# Electronic Supplementary Material (ESI) for Lab on a Chip

# Laser-Induced Microbubble Poration of Localized Single Cells

Qihui Fan,[*a] Wenqi Hu[b] and Aaron T. Ohta[b]

## Part I. Negative control tests

Negative control tests were conducted to exclude other possible causes for cell poration in the LMP system. The first negative control test replaced the optically absorbent substrate with an uncoated glass slide as the bottom of the fluidic chamber. All other experimental conditions were kept constant. With the glass substrate, no microbubble was formed, and no fluid flow or cell poration was observed (Fig. S1). This indicates the LMP is induced by the microbubble and the corresponding shear stress, but not directly by the laser energy. The second negative control test used a longer laser pulse width of 350 μs to produce larger bubbles that did not oscillate in size. Under these conditions, the nearby cells were not porated (Fig. S2). This indicates the oscillation in bubble size and the corresponding microstreaming and induced shear stress are the cause for LMP.

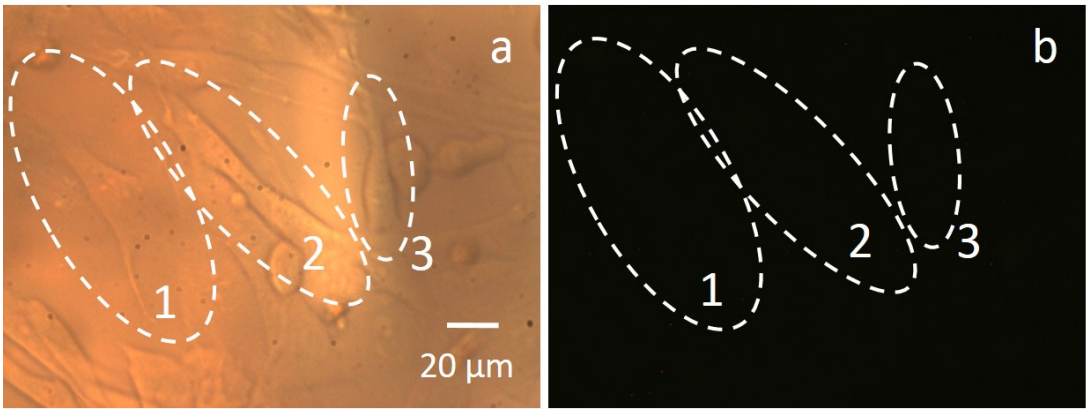


**Fig. S1** Result of the first negative control test, which replaced the optically absorbent substrate with an uncoated glass slide. (a) DIC image of the cells for which poration was attempted, which are marked by the white dashed lines. (b) Fluorescent image of the cells after the attempted poration. No cells were porated or damaged, indicated by the absence of green fluorescence from FITC or red fluorescence from EthD-1.

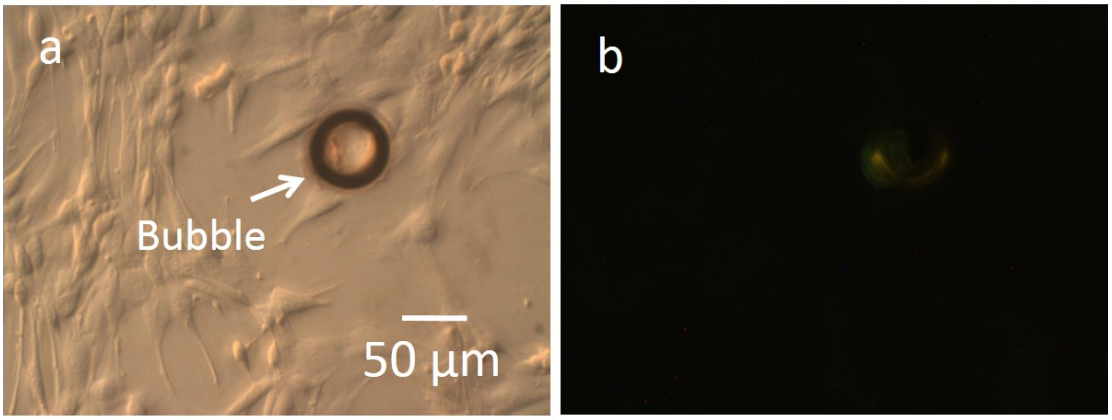


**Fig. S2** Result of the second negative control test with a large bubble that does not oscillate in size. (a) DIC image of the cells for which poration was attempted, which are the ones immediately surrounding the bubble. (b) Fluorescent image of the cells that underwent attempted poration. The cells around the bubble were not affected, as indicated by the absence of FITC or EthD-1 fluorescence. There is some weak background fluorescence visible due to FITC trapped at the bubble meniscus.

