



<b>Title</b>	<b>Sonoporation-induced endoplasmic reticulum stress: signaling pathway analysis</b>
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## Background, Motivation and Objective

To properly harness sonoporation for therapeutic applications, it is unarguably vital to characterize the fundamental biophysical processes involved. Of particular relevance are two membrane-level processes that epitomize the notion of sonoporation: 1) how membrane perforation is induced by ultrasound-microbubble interactions, and 2) how the membrane remodels itself following an episode of sonoporation. Acquiring direct observations of these processes is however not a straightforward task (ironically, these membrane-level events have yet to be convincingly demonstrated in-situ). In this investigation, our aim is to acquire the first series of direct evidence on the time course of membrane perforation and recovery in sonoporation. In particular, we seek to unravel the time-varying surface topography of sonoporated cell membrane in-situ.

## Statement of Contribution/Methods

A real-time imaging platform for monitoring of cell-microbubble interactions was first developed, and it was a composite system that coupled a 1 MHz ultrasound module to a laser scanning confocal microscope. A nose-cone shaped waveguide (1" diameter, 7.5 cm height) was devised to align the ultrasound beam focus to the microscope's imaging plane. This waveguide was angled at 45 deg. with respect to the imaging plane normal. A custom-made cell chamber was mounted onto the imaging plane, and it housed a 20x28 mm observation window with acoustically thin top and bottom layers (<0.16 mm thick). MRC5 fetal fibroblasts were seeded onto the bottom layer of the observation window, and their plasma membrane was fluorescently labeled using the CellMask Orange dye at 2.5 ug/ml concentration. Lipid-shelled microbubbles (Targeson) were then introduced on a 1:1 cell/bubble ratio, and they were allowed to passively settle onto the fibroblast monolayer. After that, a single ultrasound pulse (1 MHz frequency, 10 cycles, 0.75 MPa in situ peak negative pressure) was applied to instigate microbubble pulsation and collapse. Over this process, the surface topography of fibroblast membrane at positions with microbubbles was imaged in real-time using the confocal microscope. Pore size and recovery time were quantified from the acquired cine-loops.

## Results/Discussion

Localized perforation of cell membrane was synchronized with the time course of microbubble collapse. The pore size was highly time-dependent: it expanded for a limited time after microbubble collapse (up to 7 um diameter), after which resealing started to take place. The pore size was generally greater than the microbubble (mean diameter: 2.2 um). Membrane striation was also evident away from the perforation site: this signifies an increased membrane tension. During recovery, the perforation site exhibited a contractile ring morphology, and the endoplasmic reticulum was found to be actively participating in the resealing process. These findings demonstrate that membrane-level processes in sonoporation are highly dynamic.

## IUS1-K3-5

### Relation between cell membrane tension and repair of membrane damaged during sonoporation

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## Background, Motivation and Objective

Repair of a cell membrane damaged during sonoporation depends on membrane tension, and a difference in membrane tension has a great effect on sonoporation efficiencies in *in vitro* and *in vivo* conditions. In this study, cell membrane tension was controlled by changing osmotic pressure of the culture medium, and the effect of cell membrane tension was investigated by fluorescence microscopic observations of cells during sonoporation.

## Statement of Contribution/Methods

Human prostate cancer cells (PC-3) were cultured on a coverslip, and the coverslip with cells facing down was attached to an observation chamber created in the bottom of a water bath (Fig. 1a). The observation chamber was filled with an isotonic or non-isotonic solution supplemented with 5 µg/ml propidium iodide (PI), which permeates only through damaged cell membranes and produces red fluorescence. Hanks' balanced salt solution was used as the isotonic solution. A hypertonic solution was prepared by addition of 0.2 g NaCl to a 50-ml isotonic solution, and a hypotonic solution was prepared by mixing the same volumes of 1.3 mM CaCl<sub>2</sub> solution and the isotonic solution. The solutions were also supplemented with the ultrasound contrast agent Levovist. After sufficient time to allow bubbles to come into contact with cells (30 min), the cells were exposed to a single shot of 10-cycle ultrasound pulse of 1 MHz in center frequency and 1.3 MPa in peak-negative pressure. Occurrence of cell membrane damage was discerned by increase in PI fluorescence inside the cells, and success or failure in membrane repair was discerned by a transient or persistent increase in fluorescence intensity. The repair rate was calculated as the number of repaired cells divided by the number of damaged cells.

## Results/Discussion

Fig. 1b shows the repair rates of cells treated under the three conditions of osmolality. The repair rate under a hypertonia condition was significantly higher than the rates under the other two conditions. These results indicate that a decrease in cell membrane tension promotes repair of membranes damaged during sonoporation. However, there was no significant difference between iso- and hypotonic conditions, suggesting that decreased Mg<sup>2+</sup> concentration in a hypotonic solution has a positive effect on membrane repair (R.A. Steinhardt, Ann. N. Y. Acad. Sci. 1066(2005) 152-165).

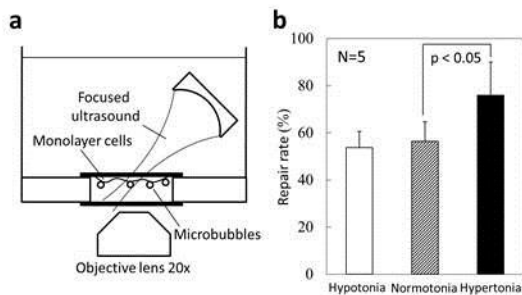


Fig. 1. (a) Experimental setup of the observation (b) Repair rates of cells treated under various conditions of osmolality. Error bar represents SD.

## IUS1-K3-6

### Sonoporation-Induced Endoplasmic Reticulum Stress: Signaling Pathway Analysis

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## Background, Motivation and Objective

