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Localized *In Vivo* Model Drug Delivery with Intravascular Ultrasound and Microbubbles

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Abstract

An intravascular ultrasound (IVUS) and microbubble drug delivery system was evaluated in both *ex vivo* and *in vivo* swine vessel models. Microbubbles with the fluorophore DiI embedded in the shell as a model drug were infused into *ex vivo* swine arteries at a physiological flow rate (105 mL/min) while a 5 MHz IVUS transducer applied ultrasound. Ultrasound pulse sequences consisted of acoustic radiation force pulses to displace DiI-loaded microbubbles from the vessel lumen to the wall followed by higher intensity delivery pulses to release DiI into the vessel wall. Insonation with both the acoustic radiation force pulse and the delivery pulse increased DiI deposition 10-fold compared to deposition with the delivery pulse alone. Localized delivery of DiI was then demonstrated in an *in vivo* swine model. The theoretical transducer beam width predicted the measured angular extent of delivery to within 11%. These results demonstrate that low frequency IVUS catheters are a viable method for achieving localized drug delivery with microbubbles.

Keywords

Intravascular Ultrasound; Microbubbles; Drug Delivery; Acoustic Radiation Force; *In Vivo*

Introduction

A variety of diseases can benefit from intravascular interventions. As a disease of the vasculature, atherosclerosis is an ideal target for intravascular interventions. Atherosclerosis is a narrowing of blood vessels due to the accumulation of plaque. Left untreated, these

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plaques may rupture, resulting in myocardial infarct or stroke (Libby et al., 2011). Although commonly treated by medical therapy, when acute symptoms are presented, surgical intervention frequently becomes necessary. Because it is a minimally invasive procedure, percutaneous coronary intervention (PCI) is performed in twice the number of patients than coronary artery bypass grafts, another surgical technique for treating atherosclerosis (Roger et al., 2011). During PCI, angioplasty is performed to expand the vessel using a balloon catheter. To support the expanded vessel, a mesh structure (i.e. a stent) is deployed at the site of balloon injury (Garg and Serruys, 2010). Following PCI, reocclusion of the vessel due to excessive vascular smooth muscle cell proliferation, termed neointimal hyperplasia, may occur. In order to minimize the risk of reocclusion, the patient can be treated using systemically administered medication, drug eluting stents, or drug eluting balloons to deliver an antiproliferative agent to the site of injury (Boden et al., 2007; Garg and Serruys, 2010; Byrne et al., 2012; Agostoni et al., 2013). Although both drug eluting stents and balloons have the ability to deliver a therapeutic agent locally, both are limited by fixed drug and dosing options (De Labriolle et al., 2009). Drug eluting stents also fail to achieve complete vessel wall coverage because drug delivery is limited to the vicinity of the stent struts (Hwang et al., 2001; Takebayashi et al., 2004).

Ultrasound-stimulated microbubbles have been widely demonstrated as a method for localized drug delivery (Unger et al., 1998; Klibanov, 2006; Ferrara et al., 2007). When ultrasound is applied to microbubbles in contact with cells, the microbubbles oscillate and induce transient cell membrane permeabilization, termed “sonoporation.” This transient permeabilization of the cell membrane enhances molecular uptake into the cell (Deng et al., 2004; Fan et al., 2012). This phenomenon has been applied to treat a number of indications, including the enhancement of angiogenesis following ischemia (Korpany et al., 2005), delivery of chemotherapy to a brain tumor (Kinoshita et al., 2006), and the prevention of neointimal formation following balloon injury (Phillips et al., 2011). *In vitro* studies have concluded that enhanced uptake is greatest at low frequencies (<5 MHz) (Meijering et al., 2007; Karshafian et al., 2009) and with the application of acoustic radiation force to direct microbubbles to the site of delivery (Shortencarier et al., 2004; Rychak et al., 2007; Patil et al., 2011).

