Controllable in vivo hyperthermia effect induced by pulsed high intensity focused ultrasound with low duty cycles

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High intensity focused ultrasound (HIFU), with its ability to accurately target and non-invasively deposit energy deeply inside the body, has drawn much attention in medical therapy for decades.1,2 HIFU can be employed in either continuous or pulsed exposures. Continuous HIFU exposures can rapidly raise the temperature higher than 70°C at the focus, permitting ablation of tumors within seconds.3,4 However, such a continuous HIFU-induced rapid temperature elevation has proven difficult to monitor and control in real-time, which could result in undesirable thermal injury to normal tissues.5 Compared to continuous HIFU, pulsed HIFU (pHIFU) with relatively low duty cycles (DCs) (<10%) can significantly reduce the heat build up in tissues through non-continuous energy deposition with lower temporal average intensity. These exposures can achieve relatively stable temperature elevation within a hyperthermia range of 39–44°C.6,7 Numerious research studies and clinical reports support the finding that ultrasound-induced hyperthermia can provide a promising and potentially much safer alternative to conventional ultrasound-based cancer therapy.1–11

Despite growing interest in utilizing pHIFU-induced hyperthermia for cancer treatment, it is also suggested that the elevated temperature at the focus should be restricted to be lower than 44°C to avoid undesirable bioeffects.5,6 However, most researchers just simply empirically select HIFU parameters for different applications. Although several numerical models have been proposed to simulate HIFU-induced temperature elevations in tissues/vessels, most numerical studies focused solely on continuous HIFU exposures and were only validated by experimental results obtained using in vitro phantoms or ex vivo tissues.12,13 There is rarely direct study performed in vivo to investigate pHIFU-induced hyperthermia responses in tissues/vessels. Especially, the dependence of hyperthermia effect on pHIFU DCs (defined as the ratio of the pulse duration to the total pulse period) has never been investigated systemically. Actually, besides peak amplitude and total treatment time, DC is another important parameter sensitive to pHIFU-induced hyperthermia effects.

The aim of the current work is to show that, with appropriate numerical modeling and thorough in vivo validations, adjusting pHIFU DCs can achieve controllable hyperthermia temperature elevations in rabbit auricular vein. Here, temperature elevations were measured using a thermocouple in the rabbit ear vein exposed to 1.17-MHz HIFU pulses at a 5300-W/cm² spatial-peak-pulse-average-intensity (I_SPPA) with varied DCs. A three-dimensional (3D) nonlinear acoustic-bioheat transfer-blood flow coupling model was applied to simulate the thermal response of tissues/vessels exposed to low DC, high I_SPPA, HIFU pulses, and then compared with measured results. The relationship between pHIFU-induced temperature change and pHIFU DCs was systematically investigated. A DC range for temperature elevated to ~39–43°C, which is sufficient to generate desirable hyperthermia bioeffects, was identified for the current system both experimentally and numerically. Next, DC thresholds corresponding to temperature elevations exceeding 44°C were investigated for varied tissue depths and inclusions based on theoretical simulations. It is expected that these results will be helpful for achieving a controllable and optimized HIFU-induced hyperthermia effect for clinic cancer treatment, while minimizing undesirable bioeffects (e.g., substantial perivascular thermal injury and significant decrease in drug release resulting from thermally induced blood vessels collapse).

Figure 1 illustrates the diagram of the experimental setup (see supplementary material I for a detailed description of the pHIFU exposure system).21 In brief, the pHIFU signals were sent from a 1.17-MHz transducer whose housing consisted of an aluminum focusing lens with a 5-cm radius of curvature. The driving electronics consisted of a waveform generator (33120A, Agilent Technologies, Palo Alto, CA) coupled to the transducer through an acoustic couplant.

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CA, USA) and a RF power amplifier (AP-400B, ENI, Rochester, NY, USA). Before the experiments, a membrane hydrophone (MHA 200A, NTR Systems, Inc., Seattle, WA, USA) was used to calibrate the spatial characteristics of the HIFU beam (~6 dB focal width of ~4 mm).

Five New Zealand white rabbits weighing 4–5 kg each were used for the acute experiments. The auricular vein is of sufficient dimension (~1–2 mm diameter) for uncomplicated thermocouple placement. All the animal preparation and anesthesia procedures were performed according to NIH guidelines and approved by the University of Washington Animal Care and Use Committee. After anesthesia, the auricular surfaces were shaved and depilated to facilitate ultrasound coupling (see supplementary material II for a detailed description of the animal anesthesia used in these studies). Before experiments, in vivo Doppler measurement (Acuson Cypress, Siemens Co., Washington, DC, USA) was performed on rabbit auricular veins to determine the blood flow velocity, whose average value was measured to be 4.25 cm/s. During the experiments, the rabbits remained anesthetized and were placed on a custom-built platform mounted to the precision 3D motion stage. The water temperature was maintained at 36–38 °C so that the baseline temperature was set at 37 °C. The rabbit ear was held on a custom-built rigid holder with a 10 × 6 cm² acoustic window such that the targeted region of the vessel could be placed reliably at the HIFU focus. Temperature measurements were acquired using an E-type thermocouple (250-μM outer diameter, Omega Engineering Inc., Stamford, CT, USA). The enlarged inset in Fig. 1 shows the positioning of the thermocouple (the black dot) in the rabbit ear vein. The change in temperature (ΔT) was determined by taking the steady-state value at 1 min of the measured temperature and subtracting it from the baseline temperature.

