INITIAL ASSESSMENT OF BOILING HISTOTRIPSY FOR MECHANICAL ABLATION OF EX VIVO HUMAN PROSTATE TISSUE

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Abstract—Boiling histotripsy (BH) is a focused ultrasound technology that uses millisecond-long pulses with shock fronts to induce mechanical tissue ablation. The pulsing scheme and mechanisms of BH differ from those of cavitation cloud histotripsy, which was previously developed for benign prostatic hyperplasia. The goal of the work described here was to evaluate the feasibility of using BH to ablate fresh ex vivo human prostate tissue as a proof of principle for developing BH for prostate applications. Fresh human prostate samples (N = 24) were obtained via rapid autopsy (<24 h after death, institutional review board exempt). Samples were analyzed using shear wave elastography to ensure that mechanical properties of autopsy tissue were clinically representative. Samples were exposed to BH using 10- or 1-ms pulses with 1% duty cycle under real-time B-mode and Doppler imaging. Volumetric lesions were created by sonicating 1/4 rectangular planes spaced 1 mm apart, containing a grid of foci spaced 1/2 mm apart. Tissue then was evaluated grossly and histologically, and the lesion content was analyzed using transmission electron microscopy and scanning electron microscopy. Observed shear wave elastography characterization of ex vivo prostate tissue (37.9 ± 22.2 kPa) was within the typical range observed clinically. During BH, hyperechoic regions were visualized at the focus on B-mode, and BH-induced bubbles were also detected using power Doppler. As treatment progressed, hypoechoic regions of tissue appeared, suggesting successful tissue fractionation. BH treatment was twofold faster using shorter pulses (1 ms vs. 10 ms). Histological analysis revealed lesions containing completely homogenized cell debris, consistent with histotripsy-induced mechanical ablation. It was therefore determined that BH is feasible in fresh ex vivo human prostate tissue producing desired mechanical ablation. The study supports further work aimed at translating BH technology as a clinical option for prostate ablation. (E-mail: verak2@uw.edu) © 2022 World Federation for Ultrasound in Medicine & Biology. All rights reserved.

Key Words: Boiling histotripsy, High-intensity focused ultrasound, Prostate.

INTRODUCTION

Histotripsy is a developmental high-intensity focused ultrasound (HIFU) technology that induces non-thermal mechanical tissue ablation (Parsons et al. 2006; Khokhlova et al. 2011, 2015; Lin et al. 2014; Eranki et al. 2018; Xu et al. 2021). Compared with existing clinical thermal HIFU regimes, histotripsy delivers sequences of shorter pulses (from microseconds to a few milliseconds) of higher acoustic power (10- to 100-fold) at low duty cycle (<1%). Central to the mechanism of histotripsy is formation of vapor/gas bubbles at the focus that leads to
mechanical effects through ultrasound—bubble interactions (Maxwell et al. 2011; Simon et al. 2012; Pahk et al. 2017). The presence of these bubbles produces hyperchogenicity during each pulse, and the ensuing mechanical destruction of tissue eliminates tissue scatterers, producing a hypoechoic cavity as treatment completes (Wang et al. 2009; Khokhlova et al. 2019a). Collectively, this allows for real-time targeting, treatment monitoring, and evaluation of treatment outcomes on B-mode ultrasound, which provides minimal real-time feedback with purely thermal HIFU. Additionally, because of the non-thermal mechanism and rapidity of bio-effects, histotripsy minimizes heat sinking and thermal spread that can limit the consistency and precision of HIFU thermal ablation. In general, the precision of thermal ablative techniques is highly dependent on the target tissue, the thermal dose, and the area over which the thermal dose is applied (Eihelf et al. 2018; Gschwend et al. 2021). However, it is generally accepted that a margin of at least 5 mm beyond a tumor region is needed to provide less frequent recurrence given the limitations of at least 5 mm beyond a tumor region is needed to provide less frequent recurrence given the limitations of contemporary tumor localization techniques (e.g., magnetic resonance imaging [MRI]). As a result, histotripsy may offer a strategy to improve HIFU ablation.

