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An IVUS Transducer for Microbubble Therapies

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Abstract

There is interest in examining the potential of modified intravascular ultrasound (IVUS) catheters to facilitate dual diagnostic and therapeutic roles using ultrasound plus microbubbles for localized drug delivery to the vessel wall. The goal of this study was to design, prototype, and validate an IVUS transducer for microbubble-based drug delivery. A 1-D acoustic radiation force model and finite element analysis guided the design of a 1.5-MHz IVUS transducer. Using the IVUS transducer, biotinylated microbubbles were displaced in water and bovine whole blood to the streptavidin-coated wall of a flow phantom by a 1.5-MHz center frequency, peak negative pressure = 70 kPa pulse with varying pulse repetition frequency (PRF) while monitoring microbubble adhesion with ultrasound. A fit was applied to the RF data to extract a time constant (τ). As PRF was increased in water, the time constant decreased ($\tau = 32.6$ s, 1 kHz vs. $\tau = 8.2$ s, 6 kHz), whereas in bovine whole blood an adhesion–no adhesion transition was found for PRFs 8 kHz. Finally, a fluorophore was delivered to an *ex vivo* swine artery using microbubbles and the IVUS transducer, resulting in a 6.6-fold increase in fluorescence. These results indicate the importance of PRF (or duty factor) for IVUS acoustic radiation force microbubble displacement and the potential for IVUS and microbubbles to provide localized drug delivery.

I. Introduction

Ultrasound and microbubbles have significant potential for localized (acoustic-focus-based) therapy and molecular imaging. The enhanced permeabilization of the cell membrane (sonoporation) resulting from the combination of ultrasound and microbubbles has been investigated in models of several diseases, including atherosclerosis [1]–[3], myocardial infarction [4]–[6], and solid cancers [7], [8], as well as drug delivery through the blood–brain barrier [7], [9], [10]. Molecularly-targeted contrast agents are being developed for imaging applications in cardiovascular disease [11] and cancer [12] in addition to their use as reagents in life science research. At low acoustic pressures, the momentum of an acoustic wave can be imparted to microbubbles, resulting in a phenomenon known as primary acoustic radiation force (ARF). This results in the displacement of the microbubbles away

from the acoustic source. The compressibility of microbubbles makes them very strong scatterers of ultrasound and also causes them to experience significant displacements caused by ARF [13], [14]. Under the influence of primary ARF, molecularly-targeted microbubbles adhere in greater numbers to a target [15]–[17] and more fluorophore has been delivered to cells [3], [18], demonstrating improvements in both molecular imaging and therapy results.

IVUS is a unique imaging modality for vascular diagnosis and therapy that provides high-resolution images, the ability to characterize tissue (e.g., lipid core, calcification, collagen) [19], [20], and easy integration with existing catheter-based interventions. Other intravascular imaging tools can be used in conjunction with IVUS to provide additional information, such as intravascular photoacoustic imaging [21], which provides cellular and molecular information, and optical coherence tomography, which provides higher resolution anatomic data with a limited penetration depth [22]. Because of their proximity to the anatomy of interest, IVUS transducers can employ high frequencies, yielding high-spatial-resolution images despite the higher acoustic attenuation at high frequencies and through anatomic barriers to ultrasound transmission (e.g., ribs and lungs) [23], [24]. Further, this proximity to the anatomy of interest greatly reduces aberration relative to transthoracic imaging. High-frequency (>10 MHz) IVUS transducers have been designed and adapted for microbubble imaging and therapy [2], [25], [26]. However, these devices are not ideal, as shown by previous *in vitro* sonoporation studies which found that lower ultrasound frequencies (<5 MHz) induce more sonoporation [27], [28]. Others have addressed the design of therapeutic IVUS transducers for thermal ablation of tumors [29], [30], renal denervation [31], and sonothrombolysis [32], demonstrating the ability to create compact low-frequency transducers. Previous work by our group demonstrated a prototype low-frequency, elongated acoustic radiation force IVUS (ARFIVUS) transducer operating at 3.5 MHz, which is much closer to typical microbubble resonance frequencies and falls within the optimal frequency range for sonoporation [33].