In the experiments, multiple vessel segments were treated along the rabbit auricular vein, and the adjacent treatment segments were separated by 1 cm. After 10-s baseline measurement without ultrasound exposure applied to each tested point, two series of experiments were performed to investigate the influence of pHIFU DC on the thermal responses in rabbit ear vein: (1) a 60-s exposure was performed at 1-Hz pulse repetition frequency (PRF), with DCs varied by changing the pulse lengths (viz., 2500, 5000, 10 000, 25 000, or 50 000 cycles, corresponding to DCs of ~0.2%, 0.4%, 0.9%, 2.1%, or 4.3%, respectively); (2) a 60-s exposure was performed at varied PRFs (viz., 1, 5, 10, 50, or 100 Hz), while the corresponding pulse lengths were also adjusted to keep constant DCs (e.g., 0.9% or 4.3%). Five replicated experiments were conducted for each measurement.

Beside in vivo experimental measurements, a 3D nonlinear acoustic-bioheat transfer-blood flow coupling model was developed by combining a Khokhlov-Zabolotskaya-Kuznetsov (KZK) equation and a bioheat transfer equation with considering parabolic blood flow, and then was used to simulate the acoustic field and heat deposition in tissues/vessels.

The KZK equation is used to describe the nonlinear ultrasound propagation in tissue, whose non-dimensional form in the rectangular coordinate is written as

\[
\frac{\partial}{\partial t} \left( \frac{\partial P}{\partial \sigma} - \frac{N}{2} \frac{\partial^2 P}{\partial t^2} - AL_{abs} P \right) = \frac{1}{4G} \left( \frac{\partial^2 P}{\partial z^2} + \frac{\partial^2 P}{\partial y^2} \right),
\]

where the normalized variables (ζ, η, σ) are related to the rectangular coordinates (x, y, z) with ζ = x/a, η = y/a, and σ = z/d, where a is the aperture radius of the transducer, d is the transducer curvature radius, z is the propagation coordinate along the beam, x and y are the transverse coordinates; τ = ω(t - z/c₀), in which ω is the angular frequency, t is the time, and c₀ is the sound speed; N = dldt, here l₃ = ρ₀c₀²/(β₀p₀) is the shock formation distance for a planar wave, ρ₀ is the density of the medium and β is the nonlinearity coefficient; G = 0.5ka²/d, where k is the wave number; A = S₀d, here S₀ is the attenuation coefficient at the fundamental frequency f₀. In the frequency domain, the sound pressure P is represented as a Fourier series expansion

\[
P = \sum_{n=-\infty}^{\infty} C_n(\zeta, \eta, \sigma)\exp(i\pi t),
\]

where \(C_n(\zeta, \eta, \sigma)\) is the complex pressure amplitude of the nth harmonic component at (ζ, η, σ). The operator \(L_{abs}\) accounts for frequency-dependent absorption properties of the medium and is solved as

\[
C_n(\zeta, \eta, \sigma + d\sigma) = C_n(\zeta, \eta, \sigma)\exp(d\sigma \cdot A \cdot L_{abs}(n)),
\]

where \(L_{abs}(n) = -n^2\) for water and \(L_{abs}(n) = -n^d\) for tissue, when the dispersion is neglected.

The temperature rise in tissues containing blood vessels is modeled by the Pennes bioheat transfer equation (BHTE). The temperature field is divided into two domains and can be expressed as

\[
\frac{\partial T}{\partial t} = \frac{K_t}{\rho_t C_t} \nabla^2 T + \frac{Q_t}{\rho_t C_t} (\text{tissue domain}),
\]
\[
\frac{\partial T}{\partial t} = \frac{K_b}{\rho_b C_b} \nabla^2 T - \frac{\rho_b C_b}{\rho_c C_t} (\bar{u} \cdot \nabla T) + \frac{\bar{Q}_v}{\rho_c C_t} \quad \text{(blood vessel domain),}
\]

where \( T \) is the temperature, \( \rho, C, \) and \( K \) are the ambient density, specific heat, and thermal conductivity, and the subscripts \( t \) and \( b \) refer to tissue and blood, respectively (\( K_t = K_b = 0.5 \, \text{W/m°C}, \, C_t = C_b = 3800 \, \text{W/m³°C} \)). \( \bar{Q}_v = \frac{1}{\rho_b(r^0)} \sum_{n=1}^{N} 4\pi n^2 |C_n|^2 \) is the heat deposition source due to the acoustic field.\(^{15} \)

