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***In-vitro* Comminution of Model Renal Calculi using Histotripsy**

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Abstract

Shock wave lithotripsy (SWL) suffers from the fact that it can produce residual stone fragments of significant size (>2 mm). Mechanistically, cavitation has been shown to play an important role in the reduction of such fragments to smaller debris. In this study we assessed the feasibility of using cavitational-based pulsed ultrasound therapy (histotripsy) to erode kidney stones. Previous work has shown that histotripsy is capable of mechanically fractionating soft tissue into fine, acellular debris. Here, we investigated the potential for translating this technology to renal calculi through the use of a commonly accepted stone model, Ultracal-30 cement. Stones were sonicated using a 1-MHz focused transducer, with 5-cycle pulses delivered at a rate of 1 kHz. Pulses having peak negative pressures ranging from 3–21 MPa were tested. Results indicate that histotripsy is capable of effectively eroding the Ultracal-30 model, achieving an average stone erosion rate of 26 mg/min at maximum treatment pressure; substantial stone erosion was only observed in the presence of a dense cavitational bubble cloud. Sequential sieving of residual stone fragments indicated that debris produced by histotripsy was smaller than 100 μ m in size, and treatment monitoring showed that both the cavitational bubble cloud and model stone appear as hyperechoic regions on B-mode imaging. These preliminary results indicate that histotripsy shows promise in its use for stone comminution, and an optimized erosion process may provide a potential adjunct to conventional SWL procedures.

I. INTRODUCTION

Shock wave lithotripsy (SWL) has been in clinical use for nearly 30 years, representing one of several first-line therapies for the treatment of stones located in the kidneys and ureter. This technique utilizes focused shock waves to shatter stones into smaller fragments that are subsequently passed in the urine. It is likely that a combination of stress waves and cavitational effects are involved in stone comminution, with stress waves predominating for the initial subdivision of a primary calculus into large pieces [1, 2]. Cavitation has been shown to play an important role in subsequent fragment reduction, as studies of lithotripsy under over-pressure [3] and in cavitation suppressing fluids [1] have displayed reduced stone comminution.

Although effective, SWL suffers from the fact that it can produce stone fragments of significant size [4–7]. Traditional clinical perspective deems residual fragments less than 2 mm as clinically insignificant, since they are likely to pass with minimal difficulty [8].

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Fragments in excess of 2 mm can lead to ureteral obstruction during passage, and when retained, may serve as a nidus for recurrent stone growth [9]. The production of such fragments is a relatively common occurrence; in studies using the Dornier HM-3 lithotripter with a low rate (< 10%) of retreatment, reported stone free rates at 3 months are approximately 70–80% [7, 10, 11]. Similar rates have been reported for other lithotripters, but often include much higher rates of retreatment (up to 50%) [4–6].

Novel forms of lithotripsy have been the subject of recent investigative work. Ikeda and Yoshizawa have explored the use of high intensity focused ultrasound (HIFU) lithotripsy with cavitating microbubbles [12, 13]. This technique utilizes the collapse of a cavitation cloud to fragment stones. In order to control cloud cavitation, the group has designed a two-frequency waveform consisting of a high frequency ultrasound burst to initiate a cavitation cloud followed by a low frequency burst to force the cloud into collapse. Results indicate that application of HIFU lithotripsy to natural stones produces small stone fragments (<1 mm) as a result of localized cavitation on the stone surface. This characteristic of comminution via cavitation erosion is an attractive outcome with respect to minimizing the size of stone fragments produced during treatment.

Histotripsy is a technique for the mechanical fractionation of tissue structures which utilizes focused ultrasound pulses to control cavitation activity. Previous work with histotripsy has demonstrated the feasibility of mechanical tissue fractionation via controlled cavitation [14–17], resulting in tiny acellular debris after several minutes of treatment. The initiation and maintenance of a cavitation bubble cloud can be directed via proper ultrasound pulse sequences, which typically include very short (<50 cycles), high peak negative pressure (>6 MPa), nonlinear pulses delivered at low duty cycles (0.1–5%). In contrast to thermal HIFU ablation, the low duty cycle in histotripsy manifests in the damage being mechanical rather than thermal in nature. Considering previous work that indicates the importance of cavitation in producing fine debris during the stone comminution process [1, 3, 12, 13], we hypothesize that cavitation control with histotripsy may provide a potential adjunct to conventional SWL procedures. The present study aims to evaluate the feasibility of using histotripsy to treat urinary calculi through the use of an Ultracal-30 model.

II. METHODS

The preliminary feasibility of using histotripsy to treat renal calculi was evaluated with model stones composed of Ultracal-30 gypsum cement. Statically anchored stones were sonicated for five minutes, and the resulting erosion rate and fragment sizes were assessed. The mechanism of histotripsy-induced stone damage was investigated by correlating stone erosion with cavitation activity. In order to examine the characteristics of stone comminution distinguishable on an ultrasound image, stone treatments were monitored in real time via B-mode ultrasound imaging.

