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Pilot *ex vivo* study on non-thermal ablation of human prostate adenocarcinoma tissue using boiling histotripsy

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ABSTRACT

Focused ultrasound technologies are of growing interest for noninvasive ablation of localized prostate cancer (PCa). Here we present the results of the first case study evaluating the feasibility of non-thermal mechanical ablation of human prostate adenocarcinoma tissue using the boiling histotripsy (BH) method on *ex vivo* tissue. High intensity focused ultrasound field was generated using a 1.5-MHz custom-made transducer with nominal F#=0.75. A sonication protocol of 734 W acoustic power, 10-ms long BH-pulses, 30 pulses per focal spot, 1 % duty cycle, and 1 mm distance between single foci was tested in an *ex vivo* human prostate tissue sample containing PCa. The protocol used here has been successfully applied in the previous BH studies for mechanical disintegration of *ex vivo* prostatic human tissue with benign hyperplasia. BH treatment was monitored using B-mode ultrasound. Post-treatment histologic analysis demonstrated BH produced liquefaction of the targeted tissue volume. BH treated benign prostate parenchyma and PCa had similar tissue fractionation into subcellular fragments. The results of the study demonstrated that PCa tumor tissue can be mechanically ablated using the BH method. Further studies will aim on optimizing protocol parameters to accelerate treatment while maintaining complete destruction of the targeted tissue volume into subcellular debris.

1. Introduction

High-intensity focused ultrasound (HIFU) is an established technology for noninvasive thermal ablation of localized prostate cancer (PCa) [1–4]. This approach serves as an alternative to traditional first line treatments such as radical prostatectomy, brachytherapy, and external beam radiation, which have significant morbidity and may decrease the quality of life after the procedure [5,6].

Recently, a developmental HIFU technique, termed histotripsy, has been proposed to mechanically liquefy tissue with the aim to improve HIFU treatments [7-11]. There are two major types of histotripsy:

cavitation cloud histotripsy (CH) and boiling histotripsy (BH), both of which result in mechanical disintegration of tissue into subcellular fragments with negligible thermal damage [12,13]. Both histotripsy techniques are based on pulse-periodic treatment protocols that use microsecond-long (CH) or millisecond-long (BH) bursts of nonlinear ultrasonic waves with shocks delivered at low duty cycle (DC) of about 1 %. The CH protocol relies on the shock-scattering mechanism to generate a dense destructive bubble cloud in the focal region [14]. The BH protocol is initiated by localized boiling due to shock-wave heating and formation of a millimeter-sized vapor cavities at the focus [15]. The remaining incident shocks interact with these cavities causing formation

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of bubble clouds in front of the cavity and tissue microfountaining directed inside the cavity with subsequent tissue atomization [8,16].

Compared to conventional thermal HIFU, histotripsy may have certain clinical advantages. First, its non-thermal mechanism minimizes heat-diffusion and heat-sinking effects that limit the precision of thermal ablation [17,18]. Second, due to formation of bubbles at the focus during histotripsy, hyperechoic regions appear on B-mode ultrasound images allowing for precise real-time treatment monitoring as opposed to thermal HIFU exposure with limited B-mode feedback. Additionally, the lack of tissue scatters in the liquefied BH lesion results in appearance of hypoechoic regions of successful treatment. Third, the liquefied content of the lesion can potentially be evacuated through the orifices of the prostatic ducts into the prostatic portion of the urethra thus shortening a post-surgery recovery.

Initial attempts of performing prostatic tissue destruction with histotripsy were via a transabdominal approach using CH in an *in vivo* canine model [19,20]. CH treatment produced reliable prostate debulking and was well tolerated in canines, could successfully spare the urethra, and ablate implanted ACE-1 canine prostate cancer tumors [21–23]. Ensuing phase 1 clinical trials evaluating CH for treatment of benign prostatic hyperplasia (BPH) were performed through a transperineal acoustic window in a group of 25 men. Despite a significant subjective improvement of lower urinary tract symptoms for up to 6 months after treatment, there were no objective improvements in prostate volume, flow rate, or post-void residual suggesting failure to effectively ablate human prostate tissue with this approach [24].