In the blood vessel domain, the blood flow field is composed by a fully developed parabolic flow, whose flow velocity \( \bar{u} \) in x direction is \( \bar{u} = 2U_0[1 - (r/r_0)^2] \), where \( U_0 \) is the average velocity, \( r \) is the radial distance from the flow axis, and \( r_0 \) is the vessel radius. The flow velocity is zero in y and z direction is zero.

The 3D schematic geometry of the computational model is shown in Fig. 2. A blood vessel (1.5-mm diameter) sits in the tissue surrounded by water. The tissue thickness above the proximal vessel wall is 0.5 mm and the tissue depth under the distal vessel wall is 1.0 mm. The origin point is set at the center of HIFU transducer surface. The focal distance of the HIFU transducer is 5 cm, and the focus is located in the middle of the vessel. Since in the \textit{in vivo} experiments the tip of the thermocouple was placed within the lumen of the auricular vein against the distal vessel wall, here the distance between the measured point and the distal vessel wall is set to be 100 \( \mu \)m. The values of the physical constants used here are \( \rho_b = 1000 \, \text{kg/m}^3, \, C_b = 1486 \, \text{m/s}, \, \beta_b = 3.5, \, c_b = 0.025 \, \text{Np/m at 1 MHz}, \, \mu_b = 2 \) for water; \( \rho_t = 1050 \, \text{kg/m}^3, \, c_t = 1596 \, \text{m/s}, \, \beta_t = 6.0, \, c_t = 4.5 \, \text{Np/m at 1 MHz}, \, \mu_t = 1.2 \) for tissue; and \( \rho_t = 1057 \, \text{kg/m}^3, \, c_t = 1575 \, \text{m/s}, \, \beta_t = 3.9, \, c_b = 2.0 \, \text{Np/m at 1 MHz}, \, \mu_b = 1.4 \) for blood. All the programs were written in MATLAB (MathWorks, Natick, MA, USA).

Figure 3(a) illustrates the numerically estimated and experimentally measured temperature evolution of the temperature at the measured point against the distal wall of the rabbit auricular vein exposed to pHIFU exposures with varied DCs. “Saw-like” profiles are observed for temperature elevation curves (Fig. 3(a)) because the heat deposition resulting from HIFU pulses would dissipate gradually during the “off-time” period, which agrees with previous reports.\(^{7} \) Figure 3(b) plots the temperature change (\( \Delta T \)) after 60-s pHIFU exposures as a function of DC, which confirms the consistency between the simulated results and the measured \textit{in vivo} data. Both the numerical and experimental results demonstrate that the temperature increases with the increasing DC. Temperatures elevated to \( \sim 39–43^\circ\text{C} \) can be identified for a DC range of 2.1%–4.3%, while no significant temperature elevation is noticed for DCs less than 0.4%. Of course, the simulation results are calculated for a single point in the vein, while the thermocouple measurements report temperatures obtained along the entire length of its sensing element, which might induce small measurement errors. However, the radius of the thermocouple used here is \( \sim 125 \, \mu \)m and the calculation step size is 100 \( \mu \)m, so it is thus reasonable to accept the reliability of the comparison results. Meanwhile, since the DC could be adjusted by changing either pulse length or PRF, Fig. 4 illustrates the experimentally measured and numerically estimated

\[ \Delta T \]
temperature changes after 60-s pHIFU exposures with varied PRF/pulse length. It is noteworthy that, as long as the DCs keep constant, the variation of PRF/pulse length does not make significant differences to thermal responses in the vein. A possible explanation might lie in the low DCs. At such low DCs (≤4.3%), the sufficient “off-time” would limit the sharp increase of temperature resulting from specific violent ultrasound-mediated activities, and the heat accumulated in tissues/vessels might be only dominated by the fully delivered acoustic energy, which is determined by the exposure “on-time” and ISPPA. In other words, under pHIFU treatments with relatively low DCs and fixed ISPPA, the temperature elevations could be controlled by adjusting DCs.

