Objective: Biofilm formation in medical catheters is a major source of hospital-acquired infections which can produce increased morbidity and mortality for patients. Histotripsy is a non-invasive, non-thermal focused ultrasound therapy and recently has been found to be effective at removal of biofilm from medical catheters. Previously established histotripsy methods for biofilm removal, however, would require several hours of use to effectively treat a full-length medical catheter. Here, we investigate the potential to increase the speed and efficiency with which biofilms can be ablated from catheters using histotripsy.

Methods: Pseudomonas aeruginosa (PA14) biofilms were cultured in in vitro Tygon catheter mimics and treated with histotripsy using a 1 MHz histotripsy transducer and a variety of histotripsy pulsing rates and scanning methods. The improved parameters identified in these studies were then used to explore the bactericidal effect of histotripsy on planktonic PA14 suspended in a catheter mimic.

Results: Histotripsy can be used to remove biofilm and kill bacteria at substantially increased speeds compared with previously established methods. Near-complete biofilm removal was achieved at treatment speeds up to 1 cm/s, while a 4.241 log reduction in planktonic bacteria was achieved with 2.4 cm/min treatment.

Conclusion: These results represent a 500-fold increase in biofilm removal speeds and a 6.2-fold increase in bacterial killing speeds compared with previously published methods. These findings indicate that histotripsy shows promise for the treatment of catheter-associated biofilms and planktonic bacteria in a clinically relevant time frame.

Introduction

Medical catheter use is ubiquitous in the in-hospital setting. Catheters come in several forms, including devices such as urinary catheters, intravenous lines and arterial lines. Catheter placement is the most common invasive hospital procedure performed, with more than 330 million intravenous catheters, 30 million urinary catheters and 8 million arterial lines placed each year in the United States alone [1–3]. Despite the frequency with which catheters are used, they pose a significant risk of iatrogenic infection in the in-hospital setting. Infections with these devices can be broadly divided into two classes: catheter-related bloodstream infections (CRBSIs) and catheter-associated urinary tract infections (CAUTIs). It is estimated that 30,000–40,000 CRBSIs and more than 1 million CAUTIs occur annually in the United States [4,5]. CAUTIs and CRBSIs are responsible for the majority of hospital-acquired infections, particularly in critically ill, hospitalized patients. Medical catheters have been reported to be the causative factor in 95% of urinary tract infections (UTIs) and 87% of bloodstream infections in this population [6]. Infections through medical catheters have been reported to increase mortality, add to cost of care and prolong patient hospitalization [7].

The treatment and prevention of catheter-associated infections (CAIs) have improved in recent years, but most gains in this area have been in the prevention of bacterial colonization through strategies such as improved sterile technique and antibiotic- or antiseptic-infused catheters. However, these approaches have been reported to be of limited benefit in many settings, with trials revealing the most improvement in settings in which the risk of infection is particularly high, such as the intensive care unit (ICU) or when quality improvement programs fail to achieve their target objectives for infection reduction [8,9]. Once bacteria have colonized the catheter lumen, however, few options remain except to remove and replace the catheter, particularly in patients who are exhibiting symptoms of severe disease [10]. Although catheter replacement should definitively eliminate the source of a CAI, there are several reasons why this is not an optimal strategy. First, repeated catheterization of the same site is associated with an increased risk of catheter infection [11]. Second, catheter placement is an invasive and often uncomfortable procedure, with repeated replacement potentially causing the patient undue pain. In addition, catheter replacement combined with empiric antibiotic therapy is only an effective technique once a systemic infection has been positively identified. Numerous clinical trials...
have indicated that scheduled catheter replacement does not lower the risk of catheter infection rates and is not recommended as a preventative strategy for CAIs with both intravenous and urinary catheters [12–15].