Histotripsy mechanical ablation of human prostate is indeed difficult because, unlike the canine prostate, in which glandular elements predominate, human prostate tissue tends to have significant fibromuscular stroma with high collagen content, particularly in men with BPH making it mechanically tough. Additionally, in previous transperineal clinical trials, the angle of HIFU beam focusing was relatively small due to the limited transperineal window and depth of the targeted region in tissue. This could diminish the amplitude of shocks at the focus and thus the efficiency of administering histotripsy to the prostate [25,26]. To overcome these challenges, our group has proposed to use the BH regime for prostate tissue ablation [27,28]. As opposed to CH histotripsy, in which bubble cloud initiation is based on high peak negative pressures generated by scattering of high-amplitude shocks from cavitation bubbles, the appearance of BH-induced vapor cavity relies on the shockwave heating, which requires lower shock amplitude, lower focusing angle, and peak power requirements [12]. These lower requirements allows minimizing transducer size and using the transrectal acoustic window [29,30].

In prior initial studies, BH ablation in fresh human prostate autopsy tissue has been successfully realized [27], and subsequently the treatment protocols have been modified to provide repeatable destruction of 24 human prostate tissue samples with various elastic properties [28]. The goal of this study was to demonstrate the feasibility of ablating *ex vivo* human PCa with BH using this previously tested exposure protocol [28]. PCa tumors are known to have higher stiffness than normal prostate. Therefore, they may potentially be more resistance to BH treatments. Thus, successful mechanical ablation of *ex* vivo human PCa would serve as a proof of principal towards future translation of this technology for clinical use.

2. Materials and methods

2.1. BH field characterization

The BH transducer was a custom-built spherically focused sector array with D = 80 mm nominal aperture, O = 20 mm aperture of the central opening, F = 60 mm focal length, and $f_0 = 1.5$ MHz operating frequency. The transducer was fabricated using 12 flat piezoelectric elements; the focusing was achieved for each element using 3D-printed plastic lens [31]. All transducer elements were powered in-phase by a

custom-build class D amplifier [32]. Acoustic characterization of the transducer was performed to predict nonlinear pressure *in situ* for various amplifier operating voltages U_0 and to determine acoustic power and shock-wave field parameters *in situ* for the sonication regime used for the treatment. A combined measurement and modeling approach that has been validated in our previous studies was employed [26].

Following this approach, linear holography measurements were performed first to reconstruct the vibrational pattern at the transducer surface (Fig. 1) and spatial structure of the field it generates [33]. A hologram was measured in an $85 \times 85 \text{ mm}^2$ planar region perpendicular to the beam axis and located prior to the focus at $z_h = 45 \text{ mm}$ (Fig. 1). The pressure measurements were performed in a tank filled with degassed water (Precision Acoustics Water Treatment System, Dorset, UK), utilizing a calibrated needle hydrophone (HNA-0400 with AH-2020-025 preamplifier, Onda Corporation, Sunnyvale, CA). The hydrophone sensitivity at 1.5 MHz was 1 V/MPa, and its location was controlled using a 3-D positioner system (Precision Acoustics UMS3, Dorset, UK) with resolution < 5 μ m. The scan comprised a 171 \times 171 points grid with a step size of 0.5 mm. The measured hologram was backpropagated to the spherical surface of the array using the Rayleigh integral to determine source acoustic velocity pattern. Similarly, axial field distribution and transverse distributions in the focal plane were reconstructed.

Second, holography data were used to set a boundary condition to the field modeling in a form of an equivalent single-element circular source with geometric parameters similar to those of the real transducer but having a uniform distribution of the normal component of the vibrational velocity over its spherical surface [15,25,26]. The parameters of the equivalent source were determined by matching the solution for the axial distribution of the pressure amplitude in the focal region (within -6 dB level) for the equivalent source to the distribution reconstructed for the array transducer from the holography measurements. As demonstrated in previous studies, the nonlinear field at the focal region of an idealized equivalent source closely agrees with that of a realistic focused transducer given their focal lobe shapes, where acoustic pressure is the highest, are matched. Nonlinear effects are predominantly accumulated in regions of high amplitude, i.e. within the focal region, which further supports the match between the two fields [15,25,26]. The following analytic result for the axial pressure distribution in a field generated by an annual uniformly vibrating source was employed [34]:



Fig. 1. Vibrational pattern of the transducer surface reconstructed from holography measurements performed in the plane *S*. Dashed white contours depict the edges of the equivalent single-element source with uniform vibrational velocity distribution.

$$P_{\rm eq}(z) = \frac{P_0^{\rm eq}}{|1 - z/F_{\rm eq}|} \left(\exp\left[ikR_{\rm max}(z, F_{\rm eq}, O_{\rm eq})\right] - \exp\left[ikR_{\rm max}(z, F_{\rm eq}, D_{\rm eq})\right] \right)$$
(1)

Here $P_0^{\text{eq}} = \rho_0 c_0 V_0^{\text{eq}}$ is the characteristic pressure amplitude at the source surface and V_0^{eq} is the vibrational velocity amplitude, *z* is the axial coordinate, F_{eq} is the focal distance, D_{eq} is the source external diameter, O_{eq} is the diameter of the central opening,

$$R_{\rm max} = F_{\rm eq} \sqrt{1 + (1 - z/F_{\rm eq})^2 - 2(1 - z/F_{\rm eq})} \sqrt{1 - O_{\rm eq}^2/4F_{\rm eq}^2}$$
(2)

Corresponding inner and outer edges of the source are depicted in Fig. 1 by dashed contours.

Once parameters of the equivalent source are defined, an analytic solution for the acoustic pressure amplitude along the radial coordinate in the focal plane z = F can be obtained as follows [34]:

$$P_{\rm eq}(r) = k P_0^{\rm eq} \left[\Pi \left(D_{\rm eq} \right) - \Pi \left(O_{\rm eq} \right) \right], \tag{3}$$

where
$$\Pi(D_{\rm eq}) = 4F^2 \left(1 - \sqrt{1 - \left(D_{\rm eq}/2F\right)^2}\right) J_1(kD_{\rm eq}r/2F)/kD_{\rm eq}r, J_1$$

is the first-order Bessel function, r is the radial coordinate. The solution (3) will be used for comparing the field pattern in the focal plane generated by the equivalent source and reconstructed from the holography measurements.

Finally, axially-symmetric nonlinear field simulations were performed at increasing voltages using a freely available open-source HIFUbeam software (<u>https://limu.msu.ru/</u>) based on solving the one-way propagation Westervelt equation [35]:

$$\frac{\partial^2 p}{\partial \tau \partial z} = \frac{c_0}{2} \left(\frac{\partial^2 p}{\partial z^2} + \frac{\partial^2 p}{\partial r^2} + \frac{1}{r} \frac{\partial p}{\partial r} \right) + \frac{\beta}{2\rho_0 c_0^3} \frac{\partial^2 p^2}{\partial \tau^2} + \frac{\delta}{2c_0^3} \frac{\partial^3 p}{\partial \tau^3} + L_a(p) \tag{4}$$

Here $p(r, z, \tau)$ is the acoustic pressure, $\tau = t - z/c_0$ is the retarded time, β and δ are nonlinearity coefficient and thermoviscous diffusivity of sound in the propagation medium, respectively. The linear operator $L_a(p)$ accounts for a power law of absorption $\alpha(f) = \alpha_0 (f/f_0)^\eta$, where α_0 is the absorption coefficient at the frequency f_0 , and the exponent parameter η is typically close to unity for biological tissues [36].

Corresponding to the experimental arrangements, simulations were performed in a "water-prostate" flat-layered medium with the focus located 1 cm deep in the prostate tissue sample. The following acoustic parameters were set for the two layers: coefficient of nonlinearity $\beta_w = 3.5$, sound speed $c_0^w = 1490$ m/s, density $\rho_0^w = 997$ kg/m³; $\beta_p = 4.8$, $c_0^p = 1559.5$ m/s, $\rho_0^p = 1045$ kg/m³, absorption coefficient $\alpha_0 = 0.14$ Np/ cm@1.5 MHz and power law $\eta = 1.1$ for prostate [36,37]. The diffusivity coefficient was chosen as $\delta = 4.33 \cdot 10^{-6}$ m²/s for both water and prostate.