Atherosclerosis, the narrowing of blood vessels resulting from the accumulation of fat, cholesterol, and cell material, is a significant contributor to the development of cardiovascular disease, the number one cause of death worldwide [34]. Vessels occluded by atherosclerosis can be treated by angioplasty, a minimally invasive procedure in which a balloon catheter expands the occluded blood vessel, restoring blood flow. Following angioplasty, a stent, a mesh structure, is deployed to support the vessel. To prevent occlusion from neointimal hyperplasia, the over-growth of the smooth muscle cells, the majority of deployed stents are coated with a polymer bearing an antiproliferative drug [35]. These drug-eluting stents offer limited drug and dosing options with no mechanism for spatial control of delivery. Using IVUS, our laboratory is developing a method for drug delivery that will offer greater drug and dosing flexibility as well as spatial control of delivery.

In this study, a small-form-factor (diameter <2 mm) IVUS transducer for microbubble-based drug delivery was designed, fabricated, and validated. The compact, single-element, therapeutic IVUS transducer was designed using a finite element method (FEM) to have a center frequency of 1.5 MHz. A compact geometry was selected to produce localized sonoporation for microbubble-based drug delivery while allowing navigation of the

vasculature. Following fabrication and transducer characterization, a series of flow phantom experiments were executed to evaluate the effect of duty factor on ARF displacement and adhesion of biotinylated microbubbles to a streptavidin-coated surface by adjusting pulse repetition frequency (PRF). These experiments provide the first comparison of the optimal PRF for IVUS displacement in water and blood, demonstrating the large impact of blood on microbubble displacement with IVUS. Finally, delivery of a fluorescent model payload was performed in an *ex vivo* swine artery to demonstrate the ability of this method to localize therapeutic delivery under flow.

II. Low-Frequency IVUS Transducer Design Constraints

The design of an IVUS transducer requires a small form factor, where typical IVUS imaging catheters for coronary applications measure approximately 1 mm in diameter (i.e., 3F) [36], [37]. Conventional transducer designs use the piezoelectric ceramic thickness to control the transducer's center frequency. As transducer thickness increases, the center frequency decreases. Assuming a square cross section for the transducer, the width and thickness must be $\geq 707 \mu\text{m}$ to fit within a 1-mm-diameter catheter. To complete the transducer, a backing layer, matching/sealing layer, and electrodes are required. If these layers measure 400- μm -thick, the ceramic is constrained to a thickness of 307 μm .

Using a microbubble ARF model [14], [33], the effect of transducer center frequency on microbubble displacement was evaluated. The ARF model, based on a modified Rayleigh–Plesset model and force balance equation introduced by Dayton *et al.* [14], was implemented in Matlab (The MathWorks Inc., Natick, MA) as described previously [33]. For a 2.4- μm -diameter microbubble, this displacement was simulated during insonation with a single, 20- μs sinusoid at peak negative pressure (PNP) = 100 kPa with varying center frequency. This simulation illustrates the importance of center frequency for microbubble displacement. Much higher microbubble displacements occur at low frequencies (<5 MHz) than high frequencies (>10 MHz). The peak displacement is also markedly lower when changing the medium surrounding the microbubble from water to blood (Fig. 1).

The results of both the transducer and ARF microbubble displacement simulations illustrate the challenge of designing of an IVUS transducer for microbubble therapy: ceramic thicknesses that would produce a transducer with the appropriate center frequency for microbubble-based drug delivery are too large for coronary catheters. To design a thickness mode PZT5H-type transducer with a 1.5 MHz center frequency, a 1.35-mm-thick ceramic is required. Once a backing layer, electrodes, and a matching layer are added to this device, the total device thickness may exceed 2 mm, making the device too large to navigate the coronary vasculature.

This challenge can be addressed by designing the transducer center frequency using the lateral dimensions of a piezoelectric ceramic. Previous therapeutic IVUS catheters have been designed making use of this principal for thermal ablation [29], [30]. In this work, a 1 mm square ceramic element with a 307 μm thickness was designed and prototyped. By using the lateral dimensions to design the transducer center frequency, a thin transducer element can provide a lower center frequency than is feasible with a thickness-mode transducer.