Intravascular ultrasound (IVUS) is an ultrasound imaging mode that provides high resolution cross sectional images of the vasculature. IVUS imaging has been demonstrated to improve patient outcomes during PCI (Uren et al., 1998; Stone et al., 1999; Fitzgerald et al., 2000). Because acoustic radiation force displacement and sonoporation with microbubbles is greatest at low frequencies (Dayton et al., 2002; Karshafian et al., 2009), the high frequencies (>20 MHz) required to produce high resolution IVUS images are not suitable for microbubble-based drug delivery. There has been limited development and evaluation of low frequency IVUS transducers for therapy because low frequency thickness mode ultrasound transducers require dimensions that are incompatible with intravascular applications (Mahon et al., 2003; Herickhoff et al., 2011; Mabin et al., 2012; Patel et al., 2013; Kilroy et al., 2014). Investigations of IVUS for microbubble applications are also limited and focused on imaging applications because of the low microbubble resonance frequencies which are incompatible with current high frequency IVUS transducers (Frijlink

et al., 2006; Goertz et al., 2006). In order to improve microbubble localization under physiological flow, our group has previously published the design of an IVUS transducer for the acoustic radiation force displacement of microbubbles under physiological flow (Kilroy et al., 2012).

The present study investigates the viability of using IVUS and microbubbles for localized drug delivery in the vasculature. In order to accomplish this, a prototype low frequency IVUS transducer was designed to match the resonance frequencies of lipid shelled microbubbles while being dimensionally compatible with the vasculature. A series of *ex vivo* experiments were performed to evaluate acoustic parameters for the delivery of a fluorophore (DiI) from microbubbles using the prototype IVUS catheter in blood under physiological flow. Finally, using the acoustic parameters selected from the *ex vivo* experiments, DiI-loaded microbubbles were infused into a swine model while the prototype IVUS transducer applied ultrasound to deliver DiI-loaded microbubbles to the vessel wall. Localized and distributed delivery along the artery circumference are both demonstrated in the swine model using the prototype IVUS system.

Materials and Methods

DiI-Loaded Microbubbles

Microbubbles (MB) were formulated as described previously (Phillips et al., 2011). Briefly, microbubbles were prepared by sonicating a dispersion of phosphatidylcholine (2 mg/mL) (Avanti Lipids, Alabaster, AL, USA), polyethylene glycol stearate (2 mg/mL) (Sigma Chemical Co., St. Louis, MO, USA), and the fluorescent dye 1,1'-dioctadecyl-3,3,3'-tetramethylindocarbocyanine ($\approx 1\%$ molar ratio DiI:DSPC) (DiI, Molecular Probes, Eugene, OR, USA) in the presence of decafluorobutane (DFB, Flura, Newport, TN, USA). Prior to use, microbubbles were washed via centrifugation to remove excess lipids and DiI. For *ex vivo* experiments, microbubbles were washed by centrifuging in a 3 mL syringe containing 0.8 mL of microbubble stock in 2.2 mL of DFB saturated phosphate buffered saline (PBS) at 1000 RPM ($225 \times g$), for periods of 10, 6, and 6 minutes. In previous experiments (Phillips et al., 2011b), it was experimentally determined that 3 washes removed the majority of excess DiI and lipids from the microbubbles. After each centrifugation, the infranatant was drained and additional DFB saturated PBS added. Microbubbles were used within 6 days of washing for *ex vivo* experiments and within 24 hours of washing for *in vivo* experiments. The average microbubble diameter was $2.2 \mu\text{m}$.

Intravascular Ultrasound Transducer and System

A custom 5.1 MHz mechanically rotated single element IVUS transducer was fabricated for insonating microbubbles. Finite element analysis (PZFlex, Weidlinger Associates Inc., Mountain View, CA, USA) was performed to select the dimensions for a 5 MHz center frequency transducer that fit within a $500 \mu\text{m} \times 700 \mu\text{m}$ area. A center frequency of 5 MHz was selected match to the resonance frequency of the microbubbles and to provide a frequency capable of sonoporation (Dayton et al., 2002; Karshafian et al., 2009; Kilroy et al., 2012). A PZT-4 type ceramic (EBL#1, EBL Products Inc., East Hartford, CT, USA) was diced into $0.25 \text{ mm} \times 0.7 \text{ mm}$ elements and backfilled with non-conductive epoxy (RE2039/

