Ultrasonic Histotripsy for Tissue Therapy

K J Pahk¹, D K Dhar², M Malago³ and N Saffari¹

¹Department of Mechanical Engineering, University College London, WC1E 7JE, UK
²Institute of Liver and Digestive Health, University College London, NW3 2PF, UK
³Hepato-pancreatic-biliary and Liver Transplantation Surgery, Royal Free Hospital, University College London, NW3 2PF, UK

E-mail: k.pahk@ucl.ac.uk

Abstract. Hepatocyte transplantation has been considered and investigated as a promising and alternative method to liver transplantation for treating liver-based metabolic disorder in newborns over the past two decades. Although some clinical trials have been conducted and shown clinical benefits and outcomes, it is difficult to deliver and achieve a desired level of integration and transplantation of hepatocytes in the liver parenchyma. To overcome this problem, this work introduces an alternative method to a portal-infused-hepatocyte cell transplantation. To improve the level of engraftment of transplantable hepatocytes, these are injected directly into cavities generated by ultrasonic histotripsy. Histotripsy is an extracorporeal noninvasive technique which has been recently developed using high intensity focused ultrasound (HIFU) for inducing tissue fractionation with no coagulative necrosis. The exact mechanisms for the tissue fractionation are not well understood yet; but the possible mechanisms are thought to be a combination of nonlinear wave propagation effect, explosive bubble growth and ultrasonic atomization. The main objectives of this work are to demonstrate the feasibility of this new cell therapy and evaluate and distinguish between the different types of cavitation activity for either a thermally or a mechanically induced lesion. In the present work, numerical studies on the bubble dynamics (the Gilmore-Akulichev bubble model coupled with the Khokhlov-Zabolotskaya-Kuznetsov equation) and both ex- and in vivo liver experiments are conducted with histological analysis (haematoxylin and eosin stain). The numerical and the experimental results suggest that (a) the acoustic emissions emitted during the thermal ablation and the histotripsy exposure can be distinguished both numerically and experimentally and (b) the proposed cell therapy may potentially form an effective and safe clinical treatment for replacing and correcting disordered hepatocytes, although the transplantation was not conducted in this work.

1. Introduction
Liver transplantation is the main and ultimate clinical treatment for most patients with inherited liver diseases [1]; however donor shortage is currently the main limitation [2]. Hepatocyte transplantation has been suggested and proposed as an alternative promising method to liver transplantation over the past two decades [3], [4], [5]. Despite the significant clinical benefits of hepatocyte transplantation one of the main limitations is the low level of engraftment of transplanted hepatocytes due to the instant blood mediated inflammatory reaction (IBMIR) and the liver to body weight ratio making it difficult to achieve extensive proliferation of transplanted cells [6], [7]. To overcome such problems, this work introduces an alternative method to a portal-infused-hepatocyte cell transplantation. A proposed
method is to produce a cavity inside the liver parenchyma created by ultrasonic histotripsy which acts as a hepatocyte receptor and which facilitates the successful uptake, proliferation and integration of the transplanted cells into the disordered or diseased liver after direct injection into the cavity.

Recently, there has been significant interest in producing tissue fractionation rather than inducing coagulative necrosis using high intensity focused ultrasound (HIFU). This is defined as histotripsy [8]. A lesion produced by histotripsy contains fractionated tissue with no evidence of thermal damage around it [9]. The exact mechanisms for the tissue fractionation are not well understood yet; but the possible mechanisms are thought to be a combination of nonlinear wave propagation effect, explosive bubble growth and ultrasonic atomization [10]. Histotripsy uses high amplitude shock waves to fractionate tissue and has been demonstrated using both micrometre-sized cavitation bubbles (maximum radii of the order of a 100 μm) with microsecond HIFU pulses (cavitation cloud histotripsy) [11] and millimetre-sized boiling bubble for milliseconds of HIFU excitation (boiling histotripsy) [12].

In this paper, a single spherical bubble is modelled using the Gilmore-Akulichev (GA) equation coupled with the Khokhlov-Zabolotskaya-Kuznetsov (KZK) equation to investigate bubble dynamics (including corresponding radiated pressures) excited by both a histotripsy and a thermal ablation waveform. Ex vivo experiments are conducted to obtain acoustic emissions from a thermally and a mechanically induced lesion. Finally, an in vivo experiment is performed in rat liver to demonstrate the feasibility of the proposed cell therapy mediated by histotripsy.

2. Numerical method

2.1. Acoustic fields-The KZK equation

In HIFU applications, a commonly used equation for simulating an acoustic field is the Khokhlov-Zabolotskaya-Kuznetsov (KZK) parabolic nonlinear wave propagation equation. It is an augmented form of Burgers’ equation and accounts for the effects of diffraction, absorption and nonlinearity. The axisymmetric form of the KZK equation in terms of acoustic pressure is given by [13]

\[ \frac{\partial^2 p}{\partial z \partial \tau} = \frac{c_0}{2} \nabla^2 p + \frac{\delta}{2c_0^2} \frac{\partial^3 p}{\partial \tau^3} + \frac{\beta}{2\rho_0c_0^3} \frac{\partial^2 p^2}{\partial \tau^2} \]  