III. Methods

A. IVUS Transducer Design With Finite Element Analysis

Finite element analysis (FEA) of an IVUS transducer was performed using PZFlex (Weidlinger Associates Inc., Mountain View, CA). To evaluate different piezoelectric ceramic options, initial simulations were performed using a 2-D FEA geometry to determine a design that would yield the greatest output near the desired resonant frequency of 1 to 2 MHz consistent with the transducer size constraints. The model geometry was kept constant and the material definition for the piezoelectric ceramic element was changed for each simulation. Four materials in particular were evaluated using the FEM to explore a range of piezoelectric ceramic options (Table I). To evaluate these materials, three additional parameters were of interest: the electromechanical coupling coefficient, the Curie temperature (T_C), and the dissipation factor ($\tan\delta$). Coupling coefficient was a property of interest because of its effects on transducer bandwidth and ring-down time. Curie temperature and dissipation factor are important because they describe the ability of a ceramic to cope with the temperature rises and energy inputs of the high duty cycles required for ARF displacement of microbubbles. The aforementioned ceramics were stimulated by a 0.5 to 10 MHz chirp function to measure the response over a wide frequency range. The FEM grid spacing was $\lambda/20$ in both the x and y dimensions (λ of water was used throughout the model), with a maximum simulation frequency of 10 MHz. Absorbing boundaries were established on all four sides of the model.

Following ceramic selection, a 3-D model of the transducer stack was simulated, consisting of backing, silver epoxy electrode, piezoelectric ceramic, and sealing layers. Symmetry boundaries were applied in the $-x$ and $-z$ dimensions to reduce computation complexity. Absorbing boundary conditions were placed at the $+x$, $+z$, $-y$, and $+y$ boundaries to prevent acoustic reflections along the model edges from corrupting the results. The model direction of propagation was in the $+y$ dimension. Model parameters were selected to enable both quick computation and accurate results, as specified in previous work evaluating the settings for finite element ultrasound transducer models [42]. The FEM grid spacing was $\lambda/20$ in all dimensions (λ of water was used throughout the model), with a maximum simulation frequency of 3 MHz. The transducer assembly was composed of a PZT5H plate, with silver epoxy electrodes on top and bottom. Additional silver epoxy provided a backing layer for the transducer. The entire assembly was surrounded by nonconductive epoxy to seal the device (Fig. 2). Material properties for the silver epoxy, nonconductive epoxy, and water layers are presented in Table II.

The transducer was excited with a 3 MHz center frequency, 100% -6 -dB fractional bandwidth Gaussian pulse to evaluate the transducer response across the frequency range of interest. To improve model efficiency, a Kirchhoff extrapolation boundary was applied around the transducer geometry [43]. Using a Kirchhoff extrapolation boundary, the acoustic output pressures, spectra, and beam profile were computed 2 mm from the transducer face.

B. Device Fabrication and Characterization

PZT5H ceramic (307 μm thick) was diced into 1-mm squares using a dicing saw (DAD 3220, DISCO Hi-Tec America Inc., Santa Clara, CA). 1.56-mm-diameter tubing was cut to accommodate the transducer ceramic, and the device was assembled as illustrated in Fig. 2. A layer of nonconductive epoxy sealed the device to prevent water damage and short-circuiting (not shown in Fig. 2).

Following fabrication, transducer impedance was measured with an impedance analyzer (HP 4194, Hewlett-Packard, Palo Alto, CA) to verify resonance at the expected center frequency. For acoustic evaluation, a water tank was filled with deionized water and degassed overnight. IVUS transducers were aligned in the water tank with a capsule hydrophone (HGL-0085, Onda Corp., Sunnyvale, CA) using a motion stage (ESP 300, Newport Corp., Irvine, CA). For beam profile measurement, software implemented in Matlab controlled transducer position and captured hydrophone output via an oscilloscope with GPIB connection (LC334, LeCroy, Inc., Chestnut Ridge, NY). The transducer's frequency profile was measured by exciting with a 3 MHz center frequency, 100% –6-dB fractional bandwidth Gaussian pulse and performing frequency division to prepare an approximation of the transfer function. This wide-bandwidth, higher frequency pulse was applied to detect any spurious higher frequency modes. Transducer output PNP and beam profile were measured while exciting with a 1.5 MHz center frequency, 100% –6-dB fractional bandwidth Gaussian. Transducer input signals were generated with an arbitrary function generator (AWG2021, Tektronix Inc., Beaverton, OR) and 50-dB RF amplifier (ENI325LA, ENI, Rochester, NY).