HD3561, Henkel Corporation, City of Industry, CA, USA). PZT-4 was selected because it is a “hard” ceramic, exhibiting low dielectric losses and a high Curie temperature, making it compatible with high duty factor operation. The ceramic from a commercial IVUS catheter (Volcano Revolution, Volcano Corp., San Diego, CA, USA) was removed, a thin layer of silver epoxy was applied (CHOBOND 584, Parker Hannifin Corp., Woburn, MA, USA), and the custom ceramic cured in the catheter casing. The signal wire from the IVUS catheter was then placed on the top electrode of the custom ceramic and secured using a small quantity of silver epoxy. After curing the silver epoxy for 3 hours at 60°C, a thin layer of non-conductive epoxy (RE2039/HD3561, Henkel Corp., City of Industry, CA, USA) was applied and cured to seal the device. The final catheter diameter when enclosed in the sheath was approximately 1 mm.

An arbitrary function generator (AFG3022B, Tektronix, Inc., Beaverton, OR, USA) provided the electrical pulse and was controlled using MATLAB (The Mathworks, Inc., Natick, MA, USA) software on a personal computer to switch pulse amplitude and pulse length. The pulse was then amplified by a 55 dB RF amplifier (A150, ENI, Rochester, NY, USA), and coupled to the IVUS transducer through a custom slip ring and rotation assembly. During insonation, the transducer was rotated at a rate of 250 RPM. Prior to use in *ex vivo* and *in vivo* experiments, all transducer ultrasound pressures were measured using a calibrated hydrophone (HGL-0085, Onda Corporation, Sunnyvale, CA, USA) in a degassed water tank. A -6 dB beam width of 1 mm in elevation and 1.5 mm in azimuth was measured 2 mm from the transducer.

Ex Vivo Assessment of Delivery Parameters

Ex vivo swine carotid arteries were used to investigate the effects of different ultrasound parameters on model drug delivery. Common carotid arteries were harvested from swine at a local abattoir, immediately immersed into physiological saline solution (PSS) and put on ice. Bovine blood was collected from recently slaughtered farm cows and mixed with a solution of EDTA (195.75 mg/L). Bovine blood was used instead of swine blood because a larger volume was available. Each artery was cut to a 4 cm length and luer locks were secured to each end with sutures. The artery was then connected to a flow loop and placed in a custom chamber with an acoustically transparent window. 37°C PSS was constantly pumped through the chamber, submerging the artery. A transcutaneous ultrasound transducer was coupled to the chamber window using ultrasound coupling gel and the lumen of the artery was imaged using a research ultrasound scanner (Verasonics, Inc., Redmond, WA, USA) (Fig 1a). Ultrasound imaging was performed at 9 MHz center frequency, MI=0.04, and PRF=30 Hz. PBS was infused into the artery using a syringe pump (PHD-2000, Harvard Apparatus, Holliston, MA, USA). The IVUS catheter was then positioned at four different longitudinal locations within the artery using the ultrasound images for guidance.

A dispersion of DiI-loaded microbubbles was prepared in 40-45% hematocrit bovine blood at a concentration of 2.25×10^6 MB/mL based on previously published concentrations (Rahim et al., 2006; Phillips et al., 2010; Patil et al., 2011). Blood was chosen as a medium because previous research has demonstrated that microbubble displacement in blood requires higher duty cycle ultrasound than saline or water (Patil et al., 2011; Kilroy et al.,

2014). The dispersion was infused into the artery for one minute at a flow rate of 105 mL/min to model flow in the human coronary artery. The average artery diameter was 5.78 ± 0.1 mm, corresponding to a laminar flow velocity of 13.3 cm/s, similar to the averaged peak velocity in a human coronary artery (Hozumi et al., 1998). Following each infusion of DiI-loaded microbubbles, PBS was infused into the artery for 1 minute to remove excess microbubbles from the artery.