Simulations were performed at increasing amplifier output voltages over a wide range of 0.6–270 V with steps from 1 V to 30 V to capture both linear and strongly nonlinear sonication regimes. Experiments were performed at the voltage of 240 V. It was assumed that the output voltage of the amplifier is proportional to the initial characteristic pressure at the surface of the equivalent source. The spatial grid for the Westervelt modeling was $\Delta z = \Delta r = 25 \ \mu m$, the time step was $\Delta t = 0.5 \ ns$, and up to 1000 harmonics were used in the simulation depending on the level of nonlinear distortion of the waveform.

2.2. Autopsy tissue samples

De-identified human prostate tissue was obtained via rapid autopsy (19 h after death, IRB exempt). The anonymous subject had lifetime diagnoses of benign prostatic hyperplasia (BPH) and PCa. The prostate (Fig. 2A) was dissected to obtain two peripheral zone samples (Fig. 2B).

One sample was placed into cold (7 $^\circ$ C) degassed phosphate buffered saline solution (PBS) and additionally degassed in a desiccant chamber



Fig. 2. A photograph of an autopsy tissue sample of a human prostate tissue prepared for dissection (A) and the dissected sample before BH exposure (B).

for 1.5 h with residual pressure < 0.1 bar. After degassing, the sample was embedded into an agarose gel (Fig. 3A) which was prepared by mixing agarose powder (UltraPure Agarose, Invitrogen) with degassed distilled water (1.5 % wt./vol. agarose/water). Another control sample was fixed in 10 % neutral buffered formalin immediately after dissection without BH exposure.

2.3. BH setup and exposure protocol

For BH exposures, the agarose embedded sample was transferred into a 3D-printed sample holder attached to the positioning system and immersed in the tank filled with degassed water (Fig. 3A). The treatment was performed by delivering BH exposures to the nodes of a rectangular grid in a plane transverse to the axis *z* of the array at a depth of 10 mm in the tissue sample. The grid contained 4×4 points of foci with 1 mm spacing. Our prior study in benign autopsy prostate tissue reported 1 – 1.5 mm size of a single focal lesion in each dimension [28], so the expected size of the volumetric lesion was 4–4.5 mm in *x*- and *y*- directions and 1–1.5 mm in *z*-direction (Fig. 3A). Sonication was guided by B-mode ultrasound using two linear imaging probes (ATL, Philips, Bothell, WA, USA): one, L7-4, was positioned at the left side of the sample, another, P7-4, was placed in the central opening of the source (Fig. 3A). The use



Fig. 3. Experimental arrangement for ultrasound image-guided boiling histotripsy treatment of *ex vivo* cancerous prostate tissue (A). Diagram illustrating pressure waveforms at the focus in the BH pulsing protocol (B).

of the central imaging probe mimicked potential clinical realization of the BH exposures, while imaging at a side provided a closer view of the focus in the laboratory settings. The probes were driven by Verasonics V1 Ultrasound Engine (Kirkland, WA, USA), and B-mode images were used both for real-time treatment monitoring and evaluating the outcome.

The BH treatment pulsing protocol tested and validated in our previous study was implemented [28]: for a single position of the focus, 10ms BH pulses were insonicated at low duty cycle of 1 % (1 s delay between the pulses) and a BH dose of 30 pulses/focal spot was delivered with an output voltage of the amplifier $U_0 = 240$ V (Fig. 3B). After treating one focal spot, the positioning system moved the sample to the next point of the sonication grid until the entire 4 × 4 grid was covered.

2.4. Histology

The treated sample was removed from the agarose gel, and the side facing the BH transducer was marked with ink (HistoSafe, Russia) to maintain orientation. The marked sample was fixed in 10 % neutral buffered formalin for one week. The tissue was then cut into 2 mm thick blocks parallel to the sonication direction, processed and paraffin embedded for histological analysis. The control sample was prepared in the same way as the treated sample. Tissue sections (3 μ m thick) were stained with hematoxylin and eosin (H&E) and Masson's trichrome. Tissue sections from the treated sample were taken from the center of the treated region and at the edge of the treatment region. Slides were evaluated by a certified pathologist (initials N.V.D.) to both confirm the clinical diagnosis and to evaluate the BH lesions in the treated sample.