**Part II. Characterization of the LMP parameters**

The microbubble formation and expansion process is controllable by adjusting the parameters of the laser in the LMP system. This allows the control over the shear stress experienced by the cells, and the poration that results. There is an optimal shear stress at which the cells are porated efficiently, while subsequent cell viability is maximized.

**1. Laser focal point size, intensity, and microbubble size**

An objective lens was used to focus the 980-nm diode laser. Varying the power of the object lens affects the diameter of the laser spot on the substrate: a 5X objective lens can form a 17.6-µm spot, a 10X objective lens can form an 8.8-µm spot, and a 20X lens can form a 3.87-µm spot. The laser spot intensities from different focus lens are also varying: 42 kW/cm$^2$, 127 kW/cm$^2$, and 414 kW/cm$^2$ for 5X, 10X, and 20X objective lens, respectively. These also affect the size of the induced microbubbles.

With the 5X lens, microbubbles greater than 20 µm in diameter were abruptly formed by a long laser pulse width of 400 to 500 µs at a frequency of 50 Hz. This microbubble formation process was abrupt and vigorous, causing cell lysis (Fig. S3). The effective range of the shear stress with the 5X lens is also larger, so the microbubble porates multiple cells in the vicinity. This makes the use of the 5X lens undesirable. Microbubbles produced by the 20X lens had diameters of 5 µm or less, and the effective poration range of these bubbles was shorter, making it harder to maintain

the proper distance for cell poration. Using the 10X lens, microbubbles with a maximum diameter of 7 to 10 μm were formed with a laser pulse width of 90 to 110 μs. With this lens, single cells could be porated effectively and precisely with a much higher poration efficiency than from 20X lens, while maintaining high cell viability (Fig. S4).

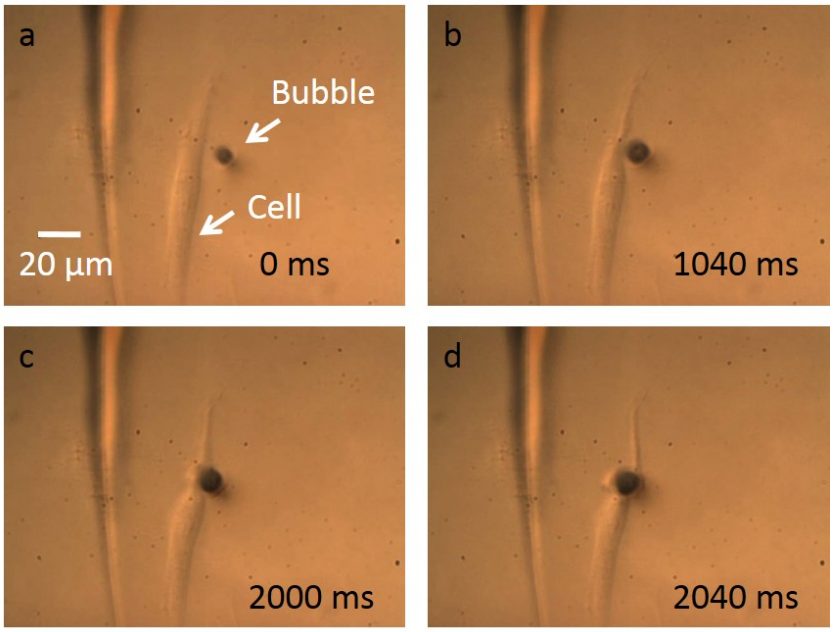


**Fig. S3** Interaction between 5X-lens-induced microbubble and a nearby cell. (a) Microbubble nearby the cell. (b) Microbubble deforming the cell. (c) The cell membrane was damaged by the microbubble. (d) The membrane was torn by the microbubble.