Four different IVUS acoustic parameter sets were applied at four different longitudinal locations within each artery (Table 1). For each treatment, two pulses are defined, the acoustic radiation force pulse (P1) and the delivery pulse (P2). The acoustic radiation force pulse was a long (500 cycle), high PRF (5 kHz), and low peak negative pressure (PNP = 600 kPa) pulse, designed to displace microbubbles out of flow to the vessel wall. The delivery pulse was a short (50 cycle), low PRF (0.5 or 1 kHz), and high PNP (1 or 2 MPa) pulse, designed to induce DiI release and uptake by cells. The first four acoustic parameter sets (Table 1a-d) were used to evaluate the effect of the delivery pulse (P2) on fluorescence intensity due to DiI uptake. The second four acoustic parameter sets (Table 1e-i) were designed to evaluate the balance between acoustic radiation force (P1) and delivery (P2) by adjusting the percentage of time dedicated to acoustic radiation force as opposed to delivery (Table 2). Ultrasound conditions were selected based on previous research by our group and others (Rahim et al., 2006; Phillips et al., 2010; Burke et al., 2012; Dixon et al., 2013). Each longitudinal location received four consecutive 15 second insonations divided between acoustic radiation force and delivery pulses as described by P1% (Table 1 - Column 1). Every acoustic parameter set was applied in a total of three arteries.

Following treatment, arteries were removed from the Luer lock connectors, cut axially, and laid flat on a glass cover slip. Arteries were imaged on a confocal microscope (LSM700, Carl Zeiss Microscopy LLC, Thornwood, NY, USA) using a 555 nm excitation. Images were collected along the circumference of the artery and stitched together to produce a mosaic in the ZEN software.

Fluorescence intensity increase along the region of treatment was measured using software created in MATLAB. A 250 μ m wide region delimited the edges of a region of interest. The logarithm of the mean fluorescence intensity increase of the treated region from the surrounding untreated artery region was determined for each acoustic setting in order to compare different acoustic parameters for treatment.

In Vivo Delivery

Prior to the *in vivo* swine model experiment, a microbubble ejection port was created in the IVUS catheter sheath 2 cm proximal from the IVUS transducer tip with a 21 G needle. The IVUS catheter and all associated tubing and connectors were assembled connecting a microbubble filled syringe to the catheter's saline infusion port. While the IVUS transducer rotated, a 296×10^6 MB/mL microbubble dispersion was infused at a rate of 1 mL/min. A sample of the microbubbles was collected from the catheter's microbubble ejection port. Microbubble concentration was then measured using a Coulter Multisizer (Multisizer 3, Beckman Coulter Inc., Brea, CA, USA) to determine the microbubble losses due to infusion. This was repeated three times and a mean microbubble loss of 60% with a standard

deviation of 8% was measured. This guided the final number of DiI microbubbles infused, which was 3 fold higher than used in *ex vivo* experiments to account for the catheter infusion losses.

A domestic Yorkshire farm pig was selected as a model for PCI in the coronary artery. Animal protocols were approved by the University of Missouri Animal Care and Use Committee in accordance with the “Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training.” The pig was anesthetized and catheterized prior to balloon injury. Balloon injury was performed in both the left circumflex (LCX) and left anterior descending (LAD) coronary arteries. Prior to injury, vessel diameters were measured by angiography (LCX = 3.1 mm, LAD = 2.8 mm) and inflation pressure was selected to inflate the balloon to 1.3× artery diameter (LCX - 11 atm, LAD - 8.5 atm). Coronary injury was induced by inflating a balloon (Maverick 15 mm length, Boston Scientific, Natick, MA, USA) three times for 30 s, with 1 min between inflations.