Transducer characterization data were processed offline using Matlab software. The transducer transfer function was estimated by performing frequency domain division of the normalized received acoustic signal and excitation pulses. Transducer beam profiles were mapped by measuring the PNP at each position and normalizing to the greatest value.

C. Flow Phantom Microbubble Displacement

Gelatin flow phantoms were prepared with vessel-mimicking channels as previously described [44]. Briefly, gelatin (6% w/v) and agar (1% w/v) were dissolved in boiling deionized water. After allowing the gelatin solution to cool before solidifying, the solution was poured into a mold which produced a 4.5-mm-diameter cylindrical channel in the phantom, mimicking the coronary artery [45]. After solidifying, tubes were removed from the mold to expose the channels and a solution of streptavidin (S0677, Sigma Aldrich, St. Louis, MO) at a concentration of 0.05 mg/mL was infused and allowed to coat the channel for a minimum of 2 h while refrigerated.

To evaluate catheter performance under flow, an IVUS transducer was inserted into the channel (Fig. 3). A dispersion of Targestar-B biotinylated microbubbles (Targeson Inc., San Diego, CA) was diluted to a concentration of 2.22×10^6 microbubbles/mL in deionized water or 3 to 3.3×10^6 microbubbles/mL in bovine whole blood (hematocrit = 40% to 50%). The microbubble dispersion was constantly stirred using a magnetic stir plate to maintain a uniform concentration. A syringe pump (PHD 2000, Harvard Apparatus, Holliston, MA)

pulled the dispersion of microbubbles from the proximal to distal end of the catheter (left to right in Fig. 3) into the channel at a 30 mL/min flow rate. Throughout the experiment, an ARF and pulse inversion (PI) imaging sequence was applied using the SonixRP (Ultrasonix, Richmond, BC, Canada) and a 64-element linear array ultrasound transducer [46]. The SonixRP-generated ARF pulse displaced the microbubbles to the bottom of the channel, preventing microbubble flotation, as shown in Fig. 3(a). All imaging and microbubble displacement with the SonixRP was performed at a center frequency of 8 MHz.

The IVUS transducer was then excited by an ARF pulse. In deionized water, the transducer was excited by a 1.5 MHz center frequency, 30 μ s, PNP = 70 kPa Gaussian ramped sinusoid with PRF = 1 to 6 kHz in 1 kHz increments, displacing microbubbles within the beam of the IVUS transducer to the top of the channel [Fig. 3(b)]. In bovine whole blood, the same pulse was applied with PNP = 155 kPa and PRF = 4 to 13 kHz in 1 kHz increments, excluding 7 kHz, to determine the optimal PRF.

Following the experiments, data were processed using Matlab software. A Chebyshev filter and a high-pass FIR filter were applied to the raw RF data to filter the tissue-mimicking phantom and IVUS transducer signals, respectively. Summing the two inverted pulses of the PI imaging sequence facilitated separation of the signal arising from the nonlinear scattering of microbubbles from signals arising from the linear scattering within the phantom. After summing the PI pulses, a slow time averaging filter was applied to reduce the signal from nonadherent microbubbles [46], [47]. A region of interest was selected in each slow time filtered video, and the change in average RF intensity for the region of interest was measured over time. The exponential decay function

$$\text{RF}_{\text{average}} = A(1 - e^{-t/\tau}), \quad (1)$$

was fit to each log-compressed RF intensity curve and the resulting coefficients (A = RF peak intensity in decibels, τ = time constant in seconds) were averaged to measure the effect of PRF on microbubble adhesion.

D. Ex Vivo Model Drug Delivery

Freshly harvested swine carotid artery was placed in a room temperature physiological saline solution (PSS) bath and Luer lock fittings were attached to each end of the artery. Tubing was then connected to the Luer lock fittings to flow a dispersion of microbubbles in 40% to 45% hematocrit bovine whole blood through the artery. Microbubble concentration in bovine whole blood was 8×10^6 microbubbles/mL. A hydrophobic fluorescent marker, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine (DiI) was embedded in the microbubble shell [48]; this enabled ready visualization of the location of delivery by epifluorescence imaging. Using an apparatus similar to that used in the flow phantom experiments, the IVUS catheter was inserted into the artery and the linear array was positioned above the artery using the PSS as a coupling medium. The SonixRP and linear array provided image guidance and prevented microbubbles from floating.

