Single cell membrane poration by bubble-induced microjets in a microfluidic chip†

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This paper demonstrates membrane poration of a single suspension cell due to a fast liquid microjet. The jet is formed during the collapse of a laser induced bubble created at a variable stand-off distance from the target cell. The cell is trapped by a converging structure within a microfluidic chip. The asymmetrical growth and collapse of the cavitation bubble next to the cell lead to the microjetting, which deforms and porates the cell membrane. In the experiments, the membrane porations of myeloma cells are probed with the uptake of trypan blue. Time-resolved studies of the diffusion of trypan blue show a marked dependency on the bubble dynamics, i.e. the stand-off distance. The penetration length of the dye increases with shorter distances. Numerical simulations of the diffusion process agree with larger pores formed on the cell membrane. This method allows for a fast, repeatable, and localized rupture of membranes of individual cells in suspension.

Introduction

Permeabilization of the cell membrane is a widely used biological technique to transport large molecules into the cell’s cytoplasm, such as gene transfection. Several techniques have been developed, including chemical poration,¹⁻³ micro-injection,⁴⁻⁷ electroporation⁸⁻¹³ and sonoporation.¹⁴⁻¹⁰ Chemical poration uses chemical stimuli to generate pores on cell membranes.¹⁻³ However, the toxicity of the chemicals and the cell-types specific uptake limits its applicability. Moreover, the spatial or temporal control of this method is difficult to realize. Microinjection uses glass capillary micropipettes to directly penetrate through the cell membranes.⁴⁻⁷ This method allows highly efficient injection of drugs into many cell lines with accurate dosage. However, the requirement for skilled technical personnel and low throughput are strong limitations of the microinjection method. In electroporation, an external pulsed electrical field is applied to porate cell membranes.⁸⁻⁹ It is an effective method for almost all cell types with high efficiency. Widely used electroporation devices have been miniaturized into the microfluidic system for single cell poration.¹⁰⁻¹² Some of the drawbacks of this method including uncontrollable size, number and location of membrane pores have been overcome by the recent demonstrated nano-electroporation (NEP),¹³ which can precisely control the dosage of biomolecules into a cell. However, a micro-/nano-electroporation device needs a relatively complex fabrication process. Sonoporation employs ultrasonic driven microbubbles to increase the transient permeability of the plasma membrane and enhance the introduction of foreign macro-molecules or DNA into cells.¹⁴⁻¹⁹ The employed ultrasound can be focused on the target cells or tissues to perform a localized therapy and enhance the uptake of the molecules with minimal invasion.¹⁸⁻¹⁹ However, the locations of the induced pores are randomly distributed on the cell membrane, which is similar to electroporation. In addition, a combination of electroporation and sonoporation has been demonstrated to yield higher efficiency.²⁰⁻²¹

Besides the ulserasonic induced bubble oscillations, cell membranes may be porated through shear forces created by laser-induced cavitation bubbles.²²⁻²⁵ For instance, a millimeter sized single cavitation bubble created at some distance from a substrate covered with adherent cells is able to porate a large group of adherent cells.²² However, the positions of the targeted cells are not controlled and therefore the parameters determining the drug delivery such as the pores’ size and location could not be controlled. Confining the bubble in a microfluidics environment allowed the delivery of drugs to suspension cells, i.e. cells floating close to the radial expanding and collapsing bubble.²³ The specificity of this method was strongly improved by using two cavitation bubbles created in tandem, which form a fast and steerable microjet.²⁴ In another method, a transient shear stress is induced by the cavitation bubble from a nano-pipette with a coated metallic
nanostructure. The latter two techniques were applied to adherent cells only.

In this paper, a new approach is devised to address the poration of single cells in suspension: individual cells are trapped first and then a single cavitation bubble next to a single trapped cell is created with a laser. Due to the trapping microstructure, a jet is generated during the shrinkage of the bubble, which is directed to the cell. Upscaling of the techniques is obtained by implementing an array of cell trapping structures within the microfluidic chips. The membrane poration is characterized by the trypan blue uptake. This method allows single suspension cell membrane poration and may have potential for single cell and biomaterial analysis in suspension.