On the basis of the results of these studies, it is clear that an improved method for reducing CAIs would be to treat the bacterial catheter colonization in situ. Unlike tissue-based infections, however, bacterial colonization of medical devices represents a unique challenge in the form of biofilm growth. Biofilms are an extracellular matrix secreted by certain microbes that allow adhesion to surfaces and resistance to harsh environmental conditions. This biofilm allows for resistance of the microbes to antibacterial treatments [16]. Antibiotic treatment of biofilm generally results in less than a 1-log reduction in colony-forming units (CFUs), rendering this a largely ineffective modality [17]. Because of a relative scarcity of effective treatment strategies, there remains a significant need for new methods for the removal of catheter-associated biofilms. Histotripsy is a non-invasive, non-ionizing, non-thermal ablation technology that uses focused ultrasound to mechanically destroy tissue through cavitation [18]. Numerous studies have previously determined that histotripsy is effective at removing bacterial biofilm from medical devices [19–22]. These studies made great improvements in the application of histotripsy for biofilm removal, with an initial focus on its use for the treatment of surgical mesh. Recently, our group conducted a study revealing the potential of histotripsy for removing biofilms and reducing viable bacteria in the lumen of medical catheters [23]. Although this study illustrated the feasibility of using histotripsy as a potential new method for the prevention and treatment of CAIs, several issues remain to be solved prior to clinical application. Most notably, the histotripsy treatment methods in this study required >8 min to treat a 1 cm length catheter for biofilm removal. This approach would therefore require between 2 and 2.5 h of treatment to remove biofilms for a standard-length (14–18 cm) central venous catheter or more than 6 h for a standard-length (44 cm) urinary catheter. Bactericidal effects required approximately triple the treatment time of biofilm removal, further limiting the direct clinical application of this technique without further optimization of the histotripsy treatment parameters.

In the study described here, we investigated methods to remove bacterial biofilms and kill bacteria in a more clinically relevant time frame using histotripsy. More specifically, this study was aimed at investigating the minimum dose of histotripsy required to remove biofilms from the lumen of medical catheters and then exploring the use of different histotripsy parameters to deliver these treatments in a more efficacious and efficient manner. To accomplish these aims, we first conducted a series of experiments to determine the minimum required dose of histotripsy to achieve biofilm ablation, using similar methods described in the previous study [23]. Next, we compared histotripsy treatments applied using discrete steps with methods that applied histotripsy using continuous motion across the length of the catheter. Several subsequent experiments then aimed to improve treatment times by altering both the speed of histotripsy application and the rate at which pulses were applied. Finally, these newly developed methods were tested to determine the amount of time required to induce a bactericidal effect.

Methods

Histotripsy pulse generation

A 16-element, 1 MHz histotripsy transducer with a geometric focus of 58 mm, aperture of 66.7 mm and f-number of 0.87 was used for all experiments in this study (Fig. 1). The histotripsy transducer was powered using a custom high-voltage pulser that generates short single-cycle pulses controlled by a field programmable gate array (FPGA) board (Altera DE0-Nano Terasic Technology, Dover, DE, USA) programmed for histotripsy pulsing, with all elements driven in phase. These pulses generated cavitation from the single large negative pressure phase of the waveform. An example of this waveform is depicted in Figure 2A. The transducer was fixed vertically and submerged in a tank of de-gassed water. A computer-guided 3-D positioning system was used to align the catheter samples at the focus of the transducer (Fig. 1). The positioning system and transducer were both controlled simultaneously using MATLAB (MATLAB, The MathWorks, Natick, MA, USA). A phased array ultrasound probe with a frequency range of 3–8 MHz (P8-3L 10SI-6, Telemed, Vilnius, Lithuania) was coaxially aligned inside the transducer for real-time treatment guidance and visualization of cavitation clouds. A diagram of the transducer and 3-D positioning system is depicted in Figure 1A. Targeting was performed by activating the histotripsy transducer in de-gassed water without a catheter present while simultaneously imaging the bubble cloud using the ultrasound probe. A target was placed on the location in the water tank where the cavitation occurred using the phased array ultrasound probe driver software. This target was subsequently used to manually aim the transducer at the center of the catheter lumen with 0.1 mm accuracy.

Biofilm culture in catheter mimics

In this study, 3 cm sections of Tygon catheter mimic were used to simulate medical catheters (Part No. 5894K31, McMaster Carr, Santa Fe Springs, CA, USA). Catheter mimics had an inner diameter of 1.16 in. and a wall thickness of 1/16 in., giving a total diameter of 3/16 in. This catheter size was selected to closely replicate the dimensions and materials found in catheters that are used clinically. This diameter closely matches the 3/16 in. diameter found in a standard 14F urinary catheter (Patient Care Medical 2021). Tygon tubing and silicone stoppers were soaked in 10% bleach for 15 min prior to inoculation. After sterilization, tubing and stoppers were flushed repeatedly with water. Sterilized catheters were then placed on a sterile Petri dish to dry.