Accordingly, histotripsy has been under development for several clinical applications including ablation of the prostate for both benign prostatic hyperplasia (BPH) and prostate cancer (PCa) (Hempel et al. 2011; Schade et al. 2012a, 2012b; Khokhlova et al. 2019b; Dubinsky et al. 2020). Initial prostate studies used “cavitation cloud” histotripsy, which relies on microsecond-duration pulses and very high peak rarefactive (negative) pressures to induce microbubbles at the focus through shock scattering (Hall et al. 2009; Maxwell et al. 2011). Early pre-clinical work showed promise, and the technology proceeded to phase 1 clinical trials for treatment of BPH using the Vortx Rx device (HistoSonics, Ann Arbor, MI, USA) through a transperineal approach. Though men demonstrated a significant subjective improvement in their 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 (Schuster et al. 2018). The relatively small bony acoustic window and significant depth of the prostate for the transperineal approach, combined with the device’s pulse parameters, may have limited the ability to produce significant ablation of BPH.

Our group has been developing an alternative histotripsy regime, termed boiling histotripsy (BH). BH uses milliseconds-long (<20 ms) pulses and non-linear propagation effects to generate high-amplitude shock fronts in acoustic pressure wavefront at the focus (Khokhlova et al. 2011). Super-efficient shock-wave focusing and shock-induced heating result in formation of a millimeter-sized vapor bubble at the focus within each pulse (Canney et al. 2010). Interaction of subsequent shock fronts with this bubble produces mechanical tissue ablation with negligible thermal effects through pre-focal cavitation, acoustic atomization, and micro-fountaining (Khokhlova et al. 2011; Maxwell et al. 2011; Simon et al. 2012; Pahk et al. 2017). Ultrasonic atomization, that is, emission of fine droplets, and micro-fountain formation are well-known phenomena that occur when a focused ultrasound wave propagating in liquid encounters an interface with air (Simon et al. 2012). Focal waveforms in BH fields are asymmetric and have very high peak positive pressures following the shock fronts. When reflected from the pressure-release boundary of the vapor-filled boiling bubble, the wave changes polarity, which leads to creation of very high negative pressures in tissue in front of the bubble. This results in pre-focal cavitation and weakening or partial disintegration of the tissue. The acoustic radiation force caused by the BH beam pushes this tissue inside the vapor cavity, forming a miniature acoustic fountain of tissue fragments, and consequent atomization occurs inside the vapor bubble. The process is repeated with each incident pulse, resulting in complete tissue liquefaction.

The BH method therefore relies on shock amplitude instead of peak rarefactive pressure and has lower power requirements, which may make it more conducive to transducer miniaturization and a transrectal application for prostate indications. In the work described here, building on prior experiments (Khokhlova et al. 2019b), we evaluated the feasibility of using BH to ablate fresh ex vivo human prostate tissue as a proof of principle for developing BH for prostate applications.

METHODS

Fresh human prostate tissue samples (N = 24) were obtained via rapid autopsy (<24 h after death, institutional review board exempt). The sizes of samples varied from 4 to 15 cm³. Tissue samples were placed in phosphate-buffered saline solution and de-gassed in a desiccant chamber for at least 1 h with residual pressure <0.1 bar. For all BH experiments, the samples then were embedded in 1% agarose gel (N = 16). For shear wave elastography measurements, the samples (N = 8) were kept in de-gassed water to perform imaging.

Shear wave elastography imaging tissue characterization

Mechanical properties of the autopsy prostate tissue were analyzed in N = 8 samples using shear wave elastography (SWE) imaging to ensure that no significant change in tissue stiffness occurred within 24 h after death. To reduce artifacts at the edges of tissue samples, which were much stronger when they were embedded in...
Agarose gel, the measurements were performed in a container filled with de-gassed water. Each tissue sample was placed in water on an absorbing silicone rubber layer, positioned so that the imaging was performed from the same direction as the BH exposure, and the areas of interest for quantitative measurement were chosen in the middle of the samples away from the artifact areas. An Aixplorer ultrasound system with gray-scale ultrasound imaging and SWE (SuperSonic Imagine, Aix-en-Provence, France) and a linear probe SL15-4 with an effective bandwidth of 4 to 15 MHz were used in the measurements.

Settings were optimized for depth of penetration using a thyroid gland pre-set with an elasticity scale of 100 kPa. SWE images were obtained and recorded in the imaging plane through the center of each sample. For each measurement, the transducer was maintained in a steady position for 4 s until the images stabilized. In each image, the elastic (Young’s) modulus was measured within three 4-mm-diameter circular regions of interest centered at depths of 6, 12, and 18 mm in the sample. Each Young’s modulus measurement was performed three times, and the average value was recorded. For analysis, the values obtained in all 24 measurements were used.