(1)

where \( p \) is the acoustic pressure, \( z \) is the propagation coordinate along the axis of the wave beam, \( c_0 \) is the small signal sound speed, \( \rho_0 \) is the ambient density, \( \beta = 1 + B/2A \) is the coefficient of nonlinearity where \( B/A \) is a parameter of the nonlinearity of the medium, \( \delta \) is the diffusivity of sound, \( \tau = t - z/c_0 \) is the retarded time variable and \( \nabla^2 \) is the transverse Laplacian operator. The sound diffusivity is given by

\[ \delta = \frac{1}{\rho_0} \left[ \mu_B + \frac{4\mu_s}{3} \right] + \kappa \left( \frac{1}{c_v} - \frac{1}{c_p} \right) \]

where \( \mu_B \) and \( \mu_s \) are bulk and shear viscosity, \( \kappa \) is thermal conductivity, \( c_v \) and \( c_p \) are specific heat capacities at constant volume and at constant pressure respectively.

The HIFU Simulator 1.2 [14] was used to compute acoustic pressures and waveform at the focus as a function of the electrical power applied to a HIFU transducer. This open source software is widely used and validated experimentally by other HIFU studies in cavitation cloud histotripsy [15], cavitation enhanced heating in bovine liver [16], HIFU beams [17], temperature measurements in ex vivo porcine muscle [18] and HIFU lesion formation in ex vivo swine tissues [19]. The KZK numerical results are illustrated in subsection 3.1.
2.2. Bubble dynamics: the Gilmore-Akulichev equation

In this work, the Gilmore-Akulichev bubble model (GA) is used for simulating gas bubble dynamics because it takes into account the compressibility of the fluid and the variation of sound velocity in the liquid (equation of state: \( P = A(\rho/\rho_o)^m - B; \partial P/\partial \rho = c^2 \) where \( A, B \) and \( m \) are material-dependent constants). The assumptions behind this model make it suitable to study single spherical bubble dynamics subjected to high acoustic pressure amplitudes such as in lithotripter shock wave pulses [20],[21]. The Gilmore-Akulichev equation is a second order differential equation which governs the dynamics of a single bubble subjected to a compressible liquid, and is given by [21]

\[
\ddot{R}R \left( 1 - \frac{\dot{R}}{C} \right) + \frac{3}{2} \left( 1 - \frac{\dot{R}}{3C} \right) \dot{R}^2 = \left( 1 + \frac{\dot{R}}{C} \right) H + \frac{\dot{R}}{C} \left( 1 - \frac{\dot{R}}{C} \right) R \frac{dH}{dR} \tag{2}
\]

and the time-varying pressure (outward) radiated by the bubble motion in the liquid, \( P_r(t) \) is [21],

\[
P_r(t) = A \left[ \frac{2}{m+1} + \frac{m-1}{m+1} \left( 1 + \frac{m+1}{r c_0^2 G} \right)^{1/2} \right]^{2m/(m-1)} - B \tag{3}
\]

where

- \( R \) – is the bubble radius;
- \( \dot{R} \) – is the velocity of the bubble wall;
- \( \ddot{R} \) – is the acceleration of the bubble wall;
- \( C = \left( c_0^2 + (m-1)H \right)^{1/2} \) – is the speed of sound at the bubble wall;
- \( H = \frac{m}{m-1} \rho_o A^{1/m} \left\{ [P(R) + B]^{(m-1)/m} - [P_\infty(t) + B]^{(m-1)/m} \right\} \) – is the enthalpy;
- \( P(R) = P_g - \frac{2\sigma}{R} - 4\mu \frac{\dot{R}}{R} \) – is the pressure at the bubble wall;
- \( P_g = \left( P_0 + \frac{2\sigma}{R_0} \right) \frac{n}{n_0} \left( \frac{R_0}{R} \right)^3 \gamma \left( \frac{R_0}{R} \right)^{3(\gamma-1)} \) – is the pressure of gas inside the bubble;
- \( R_{on} \) – is the time-varying equilibrium radius of the bubble;
- \( R_0 \) – is the initial bubble radius;
- \( n \) – is the instantaneous number of moles of gas in the bubble;
- \( n_0 \) – is the initial quantity of moles of gas in the bubble;
- \( c_0 \) – is the speed of sound in the liquid;
- \( P_0 \) – is the ambient pressure of the surrounding liquid;
- \( P_\infty \) – is the pressure at infinity;
- \( r \) – is a distance from the centre of the bubble \( (r \gg R(t) \) [22], set to 10 cm);
- \( G = R(H + \dot{R}^2/2) \) – is an invariant of the bubble motion;
- \( \mu \) – is the viscosity of the liquid;
- \( \sigma \) – is the surface tension of the liquid;
- \( \gamma \) – is the polytropic index of the gas inside the bubble.