Pseudomonas aeruginosa (PA14) was subcultured from frozen stock stored at ~80°C. A transfer loop was used to inoculate 4 mL of sterile Luria–Bertani (LB) broth in a culture tube. This culture tube was incubated at 37°C and shaken at 200 rpm for 12 h. Bacterial density was standardized via spectrophotometry (Biomate 5 Spectrophotometer, Thermo Fisher, Waltham, MA, USA) at an optical density of 600 nm to A = 0.01 by diluting overnight cultures into fresh LB. Dilutions were then confirmed using spectrophotometry. Forty microliters of PA14 standardized to A = 0.01 was used for serial plate dilution. PA14 concentration was found to be 8.819 log ≥ 0.7465 log CFU/mL. Forty microliters of standardized PA14 was then aliquoted into each catheter mimic and capped on one end. Catheter mimics were then placed upright, stopper side down, in a 96-well plate and incubated at 30°C for 48 h. After incubation, catheters were flushed three times with 1 mL of de-ionized water (diH₂O) to remove planktonic bacteria, leaving a bacterial biofilm adhered to the luminal wall of the catheter mimics.

Histotripsy treatment of catheter-based biofilms

Catheter mimics with cultured biofilms were used to test biofilm removal via histotripsy. Cultured catheter mimics were filled with 40 μL of diH₂O and capped on both ends using sterilized silicone stoppers. Catheters were then split into control and treatment groups. Catheter mimics were attached to a 3-D positioning system using a custom-made scaffold (Fig. 1C). Catheter mimics were then submerged in a tank of de-gassed water, with the focal point of the histotripsy transducer targeted in the center of the catheter mimic lumen. A pre-determined treatment zone of 25 mm was used to cover the entirety of the lumen not occupied by the silicone stoppers. A custom program was used to mechanically move the catheter through the focus across the treatment zone. A peak negative pressure of 31.6 MPa was used to induce cavitation. This negative pressure was measured using a fiberoptic probe hydrophone in de-gassed water. Initial experiments delivered histotripsy in one scan across the treatment zone in incremental steps. At each step, a pre-determined number of pulses were applied.

Machine settings as described in Childers et al. [23] were used for initial testing. Briefly, starting machine settings were 500 pulses per
point (PPP), 0.1 mm step length and a pulse repetition frequency (PRF) of 200 Hz. Dosage of histotripsy was then altered through three independent factors in separate studies; PPP, step size, and PRF. Subsequent studies used an updated method which instead applied the histotripsy dose in one continuous motion across the catheter mimic length. A visual comparison of these methods is depicted in Figure 2B and 2C. This was done both to apply histotripsy more evenly to the catheter and to increase the speed and efficiency of application. Each experiment used three catheters suspended in de-gassed water to act as controls. Dosage alterations through changes in PPP, step size, PRF and scan speed were assessed in triplicate for each experiment.

**Biofilm assay**

After treatment with histotripsy, catheter mimics were removed from the treatment apparatus and flushed three times with 1 mL of diH2O. Catheter mimics were then air dried for 10 min, and 40 µL of 0.1% crystal violet (CV) stain was added to the lumens and left for 10 min. Catheter mimics were then again flushed three times with 1 mL of diH2O. CV bound to biofilm within the catheter mimics was then solubilized with 100 µL of 33% glacial acetic acid into 96-well flat bottom plates for spectrophotometry analysis (Sectra Max Plus 384 Microtiter Plate Reader, Molecular Devices, San Jose, CA, USA). Wells were read at an absorbance of 590 nm, and the resulting optical density data were recorded. Three wells were filled with 100 µL of 33% glacial acetic acid to serve as a blank correction. The percentage of biofilm removed was calculated using

\[
B_R = \left(1 - \frac{B_T}{B_C}\right) \times 100
\]

where \(B_R\) = biofilm removed, \(B_T\) = remaining biofilm after treatment and \(B_C\) = remaining biofilm on control catheters. A one-way analysis of variance (ANOVA) was conducted to compare biofilm removals between treatment groups using statistical software (GraphPad Prism 9.0, Dotmatics, San Diego, CA, USA). Fisher’s least significant difference (LSD) test was used to determine statistical differences between individual groups. \(p\) Values <0.05 were considered to indicate significance.