Boiling histotripsy sonication

Agarose-embedded prostate tissue samples (N = 16) were placed in a custom holder submerged in de-gassed water and attached to a 3-D positioning system (Precision Acoustics, Dorchester, UK), as illustrated in Figure 1a. BH pulses were delivered using a 1.5-MHz custom-made transducer 80 mm in diameter and 60 mm in focal length, with a 24-mm diameter central opening (Fig. 1b).

To ensure successful tissue liquefaction in all samples, the transducer was operated at the highest driving voltage of 240 V provided by a custom-made electronic driving system similar to that described and characterized in our previous study (Maxwell et al. 2017). Acoustic power of the transducer and in situ focal pressures were estimated using measurement-based non-linear modeling with an equivalent single-element source as a boundary condition (Rosnitskiy et al. 2017). Pressure distributions were measured and reconstructed from acoustic holography measurements (Sapozhnikov et al. 2015) performed in a plane between the transducer and the focus using a calibrated hydrophone (HNA-0400, 1 mV/kPa at 1.5 MHz; Onda Corp., Sunnyvale, CA, USA) at low driving voltage (3 V). Geometrical parameters of an equivalent single-element source with a central opening and uniform distribution of the vibrational velocity on its surface were defined so that its axial pressure amplitude distribution matched the focal lobe (above the −6-dB level) of the corresponding experimentally obtained axial distributions as illustrated in Figure 2a. Then, assuming linear dependence of the source pressure on the driving voltage (Maxwell et al. 2017), non-linear simulations were performed for the equivalent source at the operational output (240 V) using a HIFU-beam software (Yuldashev et al. 2021). Simulations were performed in a layered medium “water—prostate” with the focus located 1 cm deep inside the prostate (Fig. 2b). Focal waveform was also derived from simulations in water, performed at lower voltage to compensate for attenuation in tissue (Khokhlova et al. 2011).

The following parameters were used in acoustic simulations in water and tissue, respectively: sound speed 1490.6 and 1559.5 m/s, density 997 and 1045 kg/m³, non-linear parameter 3.5 and 4.8, thermoviscous absorption (sound diffusivity) 4.33 × 10⁻⁶ m²/s, with additional absorption in tissue of 1.2 dB/cm at 1.5 MHz with a power law of 1.1 (Duck 1990). The aperture of the equivalent source was 65.8 mm, the focal length 56 mm and the central opening aperture 20 mm; the characteristic source pressure amplitude that corresponded to a 3-V driving voltage was 10 kPa. An acoustic output of 240 V used in BH experiments corresponded to a 734-W acoustic power of the equivalent source, 0.8-MPa characteristic pressure amplitude or 21-W/cm² intensity on its surface. Peak pressures in the focal waveform, directly simulated and derated from simulations in water, were $P_0/P_\text{in} = 122/−22$ MPa, and the shock amplitude was 135 MPa (Fig. 2c).

Boiling histotripsy exposures were delivered to a rectangular grid containing 2–11 foci in the transverse directions along the scanning plane (Fig. 3a). Depending on the size and geometry of the samples, foci were spaced 1–2 mm apart. Volumetric lesions were obtained by sonicating 1–4 scanning planes with 1 mm between them. Two BH exposure protocols with the same peak acoustic power were evaluated (N = 8 samples each). For both protocols, bubbles were detected at the focus by B-mode and Doppler imaging in all treatments, 100% of the treatment time. The first protocol (Fig. 3b) used pulses 10 ms in duration delivered at 1% duty cycle, 20–40 pulses per focus. This is the “standard” BH protocol that has been used for treatments of different tissues (Wang et al. 2018b; Khokhlova et al. 2019a), including an initial pilot study in human prostate (Khokhlova et al. 2019b). The second protocol used shorter pulses of 1-ms duration, 1% duty cycle and 75–150 pulses per sonication point, with the aim of accelerating the treatment (Fig. 3c).

In the first N = 4 samples tested for each BH pulse duration regime (N = 8 total), different spacing and number of pulses per focus were assessed to optimize BH treatments. Focus spacing and the minimum number of
pulses needed to produce uniformly liquefied lesions were selected. Subsequently, $N = 4$ samples ($N = 8$ total) were treated for each BH pulse duration with the following BH sonication parameters: 1-mm spacing between the treatment foci and between the sonication planes; 30 pulses per focus for 10-ms pulses and 150 pulses per focus for 1-ms pulses.

Real-time-imaging feedback of the BH sonications and monitoring of the BH treatments were performed using a Verasonics V1 Ultrasound Engine (Kirkland, WA, USA). A P7-4 ATL probe operating in B-mode/power Doppler was placed within the central opening of the BH transducer (Fig. 1a, 1b). Doppler sequences were triggered by the BH driving electronics to generate images immediately after the end of each BH pulse (Li et al. 2014). B-Mode images were also collected after the exposure to evaluate the outcome of the treatment.