Following each balloon injury, the IVUS transducer was positioned within the injured segment of the vessel using angiography for guidance. The RF amplifier was turned on and the optimal acoustic parameters as determined from the *ex vivo* experiments were applied (5 MHz center frequency, 500 cycles, pulse repetition frequency (PRF) = 5 kHz, PNP = 0.6 MPa). The ultrasound transducer was pulled back within the catheter sheath using a modified syringe pump at a rate of 2 mm/min in order to treat the length of the injured artery. The IVUS transducer was not rotated while treating the LCX in order to localize ultrasound treatment to a limited portion of the artery circumference. The transducer rotated at a rate of 250 RPM while treating the LAD in order to apply ultrasound to the full artery circumference. During IVUS treatment, a dispersion of DiI-loaded microbubble (8.1×10^9 MB total) was infused into the catheter lumen at a rate of 1 mL/min while oscillating the syringe to prevent buoyancy-based separation of the microbubbles. The microbubbles then exited through the ejection port at the distal end of the catheter into the blood stream. IVUS treatment was applied to a 10 mm length within the LCX and 11.33 mm in the LAD, approximately 5 minutes for each artery.

Immediately after treatment of the LAD coronary, the animal was euthanized and the treated coronary arteries were harvested, placed in vials, frozen in liquid nitrogen, embedded in “optimal cutting temperature” (OCT) compound and sliced using frozen histology. Three sections were collected every 200 μm . Artery sections were imaged with an inverted microscope (IX51, Olympus Corporation of the Americas, Center Valley, PA, USA) to detect fluorescence due to DiI (excitation = 549 nm, emission = 565 nm), collecting multiple overlapping images to complete each section. Following microscopy, images were formed into a mosaic of the entire section using custom Matlab image processing software.

The inner edge of the external elastic lamina (EEL) was traced on the bright field image of the vessel within the treated region. A mean filter was applied to the corresponding fluorescence image along this trace to measure the circumferential fluorescence intensity along the vessel lumen following angioplasty. Every 200 μm , the circumferential fluorescence of the three adjacent sections was averaged. The circumferential fluorescence along this averaged trace was median filtered circumferentially. The peak fluorescence was

marked and the range of angles with fluorescence intensity greater than the mean around this point determined the DiI treated angular range. For ultrasound transducer beam width simulations, the average vessel diameter measured by angiography was used to simulate the beam width. Using the measured vessel diameter and IVUS transducer dimensions, an acoustic field model was prepared to estimate the axial dimension -6 dB beam width of the IVUS transducer using FIELD II (Jensen and Svendsen, 1992; Jensen, 1996).

Statistical Analysis

Analysis of variance (ANOVA) was applied to compare the fluorescence intensity increase across *ex vivo* artery segments treated with different ultra-sound parameters. A significance value (p) <0.05 was considered significant.

Results

Ex Vivo Assessment of Delivery Parameters

In fluorescence microscope images of the DiI-loaded microbubble and ultrasound treated arteries, delivery was localized within the -6 dB beam width of the ultrasound transducer (1.3 mm) along the vessel wall (Fig. 2a). This demonstrated non-preferential circumferential delivery but localized longitudinal delivery of the DiI to the artery wall within the ultrasound beam. The average fluorescence intensity along the vessel circumference was up to 10-fold greater than the background fluorescence in the representative artery (Fig. 2b).

A one-way ANOVA was used to test for fluorescence intensity differences when varying PNP and PRF of the delivery pulse (Table 1 a-d) and when varying radiation force time (Table 1 e-i). These results exhibit no statistically significant difference in fluorescence intensity increase when varying the PNP and PRF of the delivery pulse, $F(3,8) = 1.37$, $p=0.3189$ (Fig. 3a). However, the application of acoustic radiation force for more than 33% of the pulsing sequence provided an increase in fluorescence intensity due to DiI delivery compared to background, $F(4,10)=20.26$, $p<0.01$ (Fig 3b). This improvement in fluorescence intensity due to DiI delivery was measured for all pulsing sequences that included acoustic radiation force, even when no delivery pulse was applied (100%).

In Vivo Delivery

Representative images of the LCX coronary artery were selected from two sections outside of the IVUS treated region (Fig. 4a,e) and three sections within the IVUS treated region (Fig. 4b-d). Fluorescence was detected along one region of the IVUS treated vessel segments, localized along the circumference due to the lack of IVUS transducer rotation during pullback and treatment.