**Bactericidal effects on suspended P. aeruginosa**

A suspension of PA14 standardized to \(A = 0.01\) as previously described was used to test the bactericidal effects of histotripsy. Three-centimeter catheter mimics containing 40 µL of suspended PA14 were split into control and treatment groups. Histotripsy was applied using repeated scans along the length of the catheter. The 3-D motion arm and transducer were programmed to apply histotripsy at a PRF of 750 Hz and a scan speed of 5 mm/s in repeated back and forth motion across the length of the catheter. As with the biofilm analysis, catheter cavitation was monitored throughout the treatment process using ultrasound. Control catheters were again suspended in de-gassed water without undergoing histotripsy treatment. Catheters were split into a control group (\(n = 3\)) and groups receiving 2 min (\(n = 3\)), 4 min (\(n = 3\)) and 6 min (\(n = 3\)) of histotripsy treatment.
After treatment, catheter mimics were removed from the histotripsy apparatus, and the 40 \( \mu \)L of suspended PA14 within the lumen was diluted into 1 mL of diH2O and labeled as stock concentration. These stock concentrations then underwent serial dilution by removing 100 \( \mu \)L of liquid and pipetting it into 900 \( \mu \)L of diH2O. Serial dilutions were carried out from stock concentration to \( 10^{-5} \) for a total of six serial dilutions. One-hundred-millimeter LB agar plates were divided into six sections. Each section was plated with 20 \( \mu \)L of the various serial dilutions using a previously established protocol [24,25]. Plating of each dilution was done in triplicate to improve count reliability. Inoculated plates were then incubated at 37°C for 24 h. After incubation, colonies of PA14 were counted for each serial dilution that had quantifiable bacteria. The number of CFU per milliliter was determined using

\[
\text{CFU}_i = 5 \cdot C_{\text{count}} \cdot 10^{d + f} \cdot D_{i}
\]

where \( \text{CFU}_i = \) colony forming units in the initial sample, \( C_{\text{count}} = \) plate colony count, \( d = \) dilution factor and \( D_{i} = \) initial dilution of the control or treatment catheter fluid. For this experiment, the initial dilution factor was 26.

Colony-forming unit counts were recorded for each catheter mimic, with concentrations calculated as CFU per milliliter using eqn (2). Statistical analysis was performed using a one-way ANOVA, with Fisher’s LSD test used to determine statistically significant differences between individual groups using GraphPad Prism 9.0.

### Results

**Minimum histotripsy dose for biofilm removal**

The first set of experiments was aimed at determining the minimum dose of histotripsy required to successfully ablate catheter biofilms. The methods developed by Childers et al. [23] called for 500 PPP applied at a step length of 0.1 mm, correlating to 50,000 pulses per centimeter (P/cm) at a peak negative pressure of 31.5 MPa. However, we hypothesize that the minimum effective dose was lower than the dose used in that first feasibility study. To test this hypothesis, a first experiment was aimed at replicating the methods established in that article, with a stepwise reduction in PPP along the catheter (while holding step length constant). As depicted in Figure 3B and 3D, cavitation was generated focally in the catheter lumen. Careful targeting using ultrasound guidance was required to avoid pre-focal cavitation preventing the propagation of ultrasound energy into the catheter lumen. An example of this effect is depicted in Figure 3C. A setting of 500 PPP produced a dosage identical to the previously established dosage. At this dose, 96.82 ± 1.63% removal of luminal biofilm was achieved. As seen in Figure 4A, a

![Figure 2](image)

**Figure 2.** (A) Graphic depiction of the cavitation waveform. (B, C) Visual representation of both the point-based (B) and continuous (C) treatment methods for histotripsy application.

![Figure 3](image)

**Figure 3.** Optical imaging of catheter lumen prior to (A) and during (B) treatment with histotripsy, revealing intraluminal bubble cloud formation. Ultrasound imaging of both pre-focal cavitation shielding the catheter lumen (C) and properly formed intraluminal cavitation (D), with blue dotted lines indicating the location of the inner and outer catheter wall. Crystal violet staining of luminal biofilms both without (E) and with (F) histotripsy treatment.
reduction in dosage in increments of 100 down to a dose of 100 PPP continued to result in near-complete removal of biofilm for all groups. Images of CV-stained biofilms both with and without treatment (Fig. 3E, 3F) reveal the effective removal of nearly all visible biofilm. These results indicate that the minimum PPP required for biofilm removal is <10,000 P/cm. Because of device limitations (i.e., minimal dwell time for our positioning system), the PPP was unable to be lowered further than this using the stepwise treatment approach.