For 30 s, the IVUS was pulsed with a 1.5 MHz center frequency, 30 μ s Gaussian ramped sinusoid with a PNP = 155 kPa at 2 mm and PRF = 10 kHz. Following the 30 s of microbubble ARF translation, a 1.5 MHz center frequency, 20% –6-dB fractional bandwidth Gaussian with a PNP = 210 kPa at 2 mm and PRF = 1 kHz was applied for 30 s to destroy the microbubbles, releasing the DiI and causing delivery. The translation and destruction pulses were performed three times to deliver DiI to the vessel wall. After each one-minute treatment, the artery was flushed with phosphate-buffered saline (PBS) and the microbubbles were subjected to a destruction sequence from the external linear array to prevent microbubble accumulation caused by flotation.

The treated artery was fixed in 4% paraformaldehyde solution for 24 h, stored in a 30% sucrose solution for up to 24 h, and frozen in optimal cutting temperature (OCT) compound to prepare for frozen sectioning. The sectioned artery was imaged with a fluorescent microscope (TE300, Nikon, Melville, NY) to detect DiI uptake (ex = 550 nm, em = 620 nm). Images were analyzed in ImageJ (National Institutes of Health, Bethesda, MD) to determine average intensity, along the vessel wall by dividing the vessel wall into 46×42 μ m segments. Segments were averaged along the artery wall, yielding 10 samples for each image, which were analyzed to determine the average fluorescence measured in arbitrary intensity units for each treated region. Three artery segments were analyzed, one in a control region without DiI delivery distal to the IVUS transducer, and two in treated regions along the same artery segment that received DiI delivery, located 180° from one another. Fluorescence images in the treated regions were only collected where DiI fluorescence was detected at 0° and 180° with respect to the front of the IVUS transducer. The resulting average intensity in the control region was used to calculate the increase in fluorescence intensity from background for the two treated segments, measured as an n -fold increase.

E. Statistical Analysis

A student's t -test was used to compare the time constant, τ , in flow phantom experiments between different PRF and the fluorescence intensity in the treated versus untreated regions of the *ex vivo* artery treated with DiI. Analysis of variance (ANOVA) was applied to determine significance among the group of PRFs used to displace microbubbles in the flow phantom experiments. $p < 0.05$ was considered significant.

IV. Results

A. Finite Element Analysis and Prototype Characterization

The most promising ceramics based on the FEM results were the PZT5H and PMN-PT ceramics [Fig. 4(a)]. The wide bandwidth characteristics of the single-crystal PMN-PT were readily apparent from these simulations, making it a good fit for applications requiring transmission across a wide range of frequencies. Both ceramics demonstrated superior performance in the desired frequency range of 1 to 2 MHz, although PZT5H has a higher bandwidth and more distinct resonance. Ultimately, PZT5H was selected instead of PMN-PT because of its higher Curie temperature and easier machinability [49], [50].

The completed IVUS transducer exhibited a lateral center frequency of 1.49 MHz with a 56% –6-dB fractional bandwidth [Fig. 4(b) and Table III]. This center frequency matched

well with the 1.42 MHz center frequency of the simulated transducer and the -6 -dB fractional bandwidth of 64%. Output pressures from IVUS transducer prototypes were as high as 200 kPa. A comparison of beam profiles also demonstrated a close match between the simulated and measured results, with experimental -6 -dB beam widths of 2.4 mm in the elevation and 3 mm in the azimuth dimension, and the simulated beam width measured to be 2.6 mm. To verify the safety of the IVUS transducer, a thermocouple was attached to the face of the transducer, the assembly submerged in a water bath, and the change in temperature measured over a 60-s period when pulsing with a 20% duty cycle and a voltage that matched the *ex vivo* experiments. This resulted in less than 1°C change in temperature.