In sections of the LAD, where the IVUS transducer was rotated, fluorescence was exhibited at points along the entire artery circumference. In Fig. 5, fluorescence was distributed along the entire circumference, with preferential delivery as indicated by the arrows.

From angiography, the LCX diameter was 3.1 mm. Selected intensity profiles measured across five sections, three treated and two at the tails of the treated region, are presented in Fig. 6. The average angular range of treatment, as measured across a total of 10 treated

artery sections was 31.6° , with a standard deviation of 11.5° . The -6 dB beam width modeled in FIELD II measured 35.4° at a distance of 1.55 mm.

Discussion

Although microbubble and ultrasound enhanced drug delivery and acoustic radiation force enhancement of microbubble localization have been demonstrated in previous studies, this study examined microbubble and ultrasound enhanced delivery using intravascular ultrasound. In addition, this study sought to evaluate the contribution of the two elements of ultrasound and microbubble enhanced drug delivery, acoustic radiation force and sonoporation, by varying the ratio of acoustic radiation force to delivery pulses under physiological flow conditions. Following *ex vivo* experiments, we determined that when operating at a 5 MHz center frequency and PNP = 600 kPa, acoustic radiation force had a greater contribution to DiI delivery from the microbubbles.

Using the *ex vivo* swine arteries, we were able to evaluate ultrasound parameters for microbubble and ultrasound enhanced delivery with IVUS under physiological flow conditions. When adjusting the sonoporation pulse PNP and PRF, there was no significant increase in fluorescence due to DiI delivery (Fig. 3a). In previous work by our group and others, a threshold for delivery was noted (Karshafian et al., 2010; Dixon et al., 2013). This previous work and the present study suggest that the PNPs tested in this study were well above the required pressure to enhance delivery. The 0.5 -1% duty cycles used for the sonoporation pulses in the present study are also well matched to previous studies which ranged from 0.3-4% (Rahim et al., 2006; Phillips et al., 2010; Burke et al., 2012; Dixon et al., 2013).

To evaluate the need for acoustic radiation force and sonoporation pulses for DiI delivery to the artery, the ratio of acoustic radiation force (high duty cycle, low PNP) versus delivery (low duty cycle, high PNP) pulses was varied. The comparison between pulsing ratios demonstrated a significant increase in fluorescence intensity when acoustic radiation force was applied (Fig 3b). The 10 fold fluorescence intensity increase measured with the application of acoustic radiation force was similar to previously published results of 10-17 fold (Shortencarier et al., 2004). However, with physiological flow, which was not present in previous studies of the acoustic radiation force to sonoporation pulse ratio, there was no statistically significant difference in fluorescence intensity increase when sonoporation pulses were included. The present result is in agreement with previous research that has suggested that microbubble destruction is not required to induce sonoporation and that it may even be beneficial for microbubbles to survive (van Wamel et al., 2006; Fan et al., 2012).

There are two differences between the present study and previous work presented by Shortencarier et al. (2004). First, the work presented here performs delivery of a fluorescent marker under physiological flow conditions, whereas the work of Shortencarier et al. (2004) uses *in vitro* static chambers. The static chamber case may diminish the importance of acoustic radiation force in the delivery sequence because the microbubbles are in the transducer field for an unlimited period of time. Secondly, the acoustic radiation force PNP

applied in this study was much higher than that applied in the Short-encarier et al. (2004) study. The present PNP used for ARF displacement of the microbubbles was high in order to displace microbubbles in blood. This may result in a combination of lipid shedding and acoustic radiation force displacement that enables delivery without a secondary sonoporation pulse (Borden and Longo, 2002; Borden et al., 2005). In order to better understand these effects, optical high speed experiments could be used to monitor microbubbles under similar acoustic and environmental conditions (i.e., in blood).