3. Results

3.1. Equivalent source and linear acoustic field parameters

Fitting the axial pressure distributions of the array transducer and the equivalent source performed within -6 dB level of the focal lobe yielded the following parameters of the single-element equivalent ringshaped source: 56 mm focal length, 66.4 mm external diameter, 22 mm diameter of the central opening, and 0.3 V/kPa relationship between the source voltage and characteristic pressure at the source surface. The outer and inner diameters of the equivalent source (dashed contours in Fig. 1) are smaller and larger, respectively, than the nominal transducer parameters (D = 80 mm, O = 20 mm), which reflects the presence of non-vibrating edges of the transducer. This feature can also be observed in the distribution of the source normal velocity amplitude reconstructed at the transducer surface as illustrated in Fig. 1. The pattern of 12 sector elements is clearly visible in the distribution, the nonuniform behavior of each element is evident, and the inner and outer edges of the source have low vibrational amplitude. The equivalent source model accounts for these features by reducing the active surface of the source and averaging the pressure amplitude over the source surface.

Fig. 4A shows axial distributions of the pressure amplitude, reconstructed from the holography measurements (solid curve), and (dashed curve) calculated for the equivalent source boundary condition using the analytical solution Eq. (1). A good agreement of the pressure amplitude distributions between the holography data for the array transducer and simulations for equivalent source is achieved within the focal lobe defined at the –6 dB level and in the radial pressure amplitude distributions in the focal plane. These distributions are depicted in (Fig. 4B) along the *x* and *y* axes at z = F: thick and solid curves represent the field of the array transducer, obtained from holography measurements, and the dashed curve corresponds to the analytic solution (3) for the radial field of the axially-symmetric equivalent source.

3.2. Nonlinear field parameters in situ

The Westervelt equation with an equivalent source boundary con-



Fig. 4. Distributions of the pressure amplitude in water obtained from lowoutput acoustic holography ("holo *z*") measurements for the array transducer used in experiments and from the analytical solutions for the equivalent ("eqv.") source: (A) on the beam axis *z* and (B) along the \times and *y* transverse axes in the focal plane z = F.

dition was solved in a "water-prostate" layered medium using HIFUbeam software at increasing voltages applied to the transducer. Shown in Fig. 5A are the results for the peak positive and negative pressures (p_+ and p_-) and shock amplitude A_s in the focal waveform, z = F, when focusing at a depth of 10 mm within the prostate tissue *versus* transducer voltage U_0 . Starting from the output level of approximately 130 V, the shock front in the waveform first appears near the positive pressure peak and grows in amplitude toward the zero pressure level. The shock is termed "developed" once its bottom edge reaches zero pressure, therefore $A_s = p_+$ as shown by a circular marker in Fig. 5A [26]. One cycle of the corresponding pressure waveform at the focus is shown in Fig. 5B (dashed curve). In this regime, the shock amplitude A_s normalized to the characteristic source pressure P_0 reaches the maximum value ($A_s/P_0 =$ max) [26].

For experimental arrangements of the current study, the fully developed shock is formed at the $U_0^{\text{dev}} = 177$ V voltage level with peak pressures (p_+ and p_-) of 115 MPa and –18 MPa, respectively, and the shock amplitude is therefore $A_s = p_+ = 115$ MPa. Though these pressure levels would potentially be sufficient for BH, the previous experiments and this study were performed at higher voltage of $U_0^{\text{BH}} = 240$ V to ensure successful tissue fragmentation [28]. The treatment regime is depicted in Fig. 5A with "square" markers, and the focal waveform in this regime is shown in Fig. 5B (solid line). The simulation predicted the following focal pressure levels: peak pressures $p_+ = 126$ MPa, $p_- = -20$



Fig. 5. (A) Simulation results for the peak positive and negative pressures (p_{\pm} - solid lines) and shock amplitude (A_{s} - dashed line) at the focus located at 1 cm depth in tissue at increasing amplifier output voltages. The "circle" marker indicates the acoustic parameters for the developed shock regime, and the "square" marker corresponds to the regime used in the experiment. (B) Two cycles of the focal waveform for the developed shock (dashed line) and sonication (solid line) regimes.