B. Flow Phantom Microbubble Displacement

Images following PI and slow time filtering from a flow-phantom microbubble displacement experiment illustrate the localized accumulation of microbubbles within the IVUS transducer beam (Fig. 5). Before microbubble infusion, the IVUS transducer was the only visible feature in the channel [Fig. 5(a)]. After 30 s, microbubbles accumulated along the upper channel wall where the IVUS transducer was applying an ARF pulse to displace the microbubbles, resulting in an increase in RF intensity [Fig. 5(b)]. Outside the IVUS beamwidth, there was also an increase in RF intensity as microbubbles accumulated along the bottom channel wall because of the SonixRP's downward ARF [Fig. 5(c)]. In a representative averaged RF intensity time plot, upon microbubble infusion and application of the IVUS ARF, image intensity in the region of interest above the IVUS transducer increased [Fig. 5(d)]. Also included in the plot is the experimental fit using (1) to determine the time constant and peak intensity coefficients for comparisons across PRF.

After completing 8 to 10 experiments for each PRF in water and 2 to 6 experiments for each PRF in bovine whole blood, the τ and peak RF intensity for each curve fit were averaged by PRF and plotted (Fig. 6). A one-way ANOVA found no significant difference between PRF when monitoring peak RF intensity in water [Fig. 6(a)]. Across PRF there was a significant difference in time constant when microbubbles were displaced in water. An underlying trend of decreasing time constant was measured with increasing PRF [Fig. 6(b)]. However, in bovine whole blood there was a significant difference in peak RF intensity [Fig. 6(c)] but no significant difference across time constants [Fig. 6(d)]. Based on the peak RF intensity parameters, no microbubble accumulation occurred from PRF = 4 to 6 kHz. Above 8 kHz, the peak RF intensities indicated that microbubble accumulation was occurring. The similar time constants for PRF = 8 kHz indicated that microbubble accumulation occurred at a similar rate for these PRF. When PRF = 8 to 13 kHz in bovine whole blood, $\tau_{\text{mean}} = 14.7 \pm 4.6$ s and when PRF = 6 kHz in water, $\tau_{\text{mean}} = 8.2 \pm 1.7$ s.

C. Ex Vivo Model Drug Delivery

Fluorescence microscopy images were collected in the swine carotid artery in treated and untreated regions (Fig. 7). A significant increase in fluorescence intensity of 665% was measured between the untreated section [Fig. 7(a)] and the treated section in the transducer beam [Fig. 7(b)] ($p < 0.001$). An increase in fluorescence intensity of 166% was measured between the treated section within the transducer beam [Fig. 7(b)] and the segment 180° from this treated region [Fig. 7(c)] on the same histological section (Fig. 8) ($p = 0.035$). The

high level of fluorescence in this untreated region of the same histological section was probably caused by acoustic energy leaking from the backing of the transducer, which had an unfilled (i.e., low acoustic loss) backing. Calibrated hydrophone measurements and FEM results verified the leaking acoustic energy (data not shown). Replacing this with a particle-filled backing or an aerogel could localize the effects of the IVUS by reducing acoustic energy emanating from the backing of the transducer [30], [51].

V. Discussion and Conclusion

A prototype IVUS transducer was designed using a 1-D ARF model for guidance and FEA to select dimensions and piezoelectric ceramic. Generally, transducer designs attempt to prevent lateral modes because of their lower coupling coefficients (for PZT5H $k_{33} = 0.78$ and $k_{31} = 0.43$) and potential to distort the frequency response of the intended thickness modes [24]. However, the transverse frequency constants of PZT ceramics are much lower than the thickness frequency constants (1410 versus $1966 \text{ m} \times \text{Hz}$ in PZT5H), and backing and matching layers are only applied in the thickness dimension, potentially limiting the thickness of the transducer. For these reasons, we designed our low-frequency IVUS transducer to operate in a lateral mode. The completed device had a center frequency of 1.49 MHz, and a 56% –6-dB fractional bandwidth, making it suitable for both localization of microbubbles with ARF and sonoporation according to the simulations of this study and previous studies [27], [28]. The center frequencies of the simulated and fabricated IVUS transducer were in agreement (1.42 MHz and 1.49 MHz, respectively) with similar bandwidths (56% and 64%, respectively). The main lobe of the beam profile of the fabricated device was wider than the simulated result. These differences between the simulated and measured transducer spectra were likely caused by differences in the geometry and thicknesses of backing materials. The beam profile differences may be accounted for by the positioning of the transducer's top electrode interconnect directly on the transducer face, resulting in an asymmetric beam profile in both the azimuthal and elevational dimensions. The narrower beam profile may be due to the difference in simulated and measured frequency response.