A. Preparation of Model Urinary Stones

Artificial stones were cast from Ultracal-30 gypsum-based cement (U.S. Gypsum, Chicago, Illinois), which was selected as a model material for urinary stones due to its previous implementation in a number of in-vitro lithotripsy assessments [18–26]. This model has been shown to have acoustic and mechanical properties near the median of those reported for naturally occurring urinary stones [18]. Dry Ultracal-30 powder was mixed 1:1 (g:ml) with tap water for 10 minutes using a magnetic stir bar. Subsequently, 0.8 ml aliquots of the slurry were distributed into the cylindrical wells of an aluminum suppository mold (Gallipot Inc., St. Paul, Minnesota) using a 1 ml syringe. Each well was 10 mm in diameter, resulting in cylindrical stones of 7.9 ± 0.2 mm (mean $\pm$ standard deviation; $n = 56$) in height and 932.0 ± 14.1 mg in weight in the hydrated state. As specified by McAteer, *et al.* [18], the slurry

was allowed to solidify for one hour, at which time additional tap water was added to each well in order to prevent dehydration of the cement. The following day, after a minimum solidification time of 12 hours, stones were removed from the mold and immersed in a covered beaker of tap water. Each stone was treated within 48 hours of removal from the mold.

B. Experimental Setup

The experimental setup used for the treatment of Ultracal-30 model stones is displayed in Fig. 1. In order to collect stone debris, each stone was statically anchored within a polypropylene container of dimensions 13 cm x 13 cm x 6 cm (length x width x height). An 11 cm x 11 cm x 0.013 cm (length x width x thickness) polycarbonate window located in the top face of the container allowed ultrasound to be transmitted to the stone with minimal attenuation. Direct measurement of the signal propagating through this window indicated that the attenuation was negligible (<1%). Stones were kept stationary via the application of a small amount of silicon sealant (DAP Inc., Baltimore, Maryland) to the bottom face of each cylindrical stone. This face was then adhered to a PVC cylinder within the treatment container in order to elevate the stone from the container's bottom surface. The container was filled with water degassed to a dissolved oxygen content of $79\% \pm 2\%$ in order to mimic that of urine [27], and was subsequently placed into a larger water tank of dimensions 76 cm x 30 cm x 32 cm which also contained water of $80\% \pm 1\%$ dissolved oxygen. These dissolved oxygen levels were measured at the beginning and end of each treatment day (n=8) using a YSI 5000 Dissolved Oxygen Meter (YSI Inc., Yellow Springs, Ohio). A therapy transducer was mounted at the top of the tank and focused downward. It contained a central hole measuring 4 cm in diameter through which a backscatter-receiving transducer was placed. Alignment of the therapy transducer focus to the stone surface was accomplished using a PC-controlled 3-axis positioning system (Velmex Inc., Bloomfield, New York) to which the stone container was mounted. Two lasers laterally positioned near the therapy transducer focus ensured that stone position was maintained on an inter-trial basis.

C. Ultrasound Generation and Treatment

Histotripsy treatment was delivered to the Ultracal-30 stones using a piezocomposite spherically-focused 1 MHz transducer measuring 15 cm in diameter and having a 15 cm focal length (Imasonic, S.A., Voray sur l'Ognon, France). The transducer was driven using a class D amplifier developed in-house. All stones were sonicated using 5-cycle pulses delivered at a pulse repetition frequency (PRF) of 1 kHz. In order to investigate the correlation between stone erosion and cavitation cloud activity, ultrasound was applied to stones at different peak negative pressures (p_-) of 3, 7, 9, 12, 14, and 21 MPa, which were measured using a fiber optic hydrophone developed in our lab [28]. The corresponding peak positive pressure (p_+) values were 4, 13, 24, 34, 45, and 76 MPa, respectively. Representative pressure waveforms are displayed in Fig. 2. The lateral and axial -6-dB beamwidths were measured on the p_- and p_+ pressure profiles at p_-/p_+ of 10/29 MPa. The lateral -6-dB beamwidths for p_- and p_+ were 2.0 mm and 0.7 mm, respectively; the axial -6-dB beamwidths for p_- and p_+ were 19.0 mm and 7.5 mm. Beamwidths at higher pressures could not be successfully measured due to the occurrence of cavitation damage to the fiber tip during the pressure profile scan.

In each treatment, the top face of the stone cylinder was positioned coincident with the uppermost portion of the ellipsoidal cavitation bubble cloud produced at the therapy transducer focus (the portion closest to the therapy transducer, approximately 5 mm pre-focal relative to the geometric focus at maximum intensity). All stones were sonicated for 300 seconds, with both the transducer focus and stone fixed in place throughout the duration

of the treatment. Eight stones were treated at each pressure level, and all treatments were performed at room temperature (21° C).

D. Measurement of Stone Erosion Rate and Fragment Size

The average rate of stone erosion over a treatment period was calculated as the difference in the initial and final mass of the stone, divided by the treatment time (5 minutes). Due to the fact that Ultracal-30 stones must remain hydrated in order to retain their desired acoustic and mechanical properties [18], the stone weights measured pre- and post-treatment represent the hydrated weights of the stones. To ensure accurate comparison between treatments, masses were measured after all standing water was gently blotted off the stone surface with tissue paper.

The size distribution of residual stone fragments following treatment is of primary clinical concern, due to the fact that fragments of substantial size (>2 mm) can result in post-operative complications within the urinary tract. To evaluate the size of the fragments produced by histotripsy, suspended debris collected in the stone container was sequentially sieved through 2 mm, 1 mm, and 100 μ m filters. The dry weight of each filter was measured both prior to and 3 days following sieving, and the dry mass of the stone debris trapped on each respective filter was calculated as the difference in these two measurements. To obtain a meaningful comparison between this dry mass and the original weight of an Ultracal-30 stone (hydrated), a conversion factor between the hydrated and dehydrated states was determined by allowing eight Ultracal-30 cylindrical stones to dry at room temperature for 72 hours. It was found that the dehydrated stone mass represents $79\% \pm 1\%$ of its hydrated counterpart.

