Sonoporation in 3D endothelialized microvascular networks

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Introduction

In vitro

1 MHz - 330 kPa - 10 cycles - multiple pulses

1 MHz - 1.6 MPa - 1000 cycles - single pulse
Introduction

In vitro
- Stationary microbubbles
- Direct microbubble-cell contact
- Cell monolayer on rigid cell culture plate

In vivo
- Microbubbles circulating in blood vessels
- Target cells in some cases beyond blood vessels
- Cells embedded in 3D structured soft tissue

→ need for more in vivo like cell models to study ultrasound induced drug delivery
**3D microvascular networks**

3D model mimicking *in vivo* vasculature allows for the study of:

- **sonoporation** and drug delivery to endothelial cells
- **drug extravasation** into tissue

in a more realistic environment

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Zheng et al., *PNAS* 109, 9342-7, 2012

**More information:** poster C10 by Eric Juang
**Experimental setup**

**US settings:** 1 MHz, 0.4-1.4 MPa, 500-1000 cycles, 5%-20% DC - 5 sec exposure time

**Microbubbles:** DPPC/DSPE-PEG in 95/5 mol% ratio, $C_4F_{10}$ core

**Model drug:**
- Sonoporation study: propidium iodide
- Extravasation study: 40 nm FITC-labeled polystyrene beads
Characterization acoustic field

1 MHz focused transducer (2 cm dia, 8 cm focus) used in the near field

Without microvascular network

With microvascular network

→ Distortion acoustic field
→ Reduction acoustic pressure
Perfusion networks with MBs

MB conc.: 2-5x10^8 MBs/mL

Syringe pump input flow rate: 8-10 μL/min

Microbubble velocity in channels:
(determined via microbubble tracking) 0.01-0.09 cm/s

Physiological capillary blood flow velocity: ~0.03 cm/s
Sonoporation endothelial cells

propidium iodide
endothelial cell
microbubble

blood vessel

% sonoporated cells

US

Hoechst
Propidium iodide

ROI

Frequency: 1 MHz
Exposure time: 5 sec

0.4 MPa - 1000 cycles - 20% DC, N=2
1.4 MPa - 500 cycles - 5% DC, N=3

Cell co
Sonoporation endothelial cells

Need for the use of targeted microbubbles?

Acoustic settings: 1 MHz - 0.4 MPa - 1000 cycles - 20% DC - 5 sec exposure time
Drug extravasation

Preliminary experiments:
Limited though significant extravasation observed

Acoustic settings: 1 MHz - 1.4 MPa - 500 cycles - 5% DC - 5 sec exposure time
Conclusions & future directions

We were able to

- create 3D endothelialized microvascular networks for the study of sonoporation
- perfuse microvessels with microbubbles
- induce sonoporation of endothelial cells under flow conditions
- perform initial studies on ultrasound-induced drug extravasation

In the future we plan to

- co-culture microvascular networks with pericytes and tumor cells
- induce angiogenesis
- study the combined effect of ultrasound and drugs in these highly specific networks
Acknowledgements