A second experiment was performed to further reduce the dose by increasing the step length. For this experiment, a PRF of 200 Hz was used with a PPP of 200. Step size was increased from 0.1 to 2 mm incrementally. Similar to the previous results, near-complete removal of the biofilms was observed for all groups (Fig. 4B). At a step length of 2 mm, approximately 1280 P/cm was applied, resulting in a significant reduction in the total treatment dose required for removing the biofilms. It is, however, important to note that the number of pulses applied cannot be calculated by multiplying the number of steps by the pulses applied per step. Although this is a valid estimate when shorter steps are used, it becomes increasingly unreliable as step size increases, primarily because the histotripsy transducer remains active during movement between steps. As step size increases, the time spent moving between steps becomes proportionately longer, reducing the validity of this calculation. Instead, P/cm was calculated using

\[ P/cm = \frac{t \times PRF}{L} \]  

where \( t \) = total treatment time and \( L \) = catheter treatment length.

Further reductions in histotripsy dose using the stepwise treatment method were impractical for our experiments because of both machine and software limitations, as well as an increasingly uneven application of histotripsy dose across the length of the catheter once step size exceeds the width of the generated bubble cloud. Increasing step size beyond this point produces relative peaks and valleys in dose application as the areas between points are only transiently treated as the histotripsy focal point traverses down the catheter mimic to the next treatment point. For this reason, a new method was developed in which the catheters were treated with histotripsy in one continuous motion, with variations in dose made by altering the PRF and scan speed. An experiment using this new method was performed to further reduce histotripsy dose (Fig. 5). At a PRF of 200 Hz, motor speed was varied to produce total scan times of between 5 and 60 s. The motor speeds used for this were between 0.42 and 5 mm/sec, corresponding to histotripsy doses ranging from 4800 to 400 P/cm. A statistically significant increase in post-treatment biofilm was observed at scan times of 10 and 5 s, corresponding to histotripsy doses of 800 and 400 P/cm, respectively. From these results, the minimum dose required to remove biofilms was therefore between 1600 and 800 P/cm.

Effects of PRF of biofilm removal

Although vast reductions in treatment time were made by changing the minimum dose of histotripsy required to ablate biofilm, further reductions in treatment time were also explored in a final set of experiments by increasing the PRF, thereby increasing the number of pulses applied in a given time frame. As seen in Figure 6, a series of experiments were conducted to determine if increasing PRF would allow for near-complete biofilm removal at scanning speeds faster than those achieved in previous testing. As seen in Figure 6B–D, near-complete biofilm removal was restored at scan speeds of 5 and 2.5 s by increasing PRF to 500, 750 and 1000 Hz. Further increases in PRF were considered impractical in the current study because of hardware limitations. In addition, there remains concern over seeding pre-focal cavitation at

![Figure 4](image1.png)

**Figure 4.** (A) Percentage of biofilm removed at various step lengths. No significant reduction in biofilm removal was observed in any of the treatment groups. (B) Percentage of biofilm removed at various pulses-per-point transducer settings. No significant differences in biofilm removal were observed in any of the treatment groups, with all groups exhibiting near-complete removal of the biofilms.

![Figure 5](image2.png)

**Figure 5.** Percentage of biofilm removed at various scan times across a 2.5 cm catheter. Nearly all biofilm was removed at all scan times longer than 10 s.
higher PRFs. Pre-focal cavitation, as depicted in Figure 3C, is a phenomenon in which a portion of the histotripsy cloud forms between the focal point and the transducer, shielding the propagation of ultrasound energy into the catheter lumen [26]. This effect has been reported to occur more often at higher PRF settings [27]. Future studies are needed to further optimize histotripsy for this application while minimizing any pre-focal cavitation. It should also be noted that no visible damage to the treated catheters was visually observed at any of the frequencies tested.