E. Cavitation Monitoring Using Acoustic Backscatter

To monitor cavitation activity, acoustic backscatter of the therapy pulses from the cavitating microbubbles was collected for each treatment. It has been previously documented that a spatially/temporally changing backscatter signal corresponds to a cluster of cavitating microbubbles (a bubble cloud) [16, 29, 30]. Passive detection of the backscatter signal employed a 5 MHz single-element transducer with a 2.5 cm aperture and 15 cm focal length (Olympus NDT, Waltham, Massachusetts). This backscatter-receiving transducer was positioned within the central hole of the therapy transducer, and the two were confocally aligned. Range-gated backscatter signals were collected every 150 ms throughout the 300 second treatment using an oscilloscope (Lecroy 9354 TM, Chestnut Ridge, New York), and subsequent analysis was performed in MATLAB (Mathworks, Natick, Massachusetts).

Previous work has shown increased spatial variability in the backscatter signal to be an excellent indicator of a cavitating bubble cloud and tissue homogenate production with histotripsy [31]. Quantification of this spatial variability was achieved using the algorithm detailed by Parsons, *et al.* [31]: first, the cross-correlation for adjacent A-lines of the backscatter signal is computed, and the lag value which yields maximum correlation between the two is determined. Next, the difference between neighboring lags is taken, and each spatial variability value is defined as the mean of five consecutive lag differences.

The bubble cloud on the surface of the stone was also imaged directly using a Photron Fastcam SA1.1 high speed camera (Photron USA, San Diego, California). Images were acquired immediately following the arrival of a therapy pulse at the transducer focus (101 μ s following transducer firing, corresponding to the propagation time for a 15 cm focal length). A frame rate of 1000 fps was used so that one image was collected per pulse (PRF= 1 kHz), and images were exposed for 20 μ s. These parameters allowed for visualization of the bubble cloud near its maximum spatial extent, as the maximum cloud size in histotripsy has

been found to occur at the end of a therapy pulse [32, 33]. A total of 201 frames were captured for each imaged treatment.

F. Ultrasound Imaging Feedback

An additional set of stone experiments was performed in which erosion progress was monitored in real-time using a LOGIQ P6 ultrasound imaging system (GE Healthcare, USA). Stones were treated with a therapy system consisting of four geometrically-aligned 1 MHz piezocomposite transducers (Imasonic, S.A., Voray sur l'Ognon, France) driven by a custom driving network. Each measured 5 cm x 5 cm in size and had a focal length of 7 cm. The resulting -6-dB beamwidth measured on the p- pressure profile for the overall system was 1.1 mm x 1.2 mm in the lateral dimension and 3.5 mm in the axial direction; the -6-dB beamwidth measured on the p+ pressure profile was 0.7 mm x 0.9 mm in the lateral dimension and 2.5 mm in the axial direction. These measurements were made at a p-/p+ of 21/31 MPa. A centrally-located 10 MHz linear imaging probe was also aligned to the focus of the therapy system. For this experiment, Ultracal-30 stones were statically adhered to the end of an optics rod using a small amount of silicon sealant (Fig. 3). To target the stone, prior to treatment a bubble cloud was generated at the therapy system focus in the empty water tank. This bubble cloud was visible as a hyperechoic zone on the ultrasound image, and the portion of the ellipsoidal cloud closest to the transducer was marked as the focus on the imager screen. Next, each cylindrical stone was aligned such that its top face was coincident with the focal marker. Stones were sonicated using 3-cycle pulses delivered at a PRF of 1 kHz and p-/p+ in excess of 23/35 MPa. Pressure values at higher intensity could not be determined due to instantaneous cavitation on the tip of the fiber optic hydrophone. B-mode ultrasound images were recorded before, during, and after histotripsy treatment.

III. RESULTS

Results show that histotripsy is capable of effectively mechanically eroding the Ultracal-30 stone model. Significant stone erosion occurred only in the presence of a dense cavitating bubble cloud. In such cases, the side of the stone facing the therapy transducer was eroded to tiny particulates from the onset of treatment. Eroded material was ejected from the stone surface in a plume of debris that was visible immediately upon application of histotripsy. Such characteristics were never observed in the absence of cavitation activity. Real-time ultrasound imaging showed that both the stone and the cavitational bubble cloud appear distinctly as hyperechoic regions on a B-mode image.

A. Stone Erosion Rate vs. Pressure

The stone erosion rate plotted as a function of peak negative pressure (p-) is displayed in Fig. 4, and the representative damage morphology at each respective pressure level is presented in Fig. 5. Control group stones (p- = 0 MPa) were handled in the same way as stones that received histotripsy treatment, but were not exposed to ultrasound. No visible damage to control stones was observed with the naked eye, although they did display an average erosion rate of 1.68 ± 0.68 mg/min assuming a 5 minute treatment period. This loss of mass is likely the result of simply handling the stone. Stones treated at a p- of 3 MPa did not display a statistically significant variation in the rate of erosion relative to control, with a value of 1.43 ± 0.23 mg/min (t-test, $P = 0.35$), and visible damage was not readily observable on the stone surface with the naked eye. There was an increase in erosion rate with peak negative pressure between 7–21 MPa (t-test, $P < 0.03$). Stones treated at a p- of 7, 9, and 12 MPa all displayed statistically significant increases in erosion rate relative to control, with values of 2.23 ± 0.43 mg/min, 3.45 ± 1.13 mg/min, and 5.05 ± 0.50 mg/min, respectively (t-test, $P = 0.04, 0.001, 0.0007$). Stone damage was only observed on the

surface of the stone facing the therapy transducer. The typical damage area, as can be seen in Fig. 5, increased with increasing the magnitude of the peak negative pressure.