The resulting RF intensity time plots from microbubble accumulation match previous results shown by Patil *et al.* [46]. By adjusting PRF for microbubble displacement in water, it was found that the time to reach peak microbubble adhesion under flow decreased with increasing PRF (Fig. 6). In contrast to this decrease in τ in water, it was found that the rate of adhesion of microbubbles under flow in bovine whole blood was binary, with PRF < 8 kHz resulting in no microbubble adhesion and τ measurements remaining the same for PRF ≥ 8 kHz. The presence of red blood cells may increase the probability of bound microbubbles being dislodged under flow. This negative feedback to microbubble adhesion may prevent secondary radiation forces from taking effect at the vessel wall at lower PRF, resulting in the all-or-nothing microbubble adhesion found in bovine whole blood at 8 kHz PRF. Upon reaching the primary and secondary acoustic radiation force requirements to overcome dislodgement caused by red blood cells, the adhesion of microbubbles reaches its maximum accumulation. It is also important to note the approximately 2-fold increase in PNP needed to displace the microbubbles in bovine whole blood as compared with water. This increase in PNP may have been necessary because of the higher acoustic attenuation in

blood or the effect of viscosity on microbubble oscillations and the force required to displace the microbubble through the medium (Fig. 1).

This comparison of microbubble accumulation in bovine whole blood and water demonstrates the importance of accounting for the increased resistance to microbubble displacement when using acoustic radiation force to localize delivery *in vivo*. If acoustic parameters are selected in water, the effectiveness of these results will not translate to blood. In fact, parameters that are adequate in water may not cause any microbubble accumulation in blood. This conclusion agrees with previous work performed by our group for transcutaneous acoustic radiation force application [44].

Using the time constant as a minimum treatment time within the IVUS beam yields a treatment time of 4 min for a 30-mm-long lesion. Adjusting pulse length, acoustic pressure, or applying a variable frequency pulse [52] may decrease the overall treatment time. Increasing blood flow velocity or vessel diameter is expected to increase the time constant. Increased turbulence will improve microbubble mixing, which could result in shorter displacement distance for some microbubbles to the vessel wall or disruption of microbubbles that have localized at the vessel wall.

Delivery of DiI to an *ex vivo* swine artery under flow was localized. This work however cannot determine if sonoporation occurred in these *ex vivo* arteries because the experiments were conducted at room temperature. However, other research has demonstrated that at comparable pressures and frequencies, sonoporation can be induced *in vitro* [53]–[55]. Future studies using *in vitro* and *in vivo* models may be performed to verify that sonoporation is induced by this system. The 6.6-fold increase in fluorescence intensity between the untreated region of the artery and the treated region demonstrates localization along the artery length. Localization along the circumference was only partially demonstrated because of the leaky backing of the ultrasound transducer, which resulted in a 4-fold fluorescence intensity increase behind the IVUS transducer. If a lossier backing is applied, an improvement in localization of delivery can be achieved along the circumference of the artery. However, this still demonstrates that IVUS designs using lateral modes can provide better localization than radially transmitting IVUS transducers that are currently available for therapeutic ultrasound [31], [32]. The microbubble dose used in this study is comparable to the concentrations used in imaging studies, which are usually on the order of 10^6 microbubbles/mL. For clinical applications, such as drug delivery, the microbubble dose will have to be tailored to the patient, using the microbubble concentration to set the dose of drug based on the concentration of drug in the microbubble shell.

One of the key potential advantages of intravascular drug delivery with IVUS and microbubbles is the ability to implement a “see, treat, and verify” model. This prototype IVUS transducer demonstrates that IVUS can provide displace and treatment capabilities under flow conditions. An IVUS and microbubble-based delivery platform has the potential to provide new treatments for atherosclerosis, brain tumors, and other diseases accessible through the vasculature.

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Fig. 1. Simulated lipid shelled microbubble displacements with varying center frequency using 1-D ARF microbubble displacement model [14]. Black lines were simulated in water; red lines were simulated in blood. Solid line = 2- μm -diameter microbubble; dashed line = 3- μm -diameter microbubble; dash-dotted line = 4- μm -diameter microbubble. The microbubbles were stimulated with a single 20 μs , PNP = 100 kPa Gaussian ramped sinusoid.

Fig. 2.