**Bactericidal effects of histotripsy**

Although the main purpose of this study was to develop improved methods for biofilm removal, a final set of experiments were also performed to determine if improvements over previously established bactericidal effects of histotripsy could be achieved. As with biofilm removal, a previous study by Childers et al. [23] determined that it was possible to achieve a bactericidal effect with histotripsy similar to that found with hydrogen peroxide sterilization, although the amount of time required to achieve this effect with histotripsy would likely render this approach not clinically viable. Using parameters developed in the biofilm ablation studies described previously, we conducted a set of bactericidal experiments using a motor speed of 5 mm/s and a PRF of 750 Hz. A PRF of 1000 Hz was not used in this experiment because of concerns regarding pre-focal cavitation, which could potentially shield parts of the lumen from receiving a uniform histotripsy dose. As it was expected that bactericidal effects would require a substantially higher dose of histotripsy than biofilm removal, the 3-D motion arm was programmed to repeatedly scan across a 2.5 cm length of catheter in a back-and-forth motion for 2, 4 and 6 min (Fig. 7). At 6 min of scanning, a 4.241 log reduction in viable bacteria was achieved, matching or exceeding the reported bactericidal effects of cleaning agents such as hydrogen peroxide [28], as well as meeting the standards set by various American and European guidelines, which define bactericidal effect as a 4–5 log reduction in viable bacteria, which equates to a 99.99%−99.999% reduction [29].

**Discussion**

In this study, we investigated methods to improve the efficacy and efficiency at which histotripsy can be used to ablate biofilms inside medical catheters. The results of these experiments support our hypothesis that histotripsy can be applied more efficiently and efficiently at a drastically lower dose than determined in previous studies. In addition, the results of our bactericidal study suggest that histotripsy can be used to produce bacterial cell death with sufficient treatment. Together, these results indicate that histotripsy shows promise as a novel, clinically applicable method for the non-invasive removal of bacterial biofilm of medical catheters. This was achieved by both cleaning (biofilm removal) and disinfecting (bacteria cell death) medical catheters in situ.

This study represents a substantial improvement over previously developed methods for biofilm removal and bacterial disinfection of medical catheters. Previously established histotripsy methods would require approximately 2–2.5 h of treatment for a standard-length (14–18 cm) central venous catheter or more than 6 h for a standard-length (44 cm) urinary catheter to remove the biofilms. Through use of the newly developed methods in this study, histotripsy would be able to treat biofilms in medical catheters in a drastically reduced time frame. These new methods have been determined to be effective at scanning speeds of up to 1 cm of catheter/s, correlating to <20 s to treat a typical central venous catheter and <1 min to treat a standard-length urinary catheter and more than 5 h for a standard-length (44 cm) urinary catheter to remove the biofilms.

**Figure 6.** Percentage of biofilm removed at various pulse repetition frequency settings. Experiment was performed at scan times of (A) 10 s, (B) 5 s and (C) 2.5 s across a 2.5 cm catheter. Biofilm removal efficacy was restored in all groups by increasing the pulse repetition frequency to ≥500.

**Figure 7.** Bactericidal effects of histotripsy at various treatment times. Results revealed significant decreases in viable bacterial counts in all treatment groups compared with control, with a dose-dependent response reducing bacterial viability further at higher dosages. CFU, colony-forming units.
catheter. These results were achieved through improvements in three key areas; reduction in histotripsy dose, increases in PRF and conversion of treatment method from point-based application to continuous scanning.

Among these improvements, reduction in histotripsy dose accounted for the vast majority of the decrease in scanning time. Although other studies have been performed to optimize histotripsy dose for biofilm removal, these studies have only explored histotripsy application on surgical mesh, which is an environment that necessitates both biofilm removal and bacterial sterilization [20]. This is due to the fact that surgical mesh, unlike medical catheters, cannot be non-invasively flushed with saline or a bactericidal agent to remove the bacterial biofilm debris after an ablative histotripsy dose. We envision a future treatment approach in which histotripsy is applied non-invasively for rapid removal of catheter-associated biofilms, followed by a lavage designed to safely remove detached bacteria from the catheter. This would involve withdrawing the fluid from the inside of the catheter immediately after treatment and replacing it with fresh catheter fluid, most commonly 0.9% normal saline, thereby removing the detached bacteria and biofilm from the catheter without allowing these to be introduced into the body. In addition to the reduction in histotripsy dose, further improvements were made by increasing the speed at which this dose was applied. This was achieved primarily by increasing the PRF, thereby applying more pulses in the same time frame. Several previously mentioned studies have explored biofilm ablation at up to 500 Hz, but this is the first publication to explore biofilm ablation at PRFs up to 1000 Hz. The change in methods from point-based treatment to continuous scanning also represents a notable improvement over previous studies. As described previously, prior studies on the use of histotripsy to ablate biofilms applied histotripsy pulses at discrete points along the length of the medical device. Although this is an effective method for ablating biofilm, the results of this study indicate it is significantly more time consuming. In addition, this method applies the histotripsy dose in a suboptimal distribution, with relative peaks and valleys in dose as the bubble cloud moves across the catheter, particularly when larger step lengths are used.