At $p_- = 14$ and 21 MPa, statistically significant increases in the erosion rate relative to the control were observed (t-test, $P < 0.0001$). Treatments performed at a p_- of 14 MPa yielded an erosion rate of 16.23 ± 1.99 mg/min, while those at 21 MPa resulted in a rate of 25.80 ± 2.68 mg/min. Visually, treatment at these intensity levels resulted in damage regions that encompassed nearly the entire face of the 10 mm diameter cylindrical stone (Fig. 5).

B. Stone Fragment Size Distribution

The size distribution of stone fragments resulting from histotripsy was evaluated by sequentially sieving suspended debris through 2 mm, 1 mm, and 100 μm filters. The change in dry weight of each filter was measured in order to assess the fraction of stone fragments corresponding to a given pore size. In all cases, no fragments were observed to be trapped by the filters with the naked eye, and the weight change of the filters did not display a statistically significant difference between control (untreated) samples and those which received ultrasound exposure (t-test, $P > 0.15$). This result suggests that stone fragments generated by histotripsy are smaller than 100 μm , and as such were not trapped by the filters used in this study.

To help verify the fragment size results indicated by sieving, samples of stone debris collected during histotripsy treatments were placed on a hemocytometer and observed using microscopy. An example of such a sample is displayed in Fig. 6. As can be viewed in the image, the particulates are all smaller than 100 μm . This result is consistent with the outcome of sieving.

C. Cavitation Monitoring

The spatial variability in the acoustic backscatter signal was calculated in order to quantify cavitation activity. Typical results at select pressure levels are presented in the lower panel of Fig. 7. In general, the magnitude of the spatial variability increased with increasing pressure, implying that the bubble cloud action on the stone surface increased as a function of pressure. Direct imaging of the cavitation activity on the surface of the stone confirmed these results, showing larger bubble clouds at higher pressures (Fig. 7, top panel).

The mean spatial variability (MSV) was also calculated for the set of treatments performed at each pressure level (Fig. 8). At p_- of 3 and 7 MPa, the resulting MSV was low, and no/minimal bubble cloud activity was observed with imaging. At a p_- of 9 MPa and above, MSV increased significantly relative to that observed at 7 MPa (t-test, $P < 0.01$) and substantial bubble activity was visualized via imaging. Most notably, treatments at p_- of 14 and 21 MPa resulted in MSVs of 0.46 ± 0.35 mm and 1.44 ± 0.39 mm, respectively. These values are approximately an order of magnitude greater than those associated with the lower intensities used in this study. Correspondingly, imaging showed that bubble cloud activity is enhanced at these uppermost pressure levels. These results are consistent with the trends in damage morphology displayed in Fig. 5¹.

D. Ultrasound Imaging Feedback

The important events associated with the real-time monitoring of stone treatment using B-mode imaging are displayed in Fig. 9. Prior to treatment, targeting of the therapy transducer

¹This paper has supplementary downloadable material available at <http://ieeexplore.ieee.org>, provided by the authors. This includes a succession of images (played at 30 fps) captured during high-speed photography of the bubble cloud on the stone surface ($P_- = 21$ MPa).

was accomplished by first generating a cavitation bubble cloud at its focus. This cloud showed as a dynamic hyperechoic zone on the ultrasound image (Fig. 9(A)), with temporally changing characteristics corresponding to the activity of the bubble cloud. Placement of a targeting marker near the end of the cloud closest to the therapy transducer allowed for consistent targeting of the stone, which was also visible as a hyperechoic region on the ultrasound image (Fig. 9(B)). Upon application of histotripsy treatment, accurate alignment of the stone was confirmed by noting the position of the hyperechoic bubble cloud relative to the stone itself (Fig. 9(C)). A visually apparent reduction in stone size could be observed as treatment progressed, discernable by the change in shape of the stone face adjacent to the bubble cloud (Fig. 9(D))².

IV. DISCUSSION

Erosion Rate-Acoustic Backscatter Correlation

In this study we used the metric of spatial variability to quantify the cavitation activity at the surface of the Ultracal-30 model stones. Fig. 8 displays the mean spatial variability (MSV) resulting from treatment at each respective pressure level. Comparing this result with the plot of the stone erosion rate as a function of peak negative pressure (Fig. 4), it is apparent that a correlation between the MSV in the backscatter signal and the erosion rate exists. Both are observed to increase as functions of peak negative pressure, and both display drastic increases at p_- of 14 and 21 MPa. In order to further assess this correlation, the erosion rate was plotted as a function of mean spatial variability (Fig. 10); subsequent linear regression analysis yielded a correlation coefficient (R) of 0.95, suggesting that the stone erosion rate is highly correlated to the degree of cavitation activity on the stone surface. We therefore conclude that the primary mechanism of stone damage with histotripsy is cavitation-induced erosion.