IVUS transducer schematic diagram. The ceramic thickness in the y -dimension yields a thickness-mode frequency of 6.6 MHz (unused), whereas the width and length in the x - and z -dimensions produce a lateral mode frequency of 1.5 MHz (therapeutic). Materials in the schematic diagram are represented by the following colors: yellow = PZT5H (3203HD, CTS Corporation, Albuquerque, NM), black = silver epoxy (CHOBOND 584, Parker Hannifin Corp., Woburn, MA), white = nonconductive epoxy (RE2039/HD3561, Henkel Corp., City of Industry, CA), blue = tubing, and gray = wires. The final sealing layer of nonconductive epoxy is omitted to make the assembly visible in the schematic diagram.

Fig. 3.

Flow phantom apparatus used for microbubble translation under IVUS radiation force experiments [46]. (a) Without the IVUS transducer to displace the microbubbles, the Sonix RP ultrasound scanner caused adhesion of microbubbles on one side of the channel. The SonixRP ultrasound scanner performed both PI imaging and ARF translation with a linear array transducer. (b) A single-element catheter inserted into the phantom channel translated microbubbles to cause microbubble adhesion to the top of the channel wall.

Fig. 4.

FEM design of a low-frequency IVUS transducer. (a) Simulated transmit spectra for four ceramics. Ceramics were excited with a chirp pulse from 0.5 to 10 MHz in the FEM. (b) Transfer function of the fabricated and FEM-simulated PZT5H transducers. (c) Experimental azimuth, experimental elevation, and simulated azimuth/elevation beam profiles measured 2 mm from the PZT5H transducer face.

Fig. 5.

ARF microbubble displacement using a 1.5 MHz center frequency, 30 μ s Gaussian ramped sinusoid with PNP = 70 kPa at 2 mm and PRF = 10 kHz. Example frames from (a) 4 s, (b) 30 s, (c) 45 s. The black rectangle indicates the region of microbubble accumulation caused by the IVUS transducer. (d) Average change in RF intensity during microbubble displacement in the region of interest—within the white rectangle in (a–c)—for ARF displacement pulses.

Fig. 6.

Average fit parameters when the PRF of the microbubble displacement pulse was varied (a–b) in deionized water and (c–d) in bovine whole blood. (a) The peak RF intensity when varying PRF. (b) The time constant when varying PRF. (c) The peak RF intensity when varying PRF. (d) The time constant when varying PRF. ($n = 8$ for water, $n = 3$ for bovine whole blood except for 6 kHz where $n = 2$. Data displayed as mean + SD. # = $p < 0.05$ compared with PRF = 1 kHz, * = $p < 0.05$ compared with PRF = 1 kHz and 2 kHz, and + = $p < 0.05$ compared with PRF = 4 to 6 kHz.)

Fig. 7. Microscope images from the IVUS and microbubble-based DiI fluorophore delivery in a swine carotid artery. (a) Untreated swine artery region. (b) Treated swine artery region within the transducer beam. (c) Untreated swine artery region, 180° from the transducer beam in (b).

Fig. 8.

Average increase in fluorescence intensity along the artery wall across from the transducer face [Fig. 7(b)] and through the transducer backing [Fig. 7(c)] as compared with an untreated segment [Fig. 7(a)], measured in Fig. 7. The average fluorescence intensity for the treated region at the transducer face and through the transducer backing were significantly higher than the untreated segment ($p < 0.05$). The increase in average fluorescence intensity for the segment facing the transducer front was significantly higher than for the segment facing the backing ($p < 0.05$).

TABLE I

Properties of Selected Piezoelectric Ceramics.

Ceramic	k_{33}	T_C (°C)	$\tan \delta$ (%)
PZT5H [38]	0.75	225	2.0
PZT4 [39]	0.69	320	0.4
PbTiO ₃ [40]	0.4	400	1.4
PMN-PT [41]	0.9	90	0.5

TABLE II

Finite Element Material Properties.

Material	c_l (m/s)	c_s (m/s)	ρ (kg/m ³)
Water	1540	0	1000
Silver epoxy	1900	950	2500
Hard epoxy	1800	800	1150

TABLE III

Fabricated Transducer Properties.

Thickness mode f_c	6.6 MHz
Lateral mode f_c	1.49 MHz
-6-dB fractional bandwidth	56%
-6-dB elevation beam width	2.4 mm
-6-dB azimuth beam width	2.6 mm