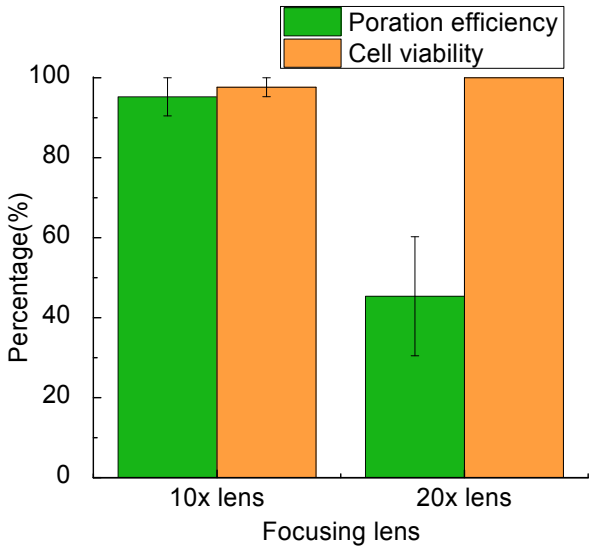

**Fig. S4** Cell poration efficiency and cell viability when using 10X and 20X laser focusing lens. More than 30 cells were tested in 3 parallel experiments for each focusing lens. Error bars show the standard error of the measurements.

## 2. Fluidic chamber height (vertical distance)

The fluidic chamber height was determined by the spacer thickness, and has an effect on the LMP performance. The microbubble was produced on the bottom of the chamber, while the cells were on the ceiling of the chamber. The spacer thickness determines the height of the chamber, which is the vertical working distance between bubble and cell. Chamber heights of 10, 20 and 30 μm were characterized (Fig. S5). For a chamber height of 10 μm, the microstreaming from the oscillating bubble forms a stronger lateral force on the ceiling, making it more likely that the cells can be detached. However, when the chamber height is 30 μm, the shear stress from the microbubble is too weak at the cell membrane. The poration efficiency is highest when using the 20-μm spacer, while the cell viability is also very high. The cell viability is reduced with the 10-μm spacer, while the poration efficiency is very low with the 30-μm spacer. Therefore, 20 μm is the optimized chamber height for cell poration.

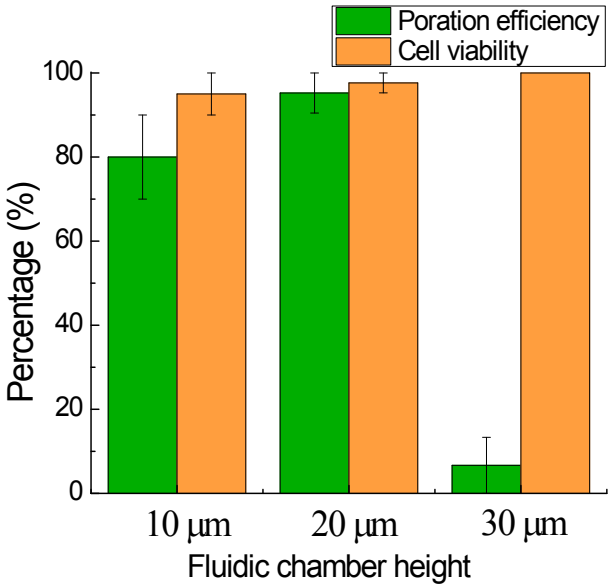


**Fig. S5** Cell poration efficiency and cell viability as a function of the chamber height. More than 30 cells were tested in 3 parallel experiments for each chamber height. Error bars show the standard error of the measurements.

### 3. Poration duration

The poration duration for each cell should be minimized to increase throughput and avoid unnecessary membrane damage. Therefore, the cells were tested at poration durations of 5 s, 15 s, and 25 s (Fig. S6). For a poration duration of 5 s, the efficiency was low. For the 15-s poration duration, both the efficiency and cell viability were high. For the 25-s poration duration, the efficiency decreased, as the prolonged exposure to the shear stress can result in cell detachment and loss during the rinsing of the FITC-Dextran solution. The cell viability was also compromised by the longer 25 s duration. Therefore, 15 s was selected as the optimized poration time.