Further examination of Fig. 10 reveals a systematic deviation from the proposed linear relationship between stone erosion rate and MSV. If a line is fitted using only the first three data points ($MSV < 0.15$ mm), the resulting slope (Rate/MSV) is 40.4 mg/mm-min; contrastingly, fitting a line to just the last three data points ($MSV > 0.15$ mm) produces a reduced slope of 14.8 mg/mm-min. The complex physics involved in the stone erosion process make it difficult to assess the validity of a mathematical model for this situation, and it is possible that a second-order polynomial may be more appropriate. Here we use a linear model for the sole purpose of assessing the correlation between erosion rate and MSV. Physically, this observation suggests that there may be a shielding phenomenon at play as a result of the high pulse repetition frequency ($PRF = 1$ kHz) used in this study. Shielding is well documented in shock wave lithotripsy, and multiple studies have demonstrated that stone breakage is improved with lowering the shock rate [34–36]. The physical phenomenon underlying this observation was investigated by Pishchalnikov, *et al.* [37]. In their study, it was concluded that an axial distribution of microscopic bubbles—i.e. cavitation nuclei—can persist between lithotripsy pulses. The negative pressure phase of subsequent pulses can then stimulate these bubbles to grow, detracting energy from the negative pressure phase of the shock wave. Hence, the shock wave reaching the stone surface will have an attenuated p_- . With respect to histotripsy, it is probable that this shielding phenomenon becomes more pronounced as the size of the bubble cloud increases in the axial dimension. This is consistent with the fact that the empirical erosion rate is lower than expected when MSV is high. It also suggests that there is a large potential for optimizing the histotripsy parameters utilized for stone treatment, with a decrease in PRF likely leading to more efficient erosion.

²This paper has supplementary downloadable material available at <http://ieeexplore.ieee.org>, provided by the authors. This includes a video of the B-mode monitoring of Ultracal-30 stone treatment.

Future optimization work will focus on evaluating the trade-off between treatment rate (high PRF) and treatment efficiency (low PRF).

An assessment of how the histotripsy stone comminution rate compares to that of conventional SWL is of obvious clinical interest. A full lithotripsy dose traditionally consists of 2000 shocks delivered at a rate of 2 Hz, corresponding to a treatment duration of 16.7 minutes. The average stone erosion rate observed at maximum treatment intensity with histotripsy was approximately 26 mg/min. Assuming that this rate is constant—as would be expected with a surface erosion phenomenon—it would take roughly 36 minutes to fully erode one of the model stones used in this study. This treatment length is slightly over twice that of traditional SWL. However, it should be noted that the histotripsy parameters utilized in this study have not yet been optimized. Because histotripsy is a surface erosion phenomenon, having a larger focal region to encompass the entire stone would be desirable. We therefore hypothesize that using lower frequency transducers will greatly enhance the rate of stone erosion; future work will examine this effect.

One inherent limitation of this initial feasibility study is the fact that it utilized model stones. Ultracal-30 is a commonly accepted stone model within the lithotripsy community; however, the extent to which it is capable of mimicking naturally-occurring renal calculi is obviously limited. For this reason, the results of this study should be regarded as relative trends as opposed to absolute in nature. The authors plan on exploring additional stone models, as well as natural human stones, in the future. However, for this preliminary evaluation of the histotripsy comminution mechanism and characteristics, we deemed the Ultracal-30 model sufficient.

Stone Fragment Size Distribution

Fragments generated upon application of histotripsy to Ultracal-30 model kidney stones were measured to be smaller than 100 μm . It is important to distinguish this debris—which is generated *from the onset* of histotripsy treatment—from the stone fragments one would expect following shock wave lithotripsy. With respect to SWL, the shock waves imparted to the stone result in a distribution of fragment sizes that is very much dependent on treatment time. Zhu, *et al.* [38] demonstrated that stone phantoms break up in a progressive manner as more and more shock waves are delivered. Specifically, following 25 to 50 shocks, it was observed that the treated stone had broken into several large pieces. The application of subsequent shocks produced more medium and small fragments, indicating progressive comminution of the initial large pieces. This mechanism of fragmentation presents the potential for the generation of residual fragments of significant size, which includes anything in excess of 2 mm in diameter by urological standards. In contrast, one of the profound characteristics of erosion induced by cavitating microbubbles in histotripsy is the fact that the stone fragments generated are of clinically insignificant size from the onset of treatment. This result is consistent with the work of Ikeda and Yoshizawa, who found that using HIFU to treat natural stones resulted in the production of small stone fragments (<1 mm) via cavitation erosion [12, 13].

From a clinical standpoint, the production of ultra-fine debris is much preferred relative to sizeable stone fragments. Residual stone pieces >2 mm may lead to ureteral obstruction, and have been shown to place patients at a higher risk for recurrent stone formation and growth [9]. Contrastingly, those <2 mm are expected to pass spontaneously without further treatment, although passage may often take several weeks [39]. It is certainly possible that in some cases these clinically insignificant residual fragments may be retained and seed new stones [40]; however, study examining patients at 12 month follow-up has shown that the probability of eliminating residual lithiasis is significantly greater in patients with “dust” than in those with significant residual fragments [41].