For simplicity, gas-rectified diffusion across the bubble wall is ignored here (i.e., let \( n = n_0 \) and \( R_{on} = R_0 \)). To solve the Gilmore-Akulichev equation numerically, it is firstly nondimensionalised according to the following scheme:

\[
\text{Mass} = \frac{P_0 R_0}{(2\pi f)^2}; \text{ Length} = R_0 \text{ and } \text{Time} = \frac{1}{2\pi f}
\]

The nondimensionalised Gilmore-Akulichev equation is then integrated numerically using an explicit Runge-Kutta method (4), (5) routine using \texttt{ode45} in MATLAB (MathWorks Inc., R2013a).
During the numerical computation, it is assumed that a single spherical gas bubble is initially at rest, the bubble remains spherical during its oscillation, there is no mass transfer and no heat diffusion at the bubble wall and the bubble pulsates adiabatically. Acoustic fields used in the Gilmore-Akulichev equation are obtained from the KZK simulation. The GA numerical results are shown in subsection 3.2.

Parameters used for both the acoustic fields and the bubble dynamics simulations are shown in table 1 and table 2 respectively.

**Table 1.** Parameters used in the KZK simulation. A 1.1 MHz (Sonic Concepts, H102) and a 2.0 MHz (Sonic Concepts, H106) HIFU transducer were employed for the *ex vivo* and the *in vivo* experiments respectively. The HIFU focus was set to 5 mm below the surface of the liver.

| Symbol | Definition | 1.1 MHz | 2.0 MHz |
|--------|------------|---------|---------|
| a      | Outer radius of HIFU transducer | 3.2 cm | 3.2 cm |
| b      | Inner radius of HIFU transducer | 1 cm | 0 cm |
| d      | Focusing depth | 6.32 cm | 6.32 cm |
| F<sub>width</sub> | Focal width | 1.37 mm | 0.73 mm |
| F<sub>depth</sub> | Focal depth | 10.21 mm | 7 mm |
| ε<sub>f</sub> | Efficiency of HIFU transducer | 85 % | 85 % |
| G      | Linear pressure gain | 35.4 | 72.9 |
| K      | Number of harmonics used in the KZK simulation | 200 | 200 |
| c<sub>0</sub> | Small-signal speed of sound | 1481 ms<sup>-1</sup> | 1568 ms<sup>-1</sup> |
| ρ<sub>0</sub> | Mass density | 1000 kg m<sup>-3</sup> | 1060 kg m<sup>-3</sup> |
| α      | Attenuation at 1 MHz | 0.217 dB m<sup>-1</sup> | 52 dB m<sup>-1</sup> |
| η      | Exponent of attenuation vs. frequency curve | 2 | 1.1 |
| β      | Nonlinearity parameter | 3.5 | 6.8 |

**Table 2.** Parameters used in the GA equation. The liver is modelled as a highly viscous human blood-like tissue.

| Symbol | Definition | Water [24] | Liver |
|--------|------------|------------|-------|
| σ      | Surface tension | 0.073 N m<sup>-1</sup> | 0.056 N m<sup>-1</sup> [25] |
| μ      | Viscosity of fluid | 1.046×10<sup>-3</sup> kg m<sup>-1</sup>s<sup>-1</sup> | 9×10<sup>-3</sup> kg m<sup>-1</sup>s<sup>-1</sup> [25] |
| γ      | Polytropic exponent of the gas | 1.4 | 1.4 |
| P<sub>0</sub> | Ambient pressure | 1.01×10<sup>5</sup> Pa | 1.01×10<sup>5</sup> Pa |
| m      | Material dependent constant | 7 at 20 °C | 5.527 (human blood) [26] |
| A      | Material dependent constant | 300.1 MPa | 614.6 MPa (human blood) [26] |
| B      | Material dependent constant | A<sub>0</sub>−P<sub>0</sub> | A<sub>0</sub>−P<sub>0</sub> |

3. Numerical results

3.1. Acoustic fields

Acoustic fields in the liver were obtained using the KZK simulation as a function of the electrical power applied to a HIFU transducer. The calculated acoustic peak positive (P<sub＋</sub>) and peak negative (P<sub－</sub>) pressures used for both thermal ablation and boiling histotripsy are shown in table 3.
Table 3. Acoustic peak positive ($P_+$) and peak negative ($P_-$) pressures at the focus. The acoustic pressures used for boiling histotripsy are within the range of required pressure values reported by other HIFU histotripsy studies [27], [28], [29].

| Electrical power [W] | $P_+$ [MPa] | $P_-$ [MPa] | Description |
|----------------------|-------------|-------------|-------------|
| 1.1 MHz HIFU transducer | 60          | 13.4        | -7.5        | Thermal ablation (for *ex vivo*) for inducing coagulative necrosis. |
|                      | 350         | 74.4        | -14.4       | Boiling histotripsy (for *ex vivo*) for producing tissue fractionation. |
| 2.0 MHz HIFU transducer | 200         | 101         | -16.7       | Boiling histotripsy (for *in vivo*) for producing tissue fractionation. |

3.2. Bubble dynamics
In boiling histotripsy, an explosive bubble growth is one of the possible mechanisms to cause tissue fractionation and that growth is thought to be led by nonlinear wave propagation effect [30]. The effect of the acoustic waveform on the bubble dynamics (i.e., sinusoidal, boiling histotripsy and thermal ablation waveforms) is numerically investigated in the absence of any heat or mass transfer across the bubble wall.