MPa and shock amplitude $A_s = 139$ MPa. It corresponded to an acoustic power of 734 W and an acoustic intensity of 21 W/cm² at the surface of the equivalent source. As shown in our previous studies, acoustic power of the equivalent source is a good estimate of that of the real source, given their geometric parameters and focal field structures are similar [26].

3.3. BH exposure

Ultrasound guided BH treatment was divided into three stages as shown in Fig. 6A–C, which provides examples of the representative Bmode images obtained with the P7-4 probe during and 15 min after the treatment (A, B) and with the L7-4 probe when the sample was taken out of the tank after the treatment (C).

The prostate sample was seen in the B-mode images surrounded by hypoechoic agarose gel. The image in Fig. 6A) was taken immediately after the first BH pulse (the sonication direction is indicated by the arrow) was delivered to the first point of the 4 \times 4 sonication grid. A bright hyperechoic locus (marked by the circle) appeared in the focal region and corresponded to the millimeter-sized vapor bubbles induced by the BH pulse. This locus provided feedback to indicate treatment initiation and identifies the position of the active treatment focus.

The post-treatment image was taken 15 min after the BH-sonication had been completed. Evidence of liquefaction is indicated by the hypoechoic region of tissue, suggesting successful homogenization of the lesion content (Fig. 6B). A higher resolution image of the lesion was also acquired using the L7-4 probe after removing the sample from the water tank (Fig. 6C).

The exposure outcome was also visualized by the macroscopic evaluation of the Masson's stained histological section after the treatment: the homogenized region is clearly visible in Fig. 6D within the rectangular contour, and four dots indicate the focus positions in the layer. The lesion dimensions and shape closely correspond to those observed in histological evaluation.

3.4. Control sample histology

Histologic evaluation of the control sample by certified pathologists (P.G. Malkov, N.V. Danilova) revealed high-grade prostatic adenocarcinoma with a Gleason score of 5 + 4 = 9 (ISUP/WHO grade group 5) [38] (Fig. 7). The sample contained large tumor nests with uniformly distributed nuclei and cords of cells. The tissue had only a few recognizable glands that were enlarged, and there was evidence of hyperplasia and fibromuscular stromal proliferation consistent with BPH.

3.5. BH exposed tissue histology

PCa with a Gleason score of 5 + 4 = 9 (ISUP/WHO grade group 5) was seen in untreated tissue surrounding the lesion, which confirmed the presence of tumor within the BH treated sample (Fig. 8 B and C). Masson's trichrome stained sections indicate a high stromal component with glandular and fibromuscular tissue, consistent with BPH, throughout the sample (Fig. 8A–C).

Histologic section taken from the center of the volumetric lesion (Fig. 8A, D) captured the whole cross-section of the ablation. The dimensions were measured to be approximately 1.5 mm along the sonication direction and 4.5 mm transversely; these values are consistent with those previously reported [28]. The demarcation between completely ablated tissue (Fig. 8D) and intact tissue measures between 200 and 500 μ m in thickness (Fig. 8F). This region contains 50–200 μ m fragments of intact collagen fibrils and smooth muscle (Fig. 8F).

Examination of the lesion content at higher magnification (Fig. 8E) revealed homogenized cell debris with some nuclear fragments and intact fibrillar collagen fragments (Fig. 8E). A section taken from the edge of the lesion (Fig. 9) clearly demonstrates PCa tumor ablation. PCa cells were observed adjacent to completely homogenized tissue, separated by a 500 μ m thick margin of partially ablated tissue (Fig. 9C and F). These results demonstrate the ability of the BH method to ablate PCa tissue.

