameters such as pulse duration and PRF in order to understand the role of mechanical and thermal effects in HIFU ablation and to develop a new type of lesion with soft tissue emulsification at the center and thermal necrosis on the boundary,
- 3) Monitored bubble activities in real-time by measuring PCD and ACD signals and temperature evaluation by thermocouple in the testing samples,
- 4) Bubble dynamics study by high speed camera imaging system to demonstrate the existence and contribution of hydrodynamic cavitation,
- 5) Evaluated the ability of hemostasis of tissue erosion by HIFU pulse in *ex vivo* porcine

kidney perfusion model.

7.2 Future Work

The novel treatment strategy of creating a new type of lesion (thermal lesion on the boundary and soft tissue emulsification in the core) in this thesis may enhance clinical application of HIFU for tissue removing safely under real-time sonography monitoring. Several future investigational directions are discussed below.

In vivo animal experiment to prove the concept of technology

Although the fresh porcine kidney perfusion model in Chapter 6 provided some hint of hemostasis ability, that model had a few limitations and further evaluation is needed in *in vivo* studies, which will be carried out in the animal experiment workshop located at Singapore General Hospital (SGH) after obtaining IRB approval. HIFU pulses with varied pulse duration and PRF would be applied to a live porcine kidney noninvasively with the pig under general anaesthesia. Urine samples will be collected from a catheter inserted into the renal ureter at the fixed time interval. RBCs numbers will be counted as an indicator of hemostasis ability, same as *ex vivo* kidney perfusion model in Chapter 6. After the experiment, the kidney samples will be collected for histological study to observe the effect of HIFU ablation on the renal vessels and tissue either mechanically or thermally. Only with the proof of hemostasis *in vivo* can we move to the next stage of development for effective large-volume tissue removal without worries of blood bleeding *in vivo*.

Real-time monitoring by cavitation monitoring technologies to estimate the progress of thermal lesion and tissue erosion

Now the most significant challenge of HIFU ablation is the lack of reliable thermometry and lesion production monitoring. MRI is one of solution. However, it is very expensive and has the problem of MR compatible. The new type of lesion created in this thesis has a mechanical ablation at the center which can be clearly shown in B-mode ultrasound image, and the echogenic spot has a good agreement with the lesion shape and size in the dissected tissue. The quantitative relationship between them needs further investigation in order to monitor the progress of mechanical erosion and then to control the ablation. The ratio of inner to outer radius of the lesion could be varied by adjusting the ultrasound parameters and this hypothesis will be evaluated in the future work.

Adaptive HIFU exposure for different types of lesion formation

In clinical application, the situation varies from one patient to another and there is no universal solution for all patients. Even during a single session, the dominant mechanism varies with the advance of ablation under the same ultrasound parameters such as pulse duration and PRF. In order to achieve a safe, controlled and efficient HIFU ablation, the ultrasound parameters should be adjusted dynamically base on the cavitation activities and temperature measurement. In addition, the initial ultrasound parameters should also be determined individually by the depth of the desired lesion and the vascular distribution from B-mode and Doppler ultrasound image, respectively.

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1. Y.F. Zhou, X.B. Gao, “*Bubble Dynamics with the Progress of Histotripsy*”, Proceedings of the 8th International Symposium on Cavitation CAV2012 , August 14-16, 2012, Singapore
2. Y.F. Zhou, X.B. Gao, “*Variation of bubble cavitation and temperature elevation during acculysis*”, invited talk at the 13th International Symposium on Therapeutic Ultrasound ISTU2013, May 12-16, 2013, Shanghai, China
3. Y.F. Zhou, X.B. Gao, “*Variations of Bubble Cavitation and Temperature Elevation during Lesion Formation by High-Intensity Focused Ultrasound*”, J Acoust Soc Am. 2013;134:pp.1683-1694.

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