3.2.1. Boiling histotripsy – Nonlinear-shocked wavefront. To study the effect of boiling histotripsy waveform on a gas bubble in a liquid, the bubble dynamics driven by both the histotripsy and the sinusoidal waveforms are compared here. The peak negative pressure of the sinusoidal wave is matched to that of the histotripsy waveform shown in figure 1.

![Figure 1](image-url)  
*Figure 1.* Histotripsy (red) and sinusoidal (blue) waveform with a frequency of 1.1 MHz. Both peak negative pressures are matched to -14.4 MPa. The histotripsy wave (nonlinear-shocked waveform) was obtained from the KZK simulation.

The initial bubble size was chosen as 20 μm [30] as this generated rectified bubble growth after only a few acoustic cycles. In figure 2, the response of the bubble is plotted for both the histotripsy ($P_+ = 74.4$ MPa and $P_- = -14.4$ MPa) and the sinusoidal waveform ($P_+ = 14.4$ MPa and $P_- = -14.4$ MPa). For the sinusoidal wave excitation, the bubble continues to grow and collapse with time; whereas the nonlinear-shocked waveform leads the bubble to undergo rectified growth within a few acoustic cycles.
These different bubble behaviours can be described by the asymmetry in the compressional and rarefractional pressure phase. In the nonlinear waveform, the positive pressure phase has a shorter duration than the negative pressure part; therefore, the bubble has relatively longer time to undergo expansion rather than contraction; resulting in the explosive growth. As the bubble gets larger over each acoustic cycle, its characteristic time becomes longer and eventually the bubble responds more to the tensile portion [30]. In the sinusoidal excitation case, it can be seen in figure 2 that the bubble behaviour is not periodic. This is likely due to the large acoustic pressure magnitude and the presence of nonlinear resonance (i.e., the ratio of the driving frequency and the linear resonance frequency approaches a rational number) [31], [32].

The rectified bubble behaviour can also be seen with the initial bubble radius of 7 μm, $P_+ = 101$ MPa and $P_- = -16.7$ MPa (used in an *in vivo* experiment) at a driving frequency of 2.0 MHz (figure 3).

![Figure 2](image1.png)

*Figure 2.* Rectified growth of a 20 μm bubble in water with a driving frequency of 1.1 MHz. The red and blue solid line represent the bubble dynamics under histotripsy ($P_+ = 74.4$ MPa and $P_- = -14.4$ MPa) and sinusoidal waveform ($P_+ = 14.4$ MPa and $P_- = -14.4$ MPa) respectively.

![Figure 3](image2.png)

*Figure 3.* Rectified growth of a 7 μm bubble in water with a driving frequency of 2.0 MHz. The red and blue solid line represent the bubble dynamics under histotripsy ($P_+ = 101$ MPa and $P_- = -16.7$ MPa) and sinusoidal waveform ($P_+ = 16.7$ MPa and $P_- = -16.7$ MPa) respectively.
Further qualitative observations may be made by adding both the mass and heat transport into the Gilmore-Akulichev equation. Kreider et al [30] showed numerically that the rectified bubble growth was observed at a high ambient temperature of 80 °C.

3.2.2. Thermal ablation waveform – a slightly distorted nonlinear wavefront. In contrast to the explosive bubble growth driven by the nonlinear-shocked-waveform shown in figure 2, no rectified bubble growth is observed with the thermal ablation waveform (figure 4b). This is most probably because the degree of nonlinearity (i.e., absence of a shock in the waveform) and the duration of the tensile part (negative pressure phase) are not sufficient to lead to such rectified behaviour. Nevertheless, the bubble growth under the thermal ablation waveform is enhanced compared with that under the sinusoidal wave since it has a slightly longer negative pressure part than that of the sine wave.

![Graph of acoustic pressure and time for thermal ablation and sinusoidal waveforms](image)

**Figure 4.** Thermal ablation waveform ($P_+ = 13.4$ MPa and $P_- = -7.5$ MPa) and the corresponding bubble dynamics at a frequency of 1.1 MHz. (a) shows the thermal ablation (red) and the sinusoidal waveform (blue). (b) The corresponding bubble dynamics with the thermal ablation and the sinusoidal waveform. A 20 μm bubble is in water. The thermal ablation waveform was obtained from the KZK simulation.

3.2.3. Bubble dynamics in the liver modelled as a highly viscous human blood-like liquid. Forced bubble oscillations in the liver-like-fluid are investigated here instead of using the Keller-Miksis [33] equation which accounts for a weakly compressible liquid. This was coupled with the linear Kelvin-Voigt viscoelastic model proposed by Yang and Church [34]. According to the numerical results
shown in figure 5 the rectified growth behaviour also appears under the boiling histotripsy waveform (figure 5b and c), whereas there is no such growth with the thermal ablation excitation (figure 5a). For both the histotripsy and the thermal ablation waveforms, the magnitude of the oscillations of the bubble is always small compared to that in water (i.e., suppressed), because of higher viscous damping in the liver.

**Figure 5.** Rectified bubble growth in the liver-like fluid. A 1.1 MHz with (a) $P_+ = 13.4$ MPa and $P_- = -7.5$ MPa; (b) $P_+ = 74.4$ MPa and $P_- = -14.4$ MPa and (c) a 2.0 MHz with $P_+ = 101$ and $P_- = -16.7$ MPa. Blue and red solid lines indicate the bubble dynamics in water and the liver respectively.

