Ultrasound in Med. & Biol., Vol. 40, No. 10, pp. 2458–2467, 2014

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2014.04.007

● *Original Contribution*

LOCALIZED *IN VIVO* MODEL DRUG DELIVERY WITH INTRAVASCULAR ULTRASOUND AND MICROBUBBLES

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(*Received* 11 *October* 2013; *revised* 8 *April* 2014; *in final form* 10 *April* 2014)

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 physiologic 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 with 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 indicate that low-frequency IVUS catheters are a viable method for achieving localized drug delivery with microbubbles. (E-mail: jh7fj@virginia.edu) © **2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **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 caused by the accumulation of plaque. Left untreated, these 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, re-occlusion of the vessel caused by excessive vascular smooth muscle cell proliferation, termed *neo-intimal hyperplasia*, may occur. To minimize the risk of re-occlusion, the patient can be treated with systemically administered medication, drug-eluting stents or drug-eluting balloons to deliver an anti-proliferative agent to the site of injury (Agostoni et al. 2013; Boden et al. 2007; Byrne et al. 2012; Garg and Serruys 2010). 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 (Ferrara et al. 2007; Klibanov 2006; Unger et al. 1998). 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

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(Deng et al. 2004; Fan et al. 2012). This phenomenon has been used to treat a number of indications, including the enhancement of angiogenesis after ischemia (Korpanty et al. 2005), the delivery of chemotherapy to a brain tumor (Kinoshita et al. 2006) and the prevention of neo-intimal formation following balloon injury (Phillips et al. 2011a). *In vitro* studies have concluded that enhanced uptake is greatest at low frequencies (<5 MHz) (Karshafian et al. 2009; Meijering et al. 2007) and with the application of acoustic radiation force to direct microbubbles to the site of delivery (Patil et al. 2011; Rychak et al. 2007; Shortencarier et al. 2004).

Intravascular ultrasound (IVUS) is an ultrasound imaging mode that provides high-resolution cross-sectional images of the vasculature. IVUS imaging has been found to improve patient outcomes during PCI (Fitzgerald et al. 2000; Stone et al. 1999; Uren et al. 1998). Because acoustic radiation force displacement and sonoporation with microbubbles are 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 (Herickhoff et al. 2011; Kilroy et al. 2014; Mabin et al. 2012; Mahon et al. 2003; Patel et al. 2013). Investigations of IVUS for microbubble applications are also limited and have focused on imaging applications because of the low microbubble resonance frequencies that are incompatible with current high-frequency IVUS transducers (Frijlink et al. 2006; Goertz et al. 2006). To improve microbubble localization under physiologic flow, our group previously published the design of an IVUS transducer for the acoustic radiation force displacement of microbubbles under physiologic flow (Kilroy et al. 2012).

The study described here investigated the viability of using IVUS and microbubbles for localized drug delivery in the vasculature. 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 physiologic flow. Finally, with 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. Both localized delivery and distributed delivery along the artery circumference are demonstrated in the swine model using the prototype IVUS system.

METHODS

DiI-loaded microbubbles

Microbubbles (MBs) were formulated as described previously (Phillips et al. 2011b). 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, St. Louis, MO, USA) and the fluorescent dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (<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 them 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*g*), for periods of 10, 6 and 6 min. In previous experiments (Phillips et al. 2011b), it was experimentally determined that three 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 h of washing for *in vivo* experiments. The average microbubble diameter was 2.2 μm.

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, Mountain View, CA, USA) was performed to select the dimensions for a 5-MHz-center-frequency transducer that fit within a 500 × 700-μm area. A center frequency of 5 MHz was selected to match 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, East Hartford, CT, USA) was diced into 0.25 × 0.7-mm elements and backfilled with non-conductive epoxy (RE2039/HD3561, Henkel, 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, San Diego, CA, USA) was removed, a thin layer of silver epoxy was applied (CHOBOND 584, Parker Hannifin, Woburn, MA, USA) and the custom ceramic was cured in the catheter casing. The signal