**Specimen processing**

After the initial $N = 4$ “optimization” BH exposures for each pulse duration regime, the samples were bisected for gross evaluation of lesion formation. Subsequently, for the optimized pulse parameters, $N = 2$ samples per pulse duration regime were formalin-fixed after BH exposures and processed for histologic assessment with Masson’s trichrome staining. Additionally, $N = 2$ samples for each regime were bisected for gross evaluation, and then the lesion content was collected for ultrastructural analysis using transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

**RESULTS**

Observed SWE imaging characterization of prostate tissue specimens was within the typical range observed...
clinically (Barr et al. 2017). Specifically, the measured Young’s moduli ranged from 11.9—91.7 kPa, with a mean ± standard deviation (SD) of 37.9 ± 22.2 kPa (Table 1). A representative photograph of an ex vivo prostate tissue sample with evidence of BPH and example B-mode and SWE images illustrating the range of tissue stiffness are provided in Figure 4.

The duration of BH treatments varied from 5 to 33 min for 1-ms pulses and from 10 to 60 min for 10-ms pulses, depending on the number of sonication points in one layer (2—11) and the number of layers (1–4). The geometry of the sonication grid was chosen with respect to the size and shape of the samples. In all treatments, hyperechoic regions were visualized during BH sonications at the focus on B-mode (Fig. 5a), and BH-induced bubbles were also detected using power Doppler mode (Fig. 5b). These areas persisted for at least 10—15 min after the BH exposure. As treatment progressed, hyperechoic regions of tissue appeared, suggesting successful tissue fractionation and liquefaction of the lesion content (Fig. 5c). On gross inspection of BH-treated tissue (Fig. 5d), lesions contained liquefied regions of a homogeneous suspension consistent with mechanical fractionation of tissue. As expected, treatment with a higher “dose” of 100 pulses, indicated by yellow dots in Fig. 5d, produced larger lesions compared with treatment with 30 pulses per focus, depicted by blue dots.

On gross inspection, in lesions produced with longer pulses (10 ms vs. 1 ms), slightly whitened tissue appeared around the lesion cavity (Fig. 6b), which may indicate some thermal effect on the surrounding tissues (Fig. 6a, 6b). Such color change is a known effect of thermal treatment of tissue. Above a specific thermal dose, tissue proteins will denature and coagulate. The changes in protein conformation and coagulation result in a color change in addition to an increase in opacity (Wang et al. 2018a; Park et al. 2019; Zhou et al. 2021).

The resulting lesion volumes closely approximated the rectangular geometry of the planned sonication grid for all treatments. With 10-ms pulses, all dimensions were ~1.5 mm larger than the grid size, and with 1-ms pulses, the dimensions were ~1 mm larger than the grid size. This expansion was expected and is consistent with the known focal lesion size (for each pulse duration) extending out from centers of the grid points at the lesion margins. Lesion evaluation performed grossly revealed that tissue was fully liquefied within the sonicated volumes.

On histological analysis, lesions containing homogenized cell debris were observed for both 1- and 10-ms pulses, consistent with histotripsy-induced mechanical ablation of glandular elements (Fig. 6). BH treatment with both pulse lengths resulted in uniform tissue homogenization containing <50-μm tissue fragments centrally. However, the use of shorter 1-ms pulses (with the same 1% duty cycle) required a larger number of pulses per focus compared with the use of 10-ms pulses (150 vs. 30, respectively) to achieve complete ablation, but resulted in a two-fold acceleration of the treatment. The treatment speed

Table 1. Shear wave elastography-measured Young’s moduli (kPa) in three locations of eight ex vivo human prostate tissue samples

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
<th>Sample 6</th>
<th>Sample 7</th>
<th>Sample 8</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>24.5</td>
<td>27.7</td>
<td>20.8</td>
<td>11.9</td>
<td>45.5</td>
<td>18.4</td>
<td>80.6</td>
<td>34.8</td>
<td>31.7 (6.7)</td>
</tr>
<tr>
<td>Central</td>
<td>29.8</td>
<td>23.6</td>
<td>12.6</td>
<td>19.8</td>
<td>47.8</td>
<td>16.6</td>
<td>82.7</td>
<td>55.2</td>
<td>29.9 (6.3)</td>
</tr>
<tr>
<td>Distal</td>
<td>40.7</td>
<td>38.4</td>
<td>24.3</td>
<td>26.7</td>
<td>59.6</td>
<td>21.9</td>
<td>54.0</td>
<td>91.7</td>
<td>49.4 (9.1)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>31.7 (6.7)</td>
<td>29.9 (6.3)</td>
<td>19.2 (4.9)</td>
<td>19.4 (6.0)</td>
<td>51.0 (6.2)</td>
<td>19.0 (2.2)</td>
<td>72.4 (13.1)</td>
<td>60.6 (23.5)</td>
<td>37.9 (22.2)</td>
</tr>
</tbody>
</table>