The extent of delivery along the artery circumference was predicted using acoustic field simulations. In the treated section of the *in vivo* swine LCX, a 35.4° region of treatment was predicted by the modeled acoustic -6 dB angular beam width. In comparison, the angular extent of the DiI delivery region in the *in vivo* artery was 31.6°. This demonstrated the ability of the IVUS and microbubble drug delivery system to localize delivery under physiological flow conditions *in vivo* with a predictable extent of delivery. Demonstrating that this delivery occurred at multiple sections within the treated region and that there was no delivery outside of the IVUS treated region further provides evidence of localization of delivery.

In the LAD, where the IVUS transducer was rotated, delivery was detected along the entire circumference of the artery (Fig. 5). However this delivery was clearly preferential, with a denser region of delivery as indicated by the arrows. We have identified two potential sources of the preferential delivery despite rotation: the microbubble infusion port position and the IVUS catheter position. Only one microbubble infusion port was created in the catheter, resulting in microbubble ejection on one side of the catheter. This may have caused greater microbubble availability for dye delivery on one side of the vessel, resulting in the preferential delivery. Alternatively, diminished ultrasound and microbubble induced bioeffects have been characterized for high microbubble concentrations due to acoustic shadowing (Song et al., 2009). Because of the increased concentration of microbubbles on one side of the vessel acoustic shadowing may have occurred, reducing microbubble shell disruption and delivery on the upper wall of the vessel. The catheter position within the vessel lumen may have also played a role, either blocking microbubble accumulation and flow along part of the vessel wall, or providing a slightly higher output pressure on one side that improved delivery. Delivery may be improved by monitoring catheter distance from the vessel wall and modulating the output pressure accordingly.

Conclusions

The demonstration of model drug delivery within an injured vessel suggests the microbubble and IVUS drug delivery system presented here may be a clinically viable method for localized drug delivery within the vasculature for applications such as PCI. The ability to localize this delivery along one segment of the artery circumference could potentially be used to treat vascular diseases with potent drugs like rapamycin. This approach has the potential to lower required drug doses and prevent cytotoxic side effects due to drug uptake by nearby cells that would be treated unintentionally by conventional drug delivery methods.

An IVUS platform, such as the one validated in this work, may provide the basis for a new approach to localized delivery of drugs, such as antiproliferatives or chemotherapeutics. Taking advantage of microbubble loading, localization of delivery with microbubbles and ultrasound, and the enhanced uptake of molecules using the combination of microbubbles and ultrasound, this technique may provide a method to lower drug doses required for therapies. Previous work by our group and others has shown that the combination of microbubbles and ultrasound allows for lower drug doses to be effective (Unger et al., 1997; Lu et al., 2003; Phillips et al., 2011). By lowering drug doses and localizing therapy more effectively, more potent pharmaceuticals and therapies known for lower uptake efficiency (e.g., gene) may become viable options (Unger et al., 1997; Kinoshita et al., 2006).

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Figure 1.

a) *Ex vivo* delivery schematic. Left - A linear array images an acoustically transparent chamber of 37°C physiological saline solution PSS containing the *ex vivo* swine artery. The IVUS transducer was positioned within the artery under guidance from the transcutaneous ultrasound (US) probe. After each treatment, the IVUS transducer was moved to a new longitudinal position within the vessel using US guidance. Right - A dispersion of DiI microbubbles was infused into the vessel lumen while the IVUS transducer rotates and transmits ultrasound to enhance DiI delivery. b) The processing of *in vivo* artery microscopy images. After collecting bright field and fluorescence images of three adjacent sections, the external elastic lamina (EEL) were identified by tracing in the bright field images. A 2-D mean filter was applied to the fluorescence images. The traced EEL boundaries from the

bright field images were then used to sample the mean filtered fluorescence images and determine fluorescence intensity along the vessel circumference. The three fluorescence intensity curves were then averaged and median filtered to determine the average EEL fluorescence for this region.

