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● *Original Contribution*

# ENHANCED INTRACELLULAR DELIVERY OF A MODEL DRUG USING MICROBUBBLES PRODUCED BY A MICROFLUIDIC DEVICE

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**Abstract—Focal drug delivery to a vessel wall facilitated by intravascular ultrasound and microbubbles holds promise as a potential therapy for atherosclerosis. Conventional methods of microbubble administration result in rapid clearance from the bloodstream and significant drug loss. To address these limitations, we evaluated whether drug delivery could be achieved with transiently stable microbubbles produced in real time and in close proximity to the therapeutic site. Rat aortic smooth muscle cells were placed in a flow chamber designed to simulate physiological flow conditions. A flow-focusing microfluidic device produced 8 $\mu$m diameter monodisperse microbubbles within the flow chamber, and ultrasound was applied to enhance uptake of a surrogate drug (calcein). Acoustic pressures up to 300 kPa and flow rates up to 18 mL/s were investigated. Microbubbles generated by the flow-focusing microfluidic device were stabilized with a polyethylene glycol-40 stearate shell and had either a perfluorobutane (PFB) or nitrogen gas core. The gas core composition affected stability, with PFB and nitrogen microbubbles exhibiting half-lives of 40.7 and 18.2 s, respectively. Calcein uptake was observed at lower acoustic pressures with nitrogen microbubbles (100 kPa) than with PFB microbubbles (200 kPa) ($p < 0.05$, $n > 3$). In addition, delivery was observed at all flow rates, with maximal delivery (>70% of cells) occurring at a flow rate of 9 mL/s. These results demonstrate the potential of transiently stable microbubbles produced in real time and in close proximity to the intended therapeutic site for enhancing localized drug delivery. (E-mail: jh7fj@virginia.edu)** **© 2013 World Federation for Ultrasound in Medicine & Biology.**

***Key Words:*** **Flow-focusing microfluidic device, Monodisperse microbubbles, Ultrasound-mediated drug delivery, Sonoporation.**

## INTRODUCTION

Ultrasound-enhanced drug and gene delivery has been investigated extensively in recent years. Early work by Fechheimer et al. (1987) demonstrated increased uptake of plasmid DNA at 20 kHz. Bao et al. (1997) extended this work by adding microbubbles and insonating at 2.25 MHz to enhance uptake of a luciferase plasmid. Since then, numerous reports studying the effect of various ultrasound (e.g., frequency, pressure, duty cycle) and microbubble (e.g., shell composition, microbubble concentration) parameters on drug and gene delivery have been published (Guzmán et al. 2001; Larina et al. 2005; Mehier-Humbert et al. 2005; Hallow et al. 2006; Hassan et al. 2010; Phillips et al. 2011b, Tartis et al., 2006).

There has been increasing interest in ultrasound-mediated drug and gene delivery to large blood vessels for the treatment of atherosclerosis (Phillips et al. 2010, 2011a); however, the effects of blood flow on ultrasound-mediated delivery have not been extensively studied. The majority of parameter optimization has been conducted *in vitro* under static conditions, but it is known that blood flow patterns alter local drug and microbubble concentrations within the vessel (Patil et al. 2009). Static conditions fail to provide a means to study the effects of blood flow rate on drug delivery, however, adding fluid flow to *in vitro* models of drug delivery may allow for better translation of optimized ultrasound parameters to *in vivo* settings.

Enhanced delivery requires precise control over both ultrasound and microbubble parameters. Although ultrasound parameters are easily controlled and models exist for the propagation of acoustic waves through tissue (Jensen 1991; Zemp et al. 2003), the properties of microbubbles are less predictable. First, the majority of current microbubble production techniques use agitation methods (Klibanov 2002), which generate microbubbles with a wide range of diameters (i.e., polydisperse)

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(Stride and Edirisinghe 2009). Populations of polydisperse microbubbles are characterized by a distribution of resonance frequencies related to the size distribution of the microbubble population. The range of resonance frequencies results in reduced imaging sensitivity when compared with imaging of monodisperse populations (Kaya et al. 2010; Streeter et al. 2010) and may affect drug delivery efficacy (Choi et al. 2010). Additional improvements in imaging sensitivity with monodisperse microbubble populations may be achieved if the microbubble resonance frequency is matched to the peak frequency of the imaging pulse (Talu et al. 2007). Second, a large percentage of microbubbles are lost following intravenous administration and circulation (Butler and Hills 1979; Lim et al. 2004; Iijima et al. 2006; Talu et al. 2008b), resulting in poor control of microbubble and drug concentrations at the therapeutic target site. Finally, while the acoustic properties of single microbubbles have been experimentally observed and modeled (Church, 1995; Morgan et al. 2000; de Jong et al. 2002; Marmottant et al. 2005; Patil et al. 2010), the interactions between clouds of oscillating microbubbles and the impact of these interactions on the processes that govern drug delivery remain unclear.