SD = standard deviation.
evaluated grossly was $9 \pm 1.7 \text{ mm}^3/\text{min}$ for 1-ms treatments and $4.5 \pm 0.7 \text{ mm}^3/\text{min}$ for 10-ms treatments. The lesion of completely ablated tissue was surrounded by a margin, measuring $<200 \ \mu\text{m}$, of incompletely ablated tissue. The margin contained regions of intact smooth muscle and collagen fibrils, which is consistent with sparing of fibromuscular elements. Beyond that margin, glandular and fibromuscular elements appeared normal, consistent with viable untreated tissue.

In TEM images of the liquefied lesion content, both treatment protocols revealed discrete regions with loss of cellular structure and indistinguishable cellular components with a gradient of remaining electron-dense matter. However, with 10-ms pulses (Fig. 7b), there were regions where the cellular debris became more condensed and electron dense versus 1-ms pulses (Fig. 7a), which may indicate some thermal effect in the liquefied lesion content (Wang et al. 2018b). In the SEM images, 10-ms pulses produced layers of fibrillar fragments of collagen covered by a thick layer of cell and protein debris, whereas 1-ms pulses resulted in thinner and cleaner fibrillar fragments with discrete globules of cellular and protein remnants.

**DISCUSSION**

Herein we report successful results after applying BH for mechanical ablation of fresh *ex vivo* human prostatic tissue. BH produced reproducible mechanical homogenization in prostate tissue samples using two pulse parameter sets, which was confirmed by histologic and ultrastructural analysis. BH treatment targeting, progression and outcomes were readily monitored in real time on B-mode and Doppler ultrasound. The presence of BH-generated bubbles resulted in the appearance of a hyperechoic region at the treatment site during each pulse, and mechanical destruction of tissue eliminated tissue scatters, producing a hypoechoic cavity as treatment was completed.

With the longer 10-ms pulses there may have been subtle thermal effects on the liquefied tissue debris seen grossly and on TEM and SEM. As the same high acoustic power output (0.7 kW) used in this study was sufficient to generate BH for both 1- and 10-ms pulses, longer tissue heating for 10 ms and initiation of boiling early within each pulse may have resulted in whitening of the lesion border and specific changes in the liquefied lesion content observed in SEM and TEM images and related to the thermal effect. Further optimization experiments will aim at minimizing the power output required for generating BH lesions with longer pulses and minimizing “dose” requirements for various pulse lengths.

This study builds on prior pre-clinical work developing cavitation cloud histotripsy as a treatment for BPH in which that technique was successfully used for...
transabdominal prostate ablation in in vivo canine studies (Hall et al. 2009; Hempel et al. 2011; Schade et al. 2012a, 2014) and ultimately led to the creation of a clinical device (Vortx, Histosonics) (Schuster et al. 2018). However, this device failed to produce objective evidence of successful prostate ablation (e.g., change in prostate volume, urine flow rate) (Schuster et al. 2018), and to date, no studies have detailed the effects of the shock scattering approach on human prostate tissue. As a result, this study represents a significant advance in the prostate histotripsy literature by providing definitive evidence of the ability of the BH approach to mechanically ablate human prostate tissue. It also offers the first ultrastructural characterization of the effects of BH on human prostate tissue on both TEM and SEM, confirming that BH can disrupt prostate tissue into subcellular debris. Additionally, similarly to what has been observed in canine prostate with cavitation cloud histotripsy (Hall et al. 2009; Hempel et al. 2011), BH ablation of ex vivo human prostate is precise with a <200-μm border.

