Quantification of Ultrasound-Mediated Drug Delivery using an *In Vitro* Flow Chamber

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Statement of Purpose: Ultrasound-mediated delivery of drugs from microbubble carriers is a promising approach for localized therapeutic delivery to targets within the vasculature. Drug delivery efficiency with this technology is primarily dependent on ultrasound parameters, microbubble design, and fluid dynamics within the blood vessel. *In vivo* optimization of ultrasound and microbubble parameters is challenging as few methods exist to accurately quantify *in vivo* drug deposition and exact fluid dynamics within the vessel of interest are often unknown. Furthermore, state of the art *in vitro* methods for optimizing these parameters evaluate drug delivery to cells in static cell culture conditions, ignoring the effects of fluid flow. Accordingly, we have developed an *in vitro* flow chamber to aid in the optimization of ultrasound and microbubble parameters under varied flow conditions for ultrasound-mediated drug delivery applications.

Methods: A custom flow chamber (Fig 1) designed to mimic laminar arterial flow conditions was constructed from cast acrylic and contained an acoustic window half-way down its length. A Thermanox coverslip containing confluent rat aortic smooth muscle cells (SMCs) was placed within the acoustic window, and the flow chamber was submerged in 1X PBS at 37°C. Flow was established by pulling PBS through the chamber using a peristaltic pump. Microbubbles were introduced by placing a microbubble-generating microfluidic device assembly within the channel, and ultrasound was applied for two minutes by a single-element transducer. Calcein, a cell-membrane impermeable fluorophore, was used as a model drug and was co-administered with the microbubbles to maintain a concentration of 0.05 mg/mL. Only cells that survived and were permeabilized by the ultrasound-mediated delivery process retained calcein.

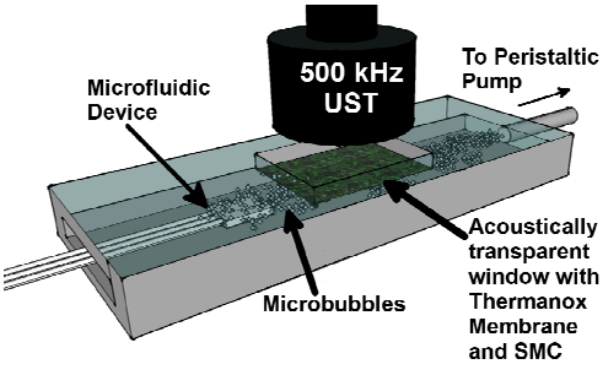

Figure 1. Schematic of the *in vitro* flow chamber

The effects of the microbubble gas core (nitrogen vs perfluorobutane (PFB)), peak ultrasound pressures (100, 200, 300 kPa), and fluid flow rate (2, 9, and 18 ml/s) on calcein internalization was assessed using the flow chamber apparatus. Calcein delivery efficiency was quantified by counting the fraction of cells on the Thermanox membrane that internalized calcien (as determined by fluorescence microscopy). Cell death as a result of the delivery process was quantified by staining with propidium iodide. All parameter permutations were performed in triplicate.

Results: Laminar flow was established within the flow chamber and fluid flow by itself did not cause calcein delivery or cell death.

(a) Effect of fluid flow rate: Calcein delivery was observed at all flow rates, with maximal delivery at 9 ml/s and maximal cell death at 2 ml/s. Results shown are with PFB-gas microbubbles and 300 kPa peak ultrasound pressure (Table 1).

Table 1:Effect of fluid flow rate on calcein delivery

	Fluid Flow Rate		
	2 ml/s	9 ml/s	18 ml/s
% Delivery	32%	78%	64%
% Death	57%	18%	16%

(b) Effect of ultrasound pressure: Minimal calcein delivery or cell death was observed below 200 kPa. Results shown are for PFB-gas microbubbles at a 9 ml/s flow rate (Table 2).

Table 2: Effect of ultrasound pressure on calcein delivery

	Peak Ultrasound Pressure			
	0 kPa	100 kPa	200 kPa	300 kPa
% Delivery	2%	2%	59%	78%
% Death	3%	5%	9%	19%

(c) Effect of microbubble gas core composition: Nitrogen-core microbubbles required higher acoustic pressures than PFB-core microbubbles to achieve calcein delivery. Results shown are at a 9 ml/s flow rate (Table 3).

Table 3: MB gas core effect on calcein delivery efficiency

	Peak Ultrasound Pressure			
	0 kPa	100 kPa	200 kPa	300 kPa
PFB-MB	2%	2%	59%	78%
Nitrogen-MB	3%	5%	9%	19%

Conclusions: This is the first demonstration of *in vitro* ultrasound-mediated drug delivery in a flow chamber. For fixed microbubble concentrations and ultrasound parameters, calcein delivery was observed to vary significantly with flow rate (Table 1). In addition, ultrasound pressure and microbubble composition also altered delivery efficiencies (Table 2,3).

Our results confirm those observed *in vivo*, as acoustic microbubble stimulation causes increased cell death in the slow flow conditions of tumor vasculature[1] and relatively little death in larger vessels.[2] Furthermore, we confirm that an ultrasound pressure threshold exists under which no delivery is observed.

Overall, the *in vitro* flow chamber provided a suitable platform for quantitative evaluation of the effects of fluid flow rate, ultrasound pressure, and microbubble composition on the ultrasound-mediated drug delivery process.

[1](Czarnota, GJ. PNAS, 2012;Epub ahead of print)
2(Phillips,LC. Art. Thro. Vasc. Bio. 2011;31:2853-2855)