3.3. Radiated pressure in the liver

The acoustic pressure radiated by an oscillating bubble driven at a frequency of 1.1 MHz was computed using equation (3) to understand the changes in the emitted acoustic signal with respect to a change in the incident acoustic field (histotripsy and thermal ablation waves) and the initial bubble radius.

In figure 6, the computed frequency spectra of the radiated pressure in the liver surrounding a 2, 5 and 10 μm bubble exposed to both the histotripsy (blue solid line, $P_+ = 74.4$ MPa; $P_- = 14.4$ MPa) and the thermal ablation waveform (red solid line, $P_+ = 13.4$ MPa; $P_- = -7.5$ MPa) are plotted.

According to figure 6, there are multiple harmonics present (of the form $nf_0$, $n$ is integer) as well as distinct frequencies at $f_0/n$ (sub-harmonics), $nf_0/m$ (ultra-subharmonics, $m$ is integer and $n < m$), $mf_0/p$ (ultra-harmonics, $p$ is integer and $m > p$) and a broadband component. In addition, these harmonics and the broadband component increase significantly with respect to a reduction of the initial radius. These differences in the frequency spectrum are because of the resonance frequency of a given initial bubble radius and the ratio of the resonance to the excitation frequency (i.e., different characteristic time scales for motion at a given bubble radius). A smaller bubble, for instance, has a higher resonance frequency (i.e., short time-scale) which results in more oscillations in a given period of time and eventually leads the bubble to grow faster and larger compared with a larger bubble with a lower resonance frequency. According to Neppiras [35] and Gaete-Garreton et al [36] the appearance of these harmonics as well as the broadband spectrum is generally considered an indicator of strong cavitation.

By comparing the frequency spectrum of the radiated pressure produced by the bubble dynamics under the histotripsy and the thermal ablation excitation over 100 acoustic cycles (figure 6d), it can be
observed that (a) multiple harmonics of larger amplitude as well as (b) the elevation of broadband emissions appear with the histotripsy wave excitation. This is likely to be due to the presence of a shock in the histotripsy waveform which contains higher multiple harmonics and pressure amplitudes, resulting in the higher degree of nonlinearity in the radial bubble oscillations and larger amplitude short-duration pressure spikes caused by inertial cavitation (i.e., combined effects of the nonlinear wave propagation and the presence of inertial cavitation).

Figure 6. Computed frequency spectra of the radiated pressure from (a) 2, (b) 5 and (c) 10 μm bubble exposed to a 1.1 MHz histotripsy (blue solid line, $P_+ = 74.4$ MPa; $P_- = -14.4$ MPa) and thermal ablation wave (red solid line, $P_+ = 13.4$ MPa; $P_- = -7.5$ MPa) over 100 acoustic cycles. (d) corresponds to a plot of (a) in a logarithmic scale on the y-axis. The multiple harmonics and the broadband component are most probably due to a nonlinear radial bubble oscillation (excited by the nonlinear waveform) and short-duration pressure spikes emitted by collapsing bubble respectively [37].

4. Ex vivo experiment

4.1. Experimental method
An ex vivo experiment was carried out to record acoustic emissions in order to evaluate and distinguish between the different types of cavitation activity for either a thermally or a mechanically induced lesion. A schematic diagram of the ex vivo experimental setup is shown in figure 7. The experiment was conducted in a water bath (50x30x22 cm) filled with 16.5 L of distilled water at room temperature (20 °C). The distilled water was then degassed using a water treatment system (Precision Acoustics, WTS). An oxygen meter (VWR, OX 4000H) was used to measure the gas concentration and the experiment was carried out once the concentration reached 20%.

A 1.1 MHz HIFU source (Sonic Concepts, H102) was used consisting of a focused single element transducer with an aperture size of 64 mm with a central hole of 20 mm in diameter (focal length of 63.2 mm). The transducer was driven by a function generator (Agilent, 33220A) via a RF power amplifier (ENI, 1040L). A computer with waveform generation software (Agilent, Agilent Waveform Builder) was used to design and trigger the function generator for various HIFU pulsing protocols. To measure the level of electrical power supplied to the transducer, a power meter (Sonic Concepts, 22A) was connected between the amplifier and the transducer. Ex vivo chicken liver was placed in water at a temperature of 20 °C. During the experiment, a 10 MHz 15-multielement ultrasound imaging probe
(Siemens, Sonoline Versa Plus) was in contact with the tissue sample. The HIFU focus was set to 5 mm below the surface of the liver (see figure 7).

For recording the acoustic emissions produced at the focus in the sample during the HIFU exposure, a 10 MHz single element focused transducer (Olympus, V327) was inserted in the middle of the HIFU source and aligned confocally with the HIFU focus. The focused hydrophone was then linked to an analogue signal filter (Kronh-Hite, 3945). The filter type was set to Butterworth-bandpass (2 to 15 MHz) and it was connected to a digital oscilloscope (LeCroy, HDO 6054).