B-Mode Imaging

Targeting is of inherent importance for safe and effective stone comminution. One potential advantage of histotripsy is the fact that it offers real-time image guidance of treatments. It was shown that both the bubble cloud and the stone itself are visible as hyperechoic zones on a B-mode image (Fig. 9(A–C)), presenting the opportunity for focal tracking mechanisms. Such tracking capability was not necessary in this preliminary feasibility study, as model stones were statically anchored in place; however, the ability to track the motion of a mobile stone and adjust the therapy transducer focus accordingly will be essential as we move forward in evaluating histotripsy stone erosion in more physiologically-relevant settings. We will pursue potential focal tracking strategies in our future work.

V. CONCLUSION

Results from our preliminary assessment of the feasibility of using histotripsy to treat renal calculi indicate that histotripsy can mechanically erode Ultracal-30 model kidney stones into fine fragments. At our maximum treatment intensity corresponding to a peak negative pressure of 21 MPa, we were able to achieve an average erosion rate of approximately 26 mg/min. Substantial stone erosion was only observed in the presence of a dense cavitating bubble cloud, as indicated by both optical imaging and the spatial variability metric. Residual stone fragments generated by histotripsy treatment appeared to be smaller than 100 μm , and both the stone and the cavitation bubble cloud were visible as hyperechoic zones on B-mode images. These results suggest that cavitation control with histotripsy may offer a potential adjunct to SWL procedures, and it is possible that a synergistic combination of the two modalities could eventually lead to faster and more complete stone comminution. Our continued work will focus on elucidating a parameter set to maximize histotripsy stone erosion, as well as exploring histotripsy stone treatment in more physiologically-relevant settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Zhu S, Cocks FH, Preminger GM, Zhong P. The role of stress waves and cavitation in stone comminution in shock wave lithotripsy. *Ultrasound Med Biol.* 2002; 28:661–71. [PubMed: 12079703]
2. Sapozhnikov OA, Maxwell AD, MacConaghy B, Bailey MR. A mechanistic analysis of stone fracture in lithotripsy. *J Acoust Soc Am.* Feb.2007 121:1190–202. [PubMed: 17348540]
3. Delius M. Minimal static excess pressure minimizes the effect of extracorporeal shock waves on cells and reduces it on gallstones. *Ultrasound Med Biol.* 1997; 23:611–7. [PubMed: 9232770]
4. Mobley TB, Myers DA, Grine WB, Jenkins JM, Jordan WR. Low energy lithotripsy with the Lithostar: treatment results with 19,962 renal and ureteral calculi. *J Urol.* Jun.1993 149:1419–24. [PubMed: 8501779]
5. Liston TG, Montgomery BS, Bultitude MI, Tiptaft RC. Extracorporeal shock wave lithotripsy with the Storz Modulith SL20: the first 500 patients. *Br J Urol.* May.1992 69:465–9. [PubMed: 1623372]
6. Coz F, Orvieto M, Bustos M, Lyng R, Stein C, Hinrichs A, San Francisco I. Extracorporeal shockwave lithotripsy of 2000 urinary calculi with the Modulith SL-20: success and failure according to size and location of stones. *J Endourol.* Apr.2000 14:239–46. [PubMed: 10795612]
7. Cass AS. Comparison of first generation (Dornier HM3) and second generation (Medstone STS) lithotripters: treatment results with 13,864 renal and ureteral calculi. *J Urol.* Mar.1995 153:588–92. [PubMed: 7861488]