To overcome some of these limitations, we propose the production of microbubbles from a catheter located within the vasculature, that is, *in situ* microbubble production, to enable local administration of a well-controlled number of monodisperse microbubbles and a controlled total drug dose. Flow-focusing microfluidic devices (FFMDs) (Gordillo et al. 2004; Garstecki et al. 2004; Hettiarachchi et al. 2007; Castro-Hernández et al. 2011) are ideal for this application, as they have a small footprint (Dhanaliwala et al. 2012) and can produce microbubbles in real time in a continuous manner. FFMDs produce microbubbles by compressing a central gas column with two liquid streams containing the microbubble shell material (Tan et al. 2006). In addition, FFMDs produce monodisperse microbubbles (Hettiarachchi et al. 2007; Stride and Edirisinghe, 2009), and microbubble diameter can be "tuned" to the requirements of specific applications by adjusting the gas and liquid input parameters. Producing microbubbles directly within the vasculature significantly reduces the number of microbubbles needed for contrast enhancement or drug delivery, as losses resulting from storage, administration and circulation are eliminated. Thus, microbubble production rates currently achieved by FFMDs, ranging from $10^3$ to $10^6$ microbubbles per second (Stride and Edirisinghe, 2009), may be sufficient to enhance drug delivery.

Another advantage of *in situ* microbubble production is the ability to investigate unconventional microbubble formulations. Microbubbles produced in the vasculature at the therapeutic target site do not require long circulation lifetimes. Unstable formulations (e.g., microbubbles with $N_2$, $CO_2$ or $O_2$ gas cores and a mild surfactant shell) that would otherwise quickly dissolve if administered systemically (Kabalnov et al. 1998; Park et al. 2010) could be insonated immediately after production. Furthermore, larger microbubbles, which provide increased acoustic contrast (Dalla Palma and Bertolotto, 1999; Gorce et al. 2000) and improved sonoporation (Deng et al. 2004; Fan et al. 2012), may be viable because quick dissolution reduces the embolic risk.

In this article, we demonstrate the feasibility of *in situ*-produced microbubbles for drug delivery *in vitro*. To better simulate *in vivo* flow conditions, we developed a flow chamber that enables the study of ultrasound-mediated drug delivery to a cell monolayer under physiological flow conditions. A FFMD was used to produce microbubbles in real time, and the effect of microbubble composition and flow rate on model drug delivery to cells was assessed by fluorescence microscopy.

## METHODS

### *FFMD fabrication*

Flow-focusing microfluidic devices were fabricated as described previously (Dhanaliwala et al. 2012). Briefly, a custom SU-8 mold was manufactured by photomicrolithography. Devices were cast in polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, Midland, MI, USA) and bound to a clean PDMS substrate using oxygen plasma. The final device had a channel height of 25 $\mu$m and a nozzle width of 8 $\mu$m, while the liquid and gas channels were 50 and 30 $\mu$m wide, respectively. The overall dimensions of the device used in this study were $10 \times 4 \times 8$ mm. Water was introduced into the microchannels immediately after binding to maintain hydrophilicity of the channels. Devices were stored in deionized water and were used within 5 d.

### *Microbubble characterization*

The liquid phase consisted of 15 mg/mL polyethylene glycol-40 stearate (PEG40S) dissolved in a solution of 10% glycerol, 10% propylene glycol and 80% phosphate buffered saline containing magnesium and calcium (v/v) (GPS). All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA). The PEG40S-GPS solution was sonicated to at least 60°C with a tip sonicator (XL2020, Misonix, Farmingdale, NY, USA) using a half-inch probe (40% power, 7 min) and sterile filtered (0.45-$\mu$m nylon flter, Fisher Scientific, Waltham, MA, USA) prior to use. The gas phase was either nitrogen (GTS Welco, Richmond, VA, USA) or perfluorobutane (PFB) (Synquest, Alachua, FL, USA). PTFE microbore tubing (Cole Parmer, Vernon Hills, IL, USA) was used