### Figure 7. A schematic diagram of the *ex vivo* experimental setup used.

4.1.1. **Signal processing for Passive Cavitation Detection (PCD)**. The sampling frequency of the oscilloscope was set to 125 MHz. The raw signals (filtered through the analogue filter during the experiment) were deconvolved by the transfer function of the focused PCD transducer using a Wiener filter. The deconvolved signals were then filtered again using a 10th order Butterworth highpass filter (2 MHz cut-off) through MATLAB (MathWorks Inc., R2013a). The signals were subsequently analysed in voltage vs time plot, an amplitude vs frequency plot (using a Fast Fourier Transform) and a frequency vs time plot (spectrogram, using a Short Time Fourier Transform).

4.1.2. **Experimental protocol-1.1 MHz HIFU transducer**. In *ex vivo* experiment, two sets of HIFU exposures were used for inducing a thermally and a mechanically induced lesion. The electrical power supplied to a 1.1 MHz HIFU source was varied between 60 W ($P_+ = 13.4$ MPa; $P_- = -7.5$ MPa) and 350 W ($P_+ = 74.4$ MPa; $P_- = -14.4$ MPa) for producing a thermally and a mechanically induced lesion respectively. A continuous 5 s HIFU excitation was used for the thermal ablation exposure, whereas the duty cycle, pulse length and pulse repetition frequency for the HIFU histotripsy insonation were chosen according to Wang *et al* [27], Khokhlova *et al* [28] and Canney *et al* [29], shown in table 4.

| Duty cycle [%] | Pulse length [ms] | Pulse repetition frequency [Hz] | Number of pulses |
|---------------|------------------|-------------------------------|-----------------|
| 1             | 10               | 1                             | 50              |

Table 4. HIFU histotripsy exposure setting used during the *ex vivo* experiment. 10 ms ON time for a pulse.
4.2. Experimental results

4.2.1. 1.1 MHz histotripsy exposure for inducing a cavity. Figure 8 shows the cross sectioned cavity produced inside the *ex vivo* liver and the corresponding ultrasound B-mode images before and after insonation. In general, the shape of a lesion generated by histotripsy is a tadpole like consisting of a “head” and a “tail” [38]; however the formation of the “head” with the absence of the “tail” is observed in figure 8a. This is most probably due to the presence of the blood vessel within the “head” boundary which the fractionated tissue travelled through during the exposure. Examining the tissue morphology around the cavity, there is no evidence of thermal damage at boundary of the cavity such as blanching.

Figure 9 illustrates the corresponding acoustic emissions recorded over period of 10 ms. There are noticeable differences in the acoustic emissions before and after 4.3 ms. In the voltage vs time plot, the amplitude is fairly uniform; it however increases significantly at 4.3 ms (figure 9a). After 4.3 ms, the peak-to-peak voltage amplitude starts to fluctuate violently. The corresponding spectrogram also indicates these changes (figure 9b). The spectrogram contains horizontal stripes from 2.2 to 9.9 MHz (multiple harmonics from second to ninth) before 4.3 ms; however stronger and more noticeable stripes with higher harmonics up to 13.2 MHz (11th harmonic) are suddenly observed at 4.4 ms. These multiple harmonics are likely to be due to the combination of the nonlinear radial bubble oscillations as well as the reflection of the incident nonlinear-shocked acoustic waves from the bubbles filled with gas and vapour due to the large acoustic impedance mismatch at their surface. Because the incident acoustic field was maintained constant during the insonation, these significant changes must be related to both the level of nonlinear radial oscillations and the surface area of the bubbles (assuming the bubble does not affect the incident acoustic field). In this context, it can be conjectured that the significant appearance of a local maximum at 4.3 ms in the voltage vs time plot, as well as in the spectrogram, is indication of the boiling bubble [29]. Because the bubble acts as stronger scatterer (due to a relatively larger surface area) and oscillates with a highly nonlinear radial behaviour, the acoustic emission is, therefore, strong and contains more multiple harmonics.

On the other hand, prior to the onset of the boiling bubble at 4.3 ms, the cavitation activity produces a lower peak-to-peak voltage amplitude and a fairly uniform frequency spectrogram. In the spectrogram, the cavitation activity encompasses multiple harmonics as well as the broadband component. Similarly, these multiple harmonics are due to the combination of the reflection of the HIFU incident wave and the nonlinear radial bubble motions, whereas the broadband component is the result of the short-duration pressure spikes caused by the inertial cavitation [37]. These experimental observations are consistent with Canney et al [29].

Figure 8. Cavity produced by a 1.1 MHz HIFU transducer. Electrical power of 350 W ($P_+ = 74.4$ MPa and $P_- = -14.4$ MPa), duty cycle of 1 %, PRF of 1 Hz, 10 ms pulse length and 50 pulses were used. (a)
Cross sectioned cavity. Ultrasound B-mode images (b) before the HIFU insonation and (c) after one pulse.

![Ultrasound B-mode images](image)

**Figure 9.** Corresponding acoustic emissions produced by the first HIFU boiling histotripsy pulse in the *ex vivo* tissue. The emission was obtained for 10 ms. (a) illustrates the filtered voltage vs time plot and (b) is the corresponding spectrogram.