8. Chaussy, C.; Schmiedt, E.; Jocham, D.; Walther, V.; Brendel, W.; Forssmann, B.; Hepp, W. Extracorporeal shock wave lithotripsy: New aspects in the treatment of kidney stone disease. Basel: Karger; 1982.
9. Fine JK, Pak CY, Preminger GM. Effect of medical management and residual fragments on recurrent stone formation following shock wave lithotripsy. *J Urol.* Jan.1995 153:27–32. discussion 32–3. [PubMed: 7966783]
10. Lingeman, JE.; Smith, LH.; Wood, JR. Urinary Calculi: ESWL, Endourology and Medical Therapy. Philadelphia: Lea & Febiger; 1989. Bioeffects and long term effects of ESWL; p. 285
11. Politis G, Griffith DP. ESWL: Stone free efficacy based upon the stone size and location. *World J Urol.* 1987; 5:255–8.
12. Ikeda T, Yoshizawa S, Tosaki M, Allen JS, Takagi S, Ohta N, Kitamura T, Matsumoto Y. Cloud cavitation control for lithotripsy using high intensity focused ultrasound. *Ultrasound Med Biol.* Sep.2006 32:1383–97. [PubMed: 16965979]
13. Yoshizawa S, Ikeda T, Ito A, Ota R, Takagi S, Matsumoto Y. High intensity focused ultrasound lithotripsy with cavitating microbubbles. *Med Biol Eng Comput.* 2009; 47:851–60. [PubMed: 19360448]
14. Parsons JE, Cain CA, Abrams GD, Fowlkes JB. Pulsed cavitation ultrasound therapy for controlled tissue homogenization. *Ultrasound Med Biol.* 2006; 32:115–29. [PubMed: 16364803]
15. Xu Z, Ludomirsky A, Eun LY, Hall TL, Tran BC, Fowlkes JB, Cain CA. Controlled ultrasound tissue erosion. *IEEE Trans Ultrason Ferroelectr Freq Control.* 2004; 51:726–36.
16. Xu Z, Fowlkes JB, Rothman ED, Levin AM, Cain CA. Controlled ultrasound tissue erosion: the role of dynamic interaction between insonation and microbubble activity. *J Acoust Soc Am.* 2005; 117:424–35. [PubMed: 15704435]
17. Xu Z, Fowlkes JB, Cain CA. Effects of acoustic parameters on bubble cloud dynamics in ultrasound tissue erosion (histotripsy). *J Acoust Soc Am.* 2007; 122:229–36. [PubMed: 17614482]
18. McAteer JA, Williams JC Jr, Cleveland RO, Van Cauwelaert J, Bailey MR, Lifshitz DA, Evan AP. Ultracal-30 gypsum artificial stones for research on the mechanisms of stone breakage in shock wave lithotripsy. *Urol Res.* Dec.2005 33:429–34. [PubMed: 16133577]
19. Kuo RL, Paterson RF, Siqueira TM Jr, Evan AP, McAteer JA, Williams JC Jr, Lingeman JE. In vitro assessment of ultrasonic lithotripters. *J Urol.* Oct.2003 170:1101–4. [PubMed: 14501701]
20. Paterson RF, Lifshitz DA, Lingeman JE, Evan AP, Connors BA, Fineberg NS, Williams JC Jr, McAteer JA. Stone fragmentation during shock wave lithotripsy is improved by slowing the shock wave rate: studies with a new animal model. *J Urol.* Nov.2002 168:2211–5. [PubMed: 12394761]
21. Chitnis PV, Cleveland RO. Quantitative measurements of acoustic emissions from cavitation at the surface of a stone in response to a lithotripter shock wave. *J Acoust Soc Am.* Apr.2006 119:1929–32. [PubMed: 16642802]
22. Sapozhnikov OA, Trusov LA, Owen NR, Bailey MR, Cleveland RO. Detecting fragmentation of kidney stones in lithotripsy by means of shock wave scattering. *AIP Conf Proc.* May.2006 829:308–312.
23. McAteer JA, Cleveland RO, Paterson RF, Rietjens DL, van APE, Connors BA, Lingeman JE, Pishchalnikov YA, Pishchalnikova IV, Williams JC Jr. Evidence that cavitation and spall contribute to stone failure in an animal model of kidney stone fragmentation by shock wave lithotripsy (SWL). *Proceedings 17th International Congress on Acoustics.* 2002; VII:202–3.
24. Mota A, Knap J, Ortiz M. Three-dimensional fracture and fragmentation of artificial kidney stones. *Journal of Physics: Conference Series.* 2006; 46:299–303.
25. Pishchalnikov YA, McAteer JA, VonDerHaar RJ, Pishchalnikova IV, Williams JC Jr. The characteristics of broad and narrow focal zone lithotripters. *AIP Conf Proc.* 2008; 1049:238–242.
26. Pishchalnikov YA, Sapozhnikov OA, Bailey MR, Williams JC Jr, Cleveland RO, Colonius T, Crum LA, Evan AP, McAteer JA. Cavitation bubble cluster activity in the breakage of kidney stones by lithotripter shockwaves. *J Endourol.* Sep.2003 17:435–46. [PubMed: 14565872]
27. Gardner EA, Fowlkes JB, Carson PL, Ivey JA, Ohl DA. Bubble Generation in Excised Canine Urinary Bladders Using an Electrohydraulic Lithotripter. 1993 IEEE Ultrasonics Symposium. 1993:905–908.

28. Parsons JE, Cain CA, Fowlkes JB. Cost-effective assembly of a basic fiber-optic hydrophone for measurement of high-amplitude therapeutic ultrasound fields. *J Acoust Soc Am.* 2006; 119:1432–40. [PubMed: 16583887]
29. Xu Z, Hall TL, Fowlkes JB, Cain CA. Optical and acoustic monitoring of bubble cloud dynamics at a tissue-fluid interface in ultrasound tissue erosion. *J Acoust Soc Am.* 2007; 121:2421–30. [PubMed: 17471753]
30. Xu Z, Raghavan M, Hall TL, Chang CW, Mycek MA, Fowlkes JB, Cain CA. High speed imaging of bubble clouds generated in pulsed ultrasound cavitation therapy- histotripsy. *IEEE Trans Ultrason Ferroelectr Freq Control.* 2007; 54:2091–101. [PubMed: 18019247]
31. Parsons JE, Cain CA, Fowlkes JB. Spatial Variability in Acoustic Backscatter as an Indicator of Tissue Homogenate Production in Pulsed Cavitation Ultrasound Therapy. *IEEE Trans Ultrason Ferroelectr Freq Control.* 2007; 54:576–90.
32. Xu Z, Raghavan M, Hall TL, Mycek MA, Fowlkes JB, Cain CA. Evolution of bubble clouds induced by pulsed cavitation ultrasound therapy - histotripsy. *IEEE Trans Ultrason Ferroelectr Freq Control.* May.2008 55:1122–32. [PubMed: 18519220]
33. Wang, T-Y.; Maxwell, AD.; Park, S.; Xu, Z.; Fowlkes, JB.; Cain, CA. Why are short pulses more efficient in tissue erosion using pulsed cavitation ultrasound (histotripsy)?. *Int Symp Therapeutic Ultrasound*; Aix en Provence, France. 2009.
34. Vallancien G, Munoz R, Borghi M, Veillon B, Brisset JM, Daudon M. Relationship between the frequency of piezoelectric shock waves and the quality of renal stone fragmentation. In vitro study and clinical implications. *Eur Urol.* 1989; 16:41–4. [PubMed: 2714316]
35. Weir MJ, Tariq N, Honey RJ. Shockwave frequency affects fragmentation in a kidney stone model. *J Endourol.* 2000; 14:547–50. [PubMed: 11030533]
36. Greenstein A, Matzkin H. Does the rate of extracorporeal shock wave delivery affect stone fragmentation. *Urology.* 1999; 54:430–2. [PubMed: 10475348]
37. Pishchalnikov YA, McAteer JA, Williams JC Jr, Pishchalnikova IV, Vonderhaar RJ. Why stones break better at slow shockwave rates than at fast rates: in vitro study with a research electrohydraulic lithotripter. *J Endourol.* Aug.2006 20:537–41. [PubMed: 16903810]
38. Zhu S, Cocks FH, Preminger GM, Zhong P. The role of stress waves and cavitation in stone comminution in shock wave lithotripsy. *Ultrasound Med Biol.* May.2002 28:661–71. [PubMed: 12079703]
39. Candau C, Saussine C, Lang H, Roy C, Faure F, Jacqmin D. Natural history of residual renal stone fragments after SWL. *Eur Urol.* 2000; 37:18–22. [PubMed: 10671779]
40. Osman MM, Alfano Y, Kamp S, Haecker A, Alken P, Michel MS, Knoll T. 5-year-follow-up of patients with clinically insignificant residual fragments after extracorporeal shockwave lithotripsy. *Eur Urol.* Jan.2005 47:860–4. [PubMed: 15925084]
41. Zanetti G, Seveso M, Montanari E, Guarneri A, Del Nero A, Nespoli R, Trinchieri A. Renal Stone fragments following shock wave lithotripsy. *J Urol.* Aug.1997 158:352–5. [PubMed: 9224301]