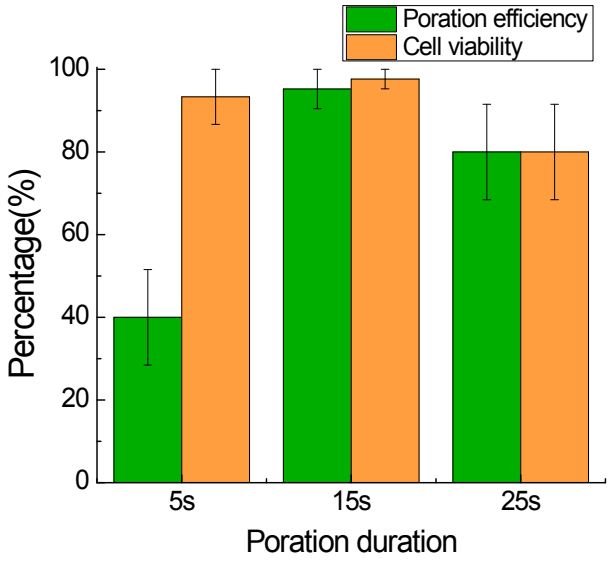


**Fig. S6** Cell poration efficiency and cell viability under different poration durations. More than 30 cells were tested in 3 parallel experiments for each poration duration. Error bars show the standard error of the measurements.

## Part III. Mechanism analysis

One important point to determine is if the size-oscillating bubble porates cells by direct contact with the cell membrane, or by the surrounding microstreaming. By looking at the geometry of the cells in the LMP system (Fig. S7), it can be hypothesized that the direct contact of the bubbles to the cells should be a rare occurrence. Therefore, the shear stress from the microstreaming surrounding the bubble should be the dominant force for cell poration.

Other data also indirectly supports the microstreaming shear stress as the poration mechanism:

when the lateral distance between the edge of the bubble and the edge of the cell is 5 μm, 30.0 ± 5.0 % of cells were still porated.

The following details the geometry of the cells in the LMP system:

For cells in suspension, which are spherical:

Diameter of suspended cells: $D_{sus\ cell}$ = 16.62 ± 1.00 μm (measured from n=10 cells)

Average cell volume: V = 2402.5 $\mu m^3$

For cells adhered on the glass slide:

Area of the cell: A = 734.18 ± 71.51 μm (measured from n=10 cells)

Thus, the average thickness cells adhered on the glass slide during the experiments is given by

$$T_{cell} = V/A = 3.27\ \mu m$$

The measured microbubble diameter (measured from n=23 bubbles) is:

$$D_{bubble} = 7.34 \pm 0.18\ \mu m$$

Under experimental conditions, where the cells are adhered to the glass slide, the resulting cell thickness is approximately 3.27 μm.

The scenario that would most likely result in bubble-cell contact would be when the bubble diameter is 9 μm (maximum measured diameter), and the cell thickness is 3.27 μm (Fig. S7). In this scenario, there is still some gap between the cell and bubble, calculated as

$$X = Chamber\ height - T_{cell} - D_{bubble}$$

Therefore, the space between the bubble edge and cell membrane is at least X = 7.73 μm. Thus, although the lateral distance between the bubble and the cell appears to be zero when viewed under the microscope, there still is a spacing between the two, preventing direct contact.

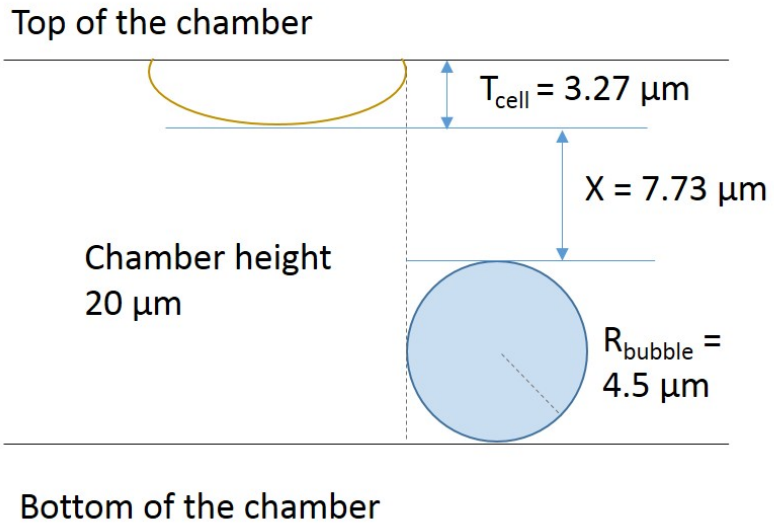


Fig. S7. Schematic view of the relative position of the bubble and cell in the fluidic chamber.