The observed consistency between the numerical simulations and our in vivo experimental measurements suggests that the appropriate 3D model simulation might be used to guide pHIFU-induced hyperthermia treatment. One important point to consider regarding the potential clinical applications of pHIFU cancer therapy is the determination of the DC range suitable for effective and safe hyperthermia treatment. Previous studies have demonstrated that 39–43°C should be regarded as an effective hyperthermia range to generate desired treatment effects.5–11 The temperature higher than 44°C is reported as a cytotoxic temperature which can make cells undergo coagulative necrosis, while essentially no significant cell death would occur below 41°C even with 1-h exposures.6,16–19 It is understandable that, due to different cell and vessel types as well as particular experimental situations, the lower temperature limit for in vitro cell toxicity and in vivo vascular damage reported in previous literatures might vary between 42 and 44°C. Here, a temperature limit of 44°C is targeted to investigate the approximate DC range for the safe hyperthermia therapy. According to numerical simulations, a DC threshold of 6.9% can be identified for the temperature exceeding 44°C at the distal side of the vein.

However, in the clinical setting, the thermal responses of the biological structures exposed to ultrasound waves

FIG. 5. The temperature elevations in a 3-mm thick tissue embedded with a blood vessel (1.5-mm diameter) sonicated with pHIFU. The profile of temperature enhancement in the (a) central transverse section (Y-Z plane, x = 0) and (b) central longitudinal section (X-Z plane, y = 0) after 60-s exposure at 1-Hz PRF and 4.3% DC. The horizontal solid and dash lines represent the boundaries of tissue and vein, respectively. The HIFU focus exactly located at the center of the vein (viz., x = 0 mm, z = 50.25 mm). The DC-dependence (c) and PRF-dependence (d) of the temperature elevations at several points along the central axis of the HIFU transducer (vertical dashed dotted line in A and B) are numerically studied. The positions of points a, b, c, d, e, and f sit at z = 49.0, 49.5, 50.2, 50.9, 51.5, and 52 mm, respectively. Point d denotes the position of the thermocouple.
would be affected by the varying depths and different inclusions in the structures. Figures 5(a) and 5(b) illustrate sample profiles of temperature changes in the central transverse (viz., \( x = 0 \)) and longitudinal sections (viz., \( y = 0 \)) of the structure sonicated with 60-s pHIFU at a DC of 4.3% (viz., 50,000 cycles, 1-Hz PRF). Furthermore, Figs. 5(c) and 5(d) demonstrate the DC- and PRF-dependence of temperature elevations at several points (viz., a, b, c, d, e, and f) along the central axis of the transducer with the increasing depth. Temperature elevations are observed in both the tissue and the vein. As shown in Fig. 5(c), for DC ≤ 6.9%, the temperatures in tissue/vein nearly linearly increase with the increasing DC at any depths, while no PRF-dependence is observed at a certain DC (Fig. 5(d)). However, the temperature in the vein (e.g., points b, c, and d) increases more slowly than that in the tissue (e.g., points e and f) due to the lower attenuation coefficient in the vein, which demonstrates an obvious “cooling” effect of blood flow. Due to the parabolic flow setting inside the vein, the lowest temperature rise is observed along the central line of the vein (point c). Since the tissue thickness above the vein (0.5 mm) is thinner than that under the vein (1.0 mm), the heat deposited in the above tissue (point a) dissipates more easily into the surrounding water, resulting in higher temperature elevations achieved in the tissue under the vein (points e and f). The temperature elevation at point e (\( z = 51.5 \) mm) is higher than that at point f (\( z = 52.0 \) mm) because point e is closer to the HIFU focus (\( z = 50.25 \) mm).

Although it has been noted above that the theoretically estimated DC threshold for the temperature at the distal vessel wall exceeding 44 °C is 6.9%, the DC thresholds will in fact vary at different positions. For instance, the estimated DC threshold for temperature higher than 44 °C at the point e is only ~4.3%. Meanwhile, at a DC = 4.3%, the temperature of the measured point d at the distal vessel wall (\( z = 50.9 \) mm) has already been raised to ~41 °C. Thus, a DC range between 2.1% and 4.3% might be more appropriate for the current system to achieve effective hyperthermia effects, while avoiding significant thermal damage to surrounding tissues.

In summary, numerical simulations based on a 3D nonlinear acoustic-bioheat transfer-blood flow coupling model and in vivo thermocouple measurements were performed to investigate thermal responses in rabbit auricular veins exposed to pHIFU with varied DCs. The results demonstrate that, at a relatively low DC (e.g., DC ≤ 6.9%), the temperature of the tissue/vein exposed to pHIFU with a given \( I_{pPA} \) will increase with the increasing DC, while hyperthermia effects will not be significantly affected by the change of PRF as long as the DC keeps constant. More importantly, the consistency between the simulations and measurements indicates that, by adjusting pHIFU DCs, it is possible to achieve controllable hyperthermia effects while minimizing perivascular thermal damage. A critical DC of 6.9% was theoretically estimated for the temperature at the distal side of the vein to exceed 44 °C. However, the DC-dependence of temperature elevations would be affected by different depths and inclusions in the tissue. Therefore, to ensure safer patient treatment, a DC range between 2.1% and 4.3% might be more appropriate for the current system to achieve desired hyperthermia effects.

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