The use of histotripsy for bactericidal effect requires a substantially higher dose than that used for biofilm ablation, with approximately 2.4 min of scanning per centimeter of catheter required to kill nearly all planktonic bacteria in the lumen in the best case tested in this study. Because of the large disparity in treatment time between biofilm ablation and effective disinfection of planktonic bacteria, it may likely be more practical to use a biofilm ablation dose of histotripsy followed by a flush with either saline or a bactericidal agent in catheters that are easily accessible. However, bactericidal doses of histotripsy would likely represent a highly effective treatment modality for disinfecting catheters that are not externally accessible or easily replaceable, such as ventricul-peritoneal shunts, ventriculo-pleural shunts and dialysis catheters. These catheters, which are permanently implanted to relieve elevated intracranial pressure, are highly susceptible to infection. Currently, no treatment option exists to disinfect these devices except surgical removal and replacement [30], suggesting that histotripsy could be an effective option for treating these infections even without further optimizations on treatment time. This would involve an initial dose of histotripsy that would remove the bacteria and biofilm from the catheter wall, followed by repeated treatments with higher doses of histotripsy that can lyse the cells of planktonic bacteria and drastically reduce their reproductive viability.

Although this study represents a substantial step toward clinical application of histotripsy for removal of biofilms from medical catheters, several hurdles remain. In this study, we elected to use PA14 to grow biofilm in the catheter mimics, as it is well established as being a common cause of catheter-based biofilms, particularly in situations where intravenous fluids are being administered [31]. It was used in this study because it is known to be a particularly virulent strain that has been very well studied and characterized as a biofilm former on a variety of medical devices [32]. Importantly, however, it is well established in the literature that multispecies biofilms are more resistant to a variety of removal methods [33]. The use of histotripsy to treat multispecies biofilms is an area that warrants further investigation.

Improvements in targetability of histotripsy to only the catheter lumen are paramount, as it is well established that histotripsy can injure tissue in the body [34,35]. Fortunately, Childers et al. [23] determined that catheter lumens, particularly from catheters made of latex, require a lower negative pressure to induce cavitation compared with free water, producing some degree of targetability. In our experiments, we found that the bubble cloud formed in free water was slightly smaller than the 3/16 in. diameter of the catheter mimics. When the transducer was targeted at a catheter, cavitation occurred primarily within the lumen, with minimal extraluminal effect observed. Current methods, however, still require the transducer to be precisely aligned within millimeters of the center of the catheter lumen using image-guided techniques. Small deviations from the target could result in extraluminal cavitation, as the peak negative pressures used in this study are sufficient to produce cavitation in free water. Maintaining the required level of accuracy along the full length of a catheter will likely be difficult to achieve using a simple handheld device in the clinical setting. It is therefore likely that robotically guided histotripsy systems will need to be developed for precise targeting of catheter biofilms, similar to the systems in development for precise tumor ablation [36–38]. Another approach to address this challenge is the use of particle-mediated histotripsy (PMH) methods, which have recently been explored by our group [39], PMH shows great promise in producing a treatment modality that is easily targetable using a handheld transducer. In the PMH process, fluid-filled or gas-filled particles reduce the histotripsy cavitation threshold [40,41], allowing for histotripsy to be applied to the catheter lumen without the need for image-guided techniques [39], This property of PMH could allow for the rapid treatment of catheter biofilms by use of a less focused transducer that can cover a larger portion of the catheter at a single time using a simple handheld device. Future work is planned to explore this possibility and test PMH methods using the optimized treatment parameters identified in this study.

Conclusions

This study determined that histotripsy is effective at both ablating catheter-based biofilms and killing luminal planktonic bacteria. Of particular importance, the histotripsy parameter improvements explored in this study have drastically improved the speed at which histotripsy can be effectively applied to medical catheters, producing a treatment that can be applied in a clinically relevant time frame. Although several challenges remain, this study represents a substantial step forward in clinical application of histotripsy for the treatment of catheter-based biofilms.

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Conflict of interest

E.V. has an ongoing research partnership with HistoSonics, Inc. No other authors have a conflict of interest to report.

Data availability statement

The data generated for use in this study are available from the corresponding author on reasonable request.