4. Discussion and conclusion

In this work, BH ablation of human PCa was successfully performed



Fig. 6. Representative B-mode ultrasound images during (A), and after (B), (C) BH treatment of the cancerous prostate sample. Images in (A - B) were acquired with P7-4 probe inside the water tank, while (C) was acquired outside with L7-4 for better image quality. The arrows indicate the direction of the BH exposure. The red contour shows a bright hyperechoic region at the focus, which appeared during sonication. (D) Masson's trichrome stained histological section of the sample after treatment. The rectangular contour shows the exposure region, red dots depict the positions of the focus.



Fig. 7. Micrograph of the control sample with prostate adenocarcinoma Gleasson Grading 10 (5 + 5). Autopsy material, <24 h after death, before BH exposure. Paraffin sections (A), (B) – H&E staining, (C), (D) – Masson's trichrome staining.



Fig. 8. Prostate adenocarcinoma Gleasson Grading 10 (5 + 5). Autopsy material, <24 h after death, after BH exposure. Masson's trichrome stained paraffin section: A – histotopogram; (B), (C) – tumor (arrowhead marker); (D) – BH lesion with a demarcation line; (E) – lesion content with intact fibrillar collagen fragments ("*" markers); (F) – line of demarcation.

in *ex vivo* tissue for the first time. BH produced the expected fractionation of targeted malignant and benign prostate tissue with loss of cellular and extracellular structures within the ablated volume. In line with previous experiments, tough benign collagenous and fibromuscular stromal components were more resistant to BH than other prostatic tissue components, particularly at the lesion margin [28]. The dimensions of the BH ablated volume were consistent with those predicted before treatment. This data demonstrates proof of principal and supports further development of BH as a clinical treatment for human PCa (and BPH).

Prior studies have demonstrated the feasibility of treating PCa with CH in an orthotopic canine PCa model [23]. Similarly, CH underwent extensive pre-clinical testing in pre-clinical BPH models and progressed to a phase 1 clinical trial in which the Vortx ® Device (Histosonics, Ann Arbor, MI USA) was used to treat men with BPH. However, in human BPH patients no objective evidence of successful tissue ablation was achieved and the device was abandoned. As a result, there are no descriptions of successful histotripsy ablation of human BPH or PCa using

CH. Consequently, this work represents the first histologic description of successful application of historipsy to human PCa tissue.

Based on these encouraging results in human PCa tissue and our work in human BPH tissue [28], our team is actively engaged in translational research to develop BH as a clinical treatment for BPH and PCa. Our work has identified two potential benefits of BH over CH that may facilitate it being a successful clinical application of histotripsy technology for prostate diseases where CH did not succeed. The first, is that due to differences in mechanism of action BH is more amenable to transducer miniaturization compared to CH [13]. As a result, we have hypothesized that a transrectal approach for BH prostate applications is possible taking advantage of a more favorable acoustic window vs. the transperineal approach used by the Vortx ® device. Accordingly, our team has successfully designed and built a preclinical transrectal BH system [29,30]. The second potential advantage of BH over CH is differences in how human prostate tissue responds to different histotripsy pulse regimes. Using a transducer with the same frequency and similar geometry to that of the Vortx ® device to administer the same number of



Fig. 9. Prostate adenocarcinoma Gleasson Grading 10 (5 + 5). Autopsy material, <24 h after death, after BH exposure. Paraffin sections, ablation region (dashed contour) with a demarcation line, (A)–(C) – H&E staining, (D)–(F) – Masson's trichrome staining. Arrowheads mark the tumor.

CH pulses (of same microseconds duration, pulse repetition frequency, and similar amplitude) as used in the phase 1 study [24] does not produce significant mechanical ablation in *ex vivo* human prostate tissue or tough prostate mimicking hydrogels [39,40]. Conversely, using longer pulses, particularly BH pulses (10 ms duration), have been found to be the most efficient and effective at completely ablating human prostate tissue [41] of the histotripsy pulse regimes that have been tested by our group.

Encouraged by the results of this study demonstrating the feasibility of BH ablation of human PCa (and other works), future studies will aim to move BH towards a clinical treatment for prostate cancer building on the results of our preclinical transrectal BH system [42].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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