The Bioheat transfer (BHT) simulator [14] was used to compute the temperature rise at the focal region in order to confirm the appearance of the boiling bubble (i.e., indication of the focal temperature of 100 °C) in the PCD measurement at 4.3 ms (figure 9). The BHT equation is given by [39]

\[
\rho C \frac{\partial T}{\partial t} = k \nabla^2 T + H - wCT
\]

where \( \rho \) is the tissue density, \( C \) is the heat capacity, \( w \) is the perfusion rate, \( k \) is the thermal conductivity and \( H \) is the heat source. The heat source can be obtained from the KZK simulation. Parameters used for the temperature simulation are shown in table 5.

**Table 5.** Parameters used in the BHT simulation.

| Symbol | Definition                      | Liver [23]                  |
|--------|---------------------------------|-----------------------------|
| \( \hat{C} \) | Heat capacity                   | 3628 [J/kg·K⁻¹]            |
| \( k \)  | Thermal conductivity            | 0.57 [W/m·K⁻¹]             |
| \( w \)  | Perfusion rate                  | 0 for the *ex vivo* liver [kg/m³·s⁻¹] |
| \( T \)  | Ambient temperature             | 20 [°C]                    |

Figure 10 shows the temperature rise of the *ex vivo* liver tissue exposed to a 1.1 MHz nonlinear-shocked waves (boiling histotripsy, \( P_+ = 74.4 \) MPa and \( P_- = -14.4 \) MPa). The computed time to reach a...
boiling temperature of 100 °C is 4 ms, which is similar to the PCD measurement (figure 9, 4.3 ms) with 7.5 % of the differences between the PCD measurement and the BHT simulation.

Figure 10. Computed temperature of an ex vivo liver tissue exposed to a 1.1 MHz nonlinear-shocked waves (boiling histotripsy, $P_+ = 74.4$ MPa and $P_- = -14.4$ MPa) over 10 ms. The time to reach a boiling temperature of 100 °C is 4 ms.

4.2.2. 1.1 MHz thermal ablation exposure for inducing coagulative necrosis. Figure 11 shows the thermally ablated ex vivo liver sample and the corresponding ultrasound B-mode images. In contrast to the cavity produced with the boiling histotripsy insonation, there is a well-defined thermal lesion (i.e., coagulative necrosis) which corresponds to the ellipsoidal shape of the HIFU focal region. In addition to the coagulative necrosis, there are three pits (blue arrows) within the lesion and these are the results of inertial cavitation [40].

Figure 11. Thermally induced lesion by a 1.1 MHz HIFU transducer. Electrical power of 60 W ($P_+ = 13.4$ MPa and $P_- = -7.5$ MPa). A continuous 5 s HIFU insonation was used. (a) Cross sectioned thermal lesion with three pits (blue arrows). Ultrasound B-mode images (b) before and (c) after the exposure.

The corresponding spectrogram of the acoustic emissions obtained over 10 ms (HIFU exposure time was 5 s) is plotted in figure 12. In contrast to the emissions produced by the histotripsy exposure shown in figure 9, there is no indication of the boiling bubble in the spectrogram. This is mainly because the magnitudes of both the peak positive and negative pressures and the shape of the incident acoustic waveform (i.e., absence of a shock) were not sufficient to produce a boiling bubble within 10 ms (i.e., the time to reach a boiling temperature of 100 °C is 2.2 s as shown in figure 13). Although there is no indication of the presence of a boiling bubble, the recorded acoustic emissions suggest that cavitation may be occurring (figure 12), but that this is not due to the large bubble formed by boiling.
Furthermore, at this acoustic pressure, no cavity was observed with the same duty cycle, pulse length, pulse repetition frequency and number of pulses used in the previous histotripsy experiments in subsection 4.2.1. Khokhlova et al [28] reported that the purely mechanical damage was only observed when both boiling and shockwaves were present at the focal region.

![Figure 12](image1.png)

**Figure 12.** Corresponding spectrogram of the acoustic emissions produced by the HIFU thermal ablation exposure in the *ex vivo* tissue. The emission was obtained only for 10 ms.

![Figure 13](image2.png)

**Figure 13.** Computed temperature of an *ex vivo* liver tissue exposed to a 1.1 MHz nonlinear waves (thermal ablation, $P_+ = 13.4$ MPa and $P_- = -7.5$ MPa). The time to reach a boiling temperature of 100 °C is 2.2 s.

4.3. *Ex vivo* experimental results vs radiated pressure simulations

In this subsection, experimentally measured acoustic emissions during the histotripsy and the thermal ablation insonations are compared with the simulations conducted in subsection 3.3.

According to the *ex vivo* PCD experimental results, two important observations can be made, as follows. During the histotripsy exposure:

- More and stronger multiple harmonics appeared;
- Higher levels of broadband emissions were observed.

Despite the computational challenges in modelling the dynamics of a single spherical bubble over 100 acoustic cycles, these experimental features are consistent with the numerical results shown in figure 14.
5. In vivo experiment

In this section, an in vivo experiment on a rat liver is described to demonstrate the feasibility of the proposed cell therapy mediated by ultrasonic histotripsy.