Fig 1.

The experimental setup for the treatment of Ultracal-30 model kidney stones consisted of a 1 MHz therapy transducer positioned at the top of a water tank, focused downward onto the stone surface. The cylindrical stones were statically anchored within a container in order to allow for collection of fragments generated during treatment. Acoustic backscatter was passively collected using a 5 MHz backscatter-receiving transducer positioned within the central hole of the therapy array.

Fig 2.

Representative pressure waveforms as recorded from a fiber optic hydrophone. The signals shown have been averaged over 1000 pulses, and are labeled corresponding to their peak negative pressure (p_-). At our maximum treatment intensity ($p_- = 21$ MPa), cavitation occurred on the fiber tip after only several pulses; as such, an averaged waveform at this intensity is not presented.

Fig 3.

B-mode imaging of stone treatments utilized a therapy system composed of four geometrically aligned 1 MHz transducers. A linear imaging probe positioned at the center of the array was also aligned to the focus. Ultracal-30 model stones were statically adhered to the end of an inverted optics rod during treatment.

Fig 4.

Stone erosion rate plotted as a function of peak negative pressure (p_-). Stones treated at p_- of 3 MPa did not exhibit an erosion rate significantly different from control, while there was an increase in erosion rate with p_- between 7-21 MPa. Drastic rate increases were observed at the upper-most pressures of 14 and 21 MPa.

Fig 5.

The stone damage morphology was observed to be dependent on the pressure level employed for the treatment. No visually apparent damage was observed in either the case of the control or 3 MPa treatments. At intensities corresponding to p– from 7-22 MPa, the typical area of damage on the face of the stone was found to increase with p–. Damage morphologies were not perfectly centered on the top surface of the stone due to slight targeting misalignments.

Fig 6. Microscope imaging of Ultracal-30 stone debris placed on a hemocytometer helped to confirm that fragments generated by histotripsy treatment were smaller than 100 μm.

Fig 7.

Bubble activity at the stone surface. The calculated spatial variability in the acoustic backscatter signal acquired during histotripsy treatments (lower panel) was observed to increase with peak negative pressure (p_-), indicating that the extent of the bubble cloud activity on the surface of the stone increases with p_- . These results were confirmed via direct imaging of the bubble activity (top panel).

Fig 8.

The mean spatial variability (MSV) is plotted as a function of peak negative pressure. Statistically significant increases in MSV above the baseline level were observed for p - of 9 MPa and greater, with the most significant increases occurring for the upper-most pressure levels of 14 and 21 MPa.

Fig 9.

B-mode images are presented from the real-time monitoring of Ultracal-30 stone treatment. The therapy transducer and imaging probe are located adjacent the top side of the images. (A) The bubble cloud showed clearly on the ultrasound image as a temporally changing hyperechoic zone. (B) Placement of a targeting marker near the proximal end of the bubble cloud facilitated alignment of the stone cylinder, which also showed clearly as a hyperechoic region on the image. (C) During treatment, accurate alignment could be confirmed by noting the positions of the stone and bubble cloud relative to one another. (D) A visually apparent reduction in stone size could be observed as treatment progressed, discernable by the change in shape of the stone face adjacent to the bubble cloud.

Fig 10.

The erosion rate achieved with histotripsy is plotted as a function of the mean spatial variability in the acoustic backscatter signal. Linear regression analysis yielded a correlation coefficient of $R=0.95$, indicating a strong correlation between the two variables. Recalling that the mean spatial variability in the backscatter signal is a measure of the bubble activity, we conclude that the stone erosion rate is highly correlated to the degree of cavitation activity on the stone surface.