Figure 2.

a) Microscope image of DiI delivery along the vessel wall. Grayscale bar measures the pixel intensity in arbitrary units of fluorescence. The dotted yellow lines denote the -6 dB beam width of the ultrasound transducer (1.3 mm). b) Fluorescence intensity increase over background in the artery plotted along the circumference in an *ex vivo* artery where delivery was performed in PBS. Plot axes were truncated to show that the fluorescence intensity increase occurs along the entire vessel wall. A maximum fluorescence intensity increase of 10x was measured in this artery with a mean of 5.3 ± 2.9 fold. This result was produced with a PNP = 2 MPa, PRF = 1 kHz sonoporation pulse. The region from 0 to 2 mm has a lower fluorescence intensity increase because the catheter was not centered and may have blocked the flow of microbubbles to this region.

Figure 3.

Fluorescence intensity increase due to DiI delivery in blood to select acoustic parameters. *Ex vivo* arteries were treated with ultrasound and DiI-loaded microbubbles and then imaged using a confocal microscope. a) Fluorescence intensity increase was measured in dB along the artery circumference following treatment for two different PNP (1 MPa, 2 MPa) and two different PRFs (0.5 kHz, 1 kHz). There was no significant difference in fluorescence intensity increase across the four acoustic parameter sets (Table 1 a-d). b) Fluorescence intensity increase was measured along the circumference of the artery following treatment

for four different ratios of acoustic radiation force to delivery pulses as listed in Table 2. (n=3, displayed as mean+standard deviation, * $p < 0.05$ compared to 0% radiation force time.)

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Figure 4.

Representative LCX artery sections of bright field microscopy with DiI fluorescence (red) overlay. a) Section 6 - no IVUS was applied to displace DiI-loaded microbubbles. b-d) Sections 9,12, and 15 where IVUS was applied without transducer rotation to displace DiI-loaded microbubbles to the vessel wall. Insets are magnified views of the fluorescence region of the EEL. e) Section 18 - no IVUS was applied to displace DiI-loaded MBs. Artifacts were found within the LCX coronary artery lumen due to residual microbubbles and red blood cells remaining in the vessel lumen. However, these artifacts are limited to the vessel lumen, enabling measurement of the extent of DiI delivery within the vessel. Scale bar = 500 μm .

Figure 5.

Representative LAD artery section of bright field microscopy with DiI fluorescence (red) overlay. Section where IVUS was applied with a rotating transducer to displace DiI-loaded microbubbles to the entire circumference of the vessel wall. The two largest continuous regions of fluorescence are indicated by the arrows in the image. Interspersed regions of fluorescence were detected along the entire artery circumference. Some fluorescence can be found within the lumen and is attributed to histological artifacts. Scale bar = 100 μm .

Figure 6.

The average fluorescence intensity profile along the inner edge of the external elastic lamina of representative artery sections, measured in arbitrary fluorescence units. Sections 9-15 are within the IVUS treatment region and sections 6 and 18 are from the tails where no treatment occurred. The simulated -6 dB angular beam width of the IVUS transducer is denoted by the two dashed red lines and the measured treatment region is denoted by the two dash-dot black lines. 0 degrees is 12 o'clock in the images of Figure 4.

Table 1

Ultrasound parameters evaluated in the *ex vivo* swine arteries

	P1 %	P1 Cycles	P1 PNP (MPa)	P1 PRF (kHz)	P2 Cycles	P2 PNP (MPa)	P2 PRF (kHz)
a	75	500	0.6	5	50	1	0.5
b	75	500	0.6	5	50	1	1
c	75	500	0.6	5	50	2	0.5
d	75	500	0.6	5	50	2	1
e	0	500	0.6	5	50	2	1
f	33	500	0.6	5	50	2	1
g	66	500	0.6	5	50	2	1
h	75	500	0.6	5	50	2	1
i	100	500	0.6	5	50	2	1

Table 2

Acoustic radiation force and sonoporation time chart. A = Acoustic radiation force pulse application. D = Delivery pulse application.

									Time (s)						
%	5	10	15	20	25	30	35	40	45	50	55	60			
0	D				D			D			D				
33	A	D		A	D	D	A	D	D	A		D			
66	A	D		A	D	D	A	D	D	A	A	D			
100	A			A			A			A					