5.1. Experimental method

A schematic diagram of the in vivo experimental setup is shown in figure 15. The experiment was conducted with a customised transducer-holder filled with degassed distilled water using a peristaltic pump (Seko, PR18). An acoustically transparent window (12 μm-thick-mylar, HiFi Industrial, PMX 980) was attached to the end of the holder (in contact with the rat). The holder coupled with a 2.0 MHz
HIFU transducer (Sonic Concepts, H106) was then linked to a manual positioner. For consistency, the HIFU focus was set to 5 mm below the surface of the liver during the experiment. The abdomen was slightly incised and then the right lobe of the liver was taken out without dissecting the hepatic portal vein (i.e., exteriorised) after anesthetisation process. Finally, the transducer-holder was placed on the surface of the liver for the experiment. After the HIFU insonation, the exposed liver was placed back into the abdomen and the suturing process was carried out. The rat was euthanized after 72 hours and morphological and histological analyses using conventional staining techniques (hematoxylin and eosin) were conducted on the liver tissue.

**Figure 15.** A schematic diagram of the *in vivo* experimental set up.

### 5.2. *In vivo* experimental protocol-2.0 MHz HIFU transducer

The electrical power supplied to a 2.0 MHz HIFU source was set to 200 W ($P_+ = 101$ MPa; $P_- = -16.7$ MPa). The duty cycle, pulse length, pulse repetition frequency, and number of pulses used in the experiment were shown in table 6.

| Duty cycle [%] | Pulse length [ms] | Pulse repetition frequency [Hz] | Number of pulses |
|---------------|-------------------|-------------------------------|-----------------|
| 1             | 10                | 1                             | 50              |

**Table 6.** HIFU histotripsy exposure setting used during the *in vivo* experiment. 10 ms ON time for a pulse.

### 5.3. Experimental results

The animal was euthanized 72 hours after the experiment for conducting the morphological and histological analyses.

#### 5.3.1. Morphological analysis

Figure 16 shows the cross sectioned cavity (filled with clotted blood as a result of the coagulation process) produced inside the liver parenchyma. The boundary of the cavity is intact, with no evidence of coagulative necrosis which means that the cavity was induced without
causing thermal damage to the cavity wall. Similarly to the *ex vivo* experimental observation (figure 8), no tadpole shaped cavity is observed (i.e., no “tail” formation).

**Figure 16.** Cavity induced inside the liver parenchyma *in vivo*. The exposed liver was cross sectioned after 72 hours of the experiment. The cavity is filled with blood clot (due to the coagulation process) and there is no evidence of thermal damage.

5.3.2. *Histological analysis.* Figure 17 shows the microscopic image of the stained right lobe exposed to a 2.0 MHz HIFU field with an electrical power of 200 W ($P_+ = 101$ MPa and $P_- = -16.7$ MPa), duty cycle of 1%, pulse length of 10 ms, pulse repetition frequency of 1 Hz and 50 pulses. There are a number of noticeable differences between the treated cavity wall and the untreated region. First, there is no sign of thermal damage around the cavity, which illustrates that the cavity was generated without causing heat damage. If thermal damage had occurred, a darker pink colour would have appeared [27]. The hepatocyte nuclei and the hepatic sinusoids are successfully located in the untreated region, whereas these are absent in the cavity as they have been destroyed by the HIFU fractionation process.

In addition, there are condensed/compressed hepatocytes (i.e., dead hepatocytes) in the cavity boundary (i.e., between the untreated region and the cavity) possibly due to the formation and expansion of the boiling bubble caused by the incident acoustic nonlinear-shocked waves.
Figure 17. Microscopic image of the stained right lobe exposed to a 2.0 MHz HIFU field with an electrical power of 200 W ($P_+ = 101$ MPa; $P_- = -16.7$ MPa), duty cycle of 1 %, pulse length of 10 ms, pulse repetition frequency of 1 Hz and 50 pulses.

6. Conclusion
The numerical studies conducted with the Gilmore-Akulichev bubble model coupled with the KZK equation confirm and demonstrate that the presence of nonlinear shocked wavefront can lead to rectified bubble growth [41] (e.g., no rectified growth observed under the thermal ablation waveform which is a slightly distorted nonlinear wavefront). Furthermore, in the numerical simulation, the rectified growth can also be seen in the liver (modelled as a highly viscous human blood-like liquid); however the magnitude of the oscillations of the bubble is always small and the time to initiate the rectification process takes longer compared to that in water, because of higher viscous damping in the liver. In the future, both mass and heat transport across the bubble wall will be added into the GA model to obtain further qualitative observations.

The acoustic emissions during the thermal ablation and the boiling histotripsy exposure can be distinguished both numerically and experimentally. According to the numerical simulations, less multiple harmonics as well as lower levels of broadband emissions were observed for the thermal ablation process. These features are consistent with the PCD ex vivo experimental measurements.

The in vivo experiment performed in this work clearly illustrates the feasibility of the proposed cell therapy mediated by boiling histotripsy. As it shows that the cavity can be generated without causing coagulative necrosis, it may act as suitable housing for cell transplantation. Although hepatocyte transplantation was not conducted in this work, this study suggests that the proposed cell therapy mediated by HIFU method can potentially form an effective and safe clinical treatment for replacing and correcting disordered hepatocytes.

Hepatocytes transplantation through a cavity (i.e., ultrasound-guided needle injection) will be carried out in the near future.

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