The results presented here were obtained using fresh human ex vivo tissue; therefore, the possibility exists that the same BH treatments would not have the same effects in patients. However, several studies by our group have obtained good correlations between ex vivo and in vivo tissue sensitivities in preclinical models evaluating BH ablation in porcine liver and kidney (Khokhlova et al. 2014, 2019a) and rat kidney (Schade et al. 2019). Additionally, the rapid autopsy tissue collected for these experiments had mechanical properties on SWE imaging similar to those that would be expected clinically in BPH and PCa patients (Barr et al. 2017; Rouvière et al. 2017). Specifically, in young patients without prostatic disorders, the stiffness of the peripheral and central zones ranges from 15 to 25 kPa, whereas the transitional zone exhibits stiffness below 30 kPa. With the development of benign BPH, the peripheral zone remains soft, whereas the transition zone becomes heterogeneous and stiff, with elasticity values ranging from 30 to 180 kPa (Barr et al. 2017). A stiffness value of the peripheral zone greater than 35 kPa is suggestive of a malignancy (Correas et al. 2015; Barr et al. 2017). Wei et al. (2018) reported an even higher stiffness of 82.6 kPa in malignant tumors compared with benign areas. All measurements presented in these articles were performed with an SE12-3 multi-frequency intracavitary probe with an effective 3- to 12-MHz bandwidth. Our studies were performed using an SL15-4 linear array transducer with an effective bandwidth of 4–15 MHz. Thus, the frequency of the transducer we used was similar to the frequency of the transducer used in clinics, and our range of Young’s moduli (37.9 ± 22.2 kPa) measured in the samples was similar to clinical values for benign tissues and certain malignancies. As a result, we anticipate that the observations and developments from this study (with respect to BH pulse parameters) will...
translate into patients though the optimal BH parameters for clinical prostate ablation remain to be determined.

Prostate cancer tumors and BPH therefore are typically stiffer mechanically (>35 kPa) (Correas et al. 2015; Barr et al. 2017; Wei et al. 2018), compared with normal prostate tissue (from 15 to 30 kPa) (Barr et al. 2017). In previous studies, it was reported that the sensitivity of tissues to histotripsy correlates with their mechanical stiffness and collagenous tissue is more resistant (Vlaisavljevich et al. 2014; Wang et al. 2018b; Khokhlova et al. 2019a, 2019b). Correspondingly, BPH and PCa tissue may have higher resistance to histotripsy; that is, these require a larger number of pulses per focus. Most of the samples used in the experiments contained BPH identified grossly and by palpation, and the BH treatment parameters were sufficient for liquefying them.

Non-thermal ablation with BH offers several potential advantages over existing minimally invasive treatment options for prostate diseases. First, BH’s mechanism of action comprising bubbles and mechanical bio-effects enables real-time ultrasound control not possible with other technologies. Second, the precision of BH ablation and lack of heat-sink and thermal diffusion effects (owing to the rapidity of its mechanism) are a major advantage over existing thermal-based techniques (HIFU, cryotherapy, etc.). We anticipate it will enable very tightly controlled ablation near critical structures such as the urinary sphincter and neurovascular bundles to minimize side effects such as urinary incontinence and erectile dysfunction when thinking about PCa focal therapy. Third, as this study demonstrated, refinement of BH pulse parameters enables the tailoring of pulse parameters and bio-effects, acceleration of treatments and facilitation of clinically relevant rates of non-thermal tissue ablation. Collectively, these advantages suggest that BH, unlike any other available minimally invasive therapy for prostate pathology, can be translated into a clinical treatment of prostate diseases.

The positive results of this study, combined with the potential advantages of the BH approach, support further investigation and development of BH for both PCa and BPH. Compared with cavitation cloud histotripsy, BH pulse parameters may be more amenable to miniaturization to facilitate the transrectal application that has been successfully used for thermal HIFU in prostate (Guillaumier et al. 2018; Huber et al. 2020). Indeed, simulations by
our group suggested that BH of the prostate is feasible using a probe with a geometry similar to that of existing thermal transrectal HIFU devices (Khokhlova et al. 2018). Following these studies, a pre-clinical system has been developed, and pilot experiments in \textit{ex vivo} human prostate tissue and suggest that BH can be accelerated without the loss of efficiency using shorter pulses of sufficient shock amplitude (1 ms vs. 10 ms). On the basis of these encouraging results, further evaluation of BH for prostate applications is underway (Schade et al. 2020).

**CONCLUSIONS**

These data represent successful application of the BH method with real-time B-mode and Doppler-type imaging in \textit{fresh} \textit{ex vivo} human prostate tissue and suggest that BH can be accelerated without the loss of efficiency using shorter pulses of sufficient shock amplitude (1 ms vs. 10 ms). On the basis of these encouraging results, further evaluation of BH for prostate applications is underway.

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