Working mechanism

Previous investigations of the cavitation bubble dynamics have shown that a fast jet is created when a laser-induced cavitation bubble collapses near a rigid boundary. In this paper, an array of single cell trapping structures are used to trap and locate cells. The structures also act as rigid boundaries to induce the microjet for cell membrane poration. The strength of the microjet is strongly affected by the stand-off distance between the bubble center and the cell center and the maximum bubble size. Thus the stand-off distance among other parameters determines the size of the pores and the cell’s viability.

Fig. 1 sketches the working mechanism of membrane poration from a single trapped suspension cell by the cavitation bubble induced microjet. Saline trypan blue solution with suspended cells is flushed into the microfluidic chip as the extracellular medium. Trypan blue is not only used as a biomarker to indicate the cell membrane poration, but also used to increase the laser absorption to facilitate bubble generation. A single cavitation bubble is created by focusing a pulsed laser beam at some location away from the cell with a stand-off distance as shown in Fig. 1(a). Both the expansion and shrinkage of the cavitation bubble are non-symmetric due to the presence of the trapping structure as shown in Fig. 1(b) and (c), respectively. During bubble expansion, the cell is pushed towards the trapping structure and flattens. Subsequently, during bubble shrinkage, the cell elongates towards the bubble. In addition, a fast microjet is formed pointing towards the trapped cell (Fig. 1(d)). After the bubble collapses, the jetting flow develops into two counter-rotating vortices (Fig. 1(e)). The first four events happen within 10–20 μs, while the deformed cell recovers its original round shape subsequently on a millisecond timescale. The diffusive uptake of trypan blue molecules into the cell’s cytosol occurs on a second timescale as shown in Fig. 1(f).

Materials and methods

Microfluidic chip design

A polydimethylsiloxane (PDMS) microfluidic chip is designed and fabricated using the standard soft lithography. The design of trapping structures follows the typical single cell trapping structures. The trapped cell within a converging microchannel in the shape of an equilateral triangle is shown in the inset of Fig. 2. Each side of the equilateral triangle \( w_1 \) is 20 μm, the width of the rectangular microchannel \( w_2 \) is 5 μm and the width \( w_3 \) is 70 μm. The height of the microfluidic chip is 27 μm. Detailed information of the microfluidic chip fabrication and single cell trapping are listed in the ESI.1

![Fig. 2 Schematic of the microfluidic chip with an array of single suspension cell trapping structures (not to scale). The inset denotes the design parameters \( w_1 \), \( w_2 \) and \( w_3 \).](image-url)
Cell culture and preparation

The myeloma cells were obtained from the American Type Culture Collection and cultured in a medium consisting of 86% (v/v) DMEM (Sigma-Aldrich, USA), 10% (v/v) fetal bovine serum (FBS, Gibco, USA), 1% (v/v) penicillin/streptomycin (Gibco, USA), 1% (v/v) sodium pyruvate, 1% (v/v) L-Glutamine and 1% (v/v) non-essential amino acid. Cells are grown in a T-75 cell culture flask (NUNC, Denmark) at 37 °C in a humidified environment with 5% CO₂. Cells in medium are centrifuged at 2000 rpm for 2 min (Velocity 18R, Dynamica). Then, the culture medium is directly replaced by a 0.4% trypan blue saline solution (T5184, Sigma-Aldrich, prepared in 0.81% sodium chloride and 0.06% potassium phosphate, dibasic). The trypan blue solution with myeloma cells at 1 × 10⁶ cells/ml concentration is pumped into the designed microfluidic chip with a syringe pump (New Era 1000, USA) with a flow rate of 0.5 μl min⁻¹.

Bubble creation and high-speed imaging

The optical setup used to create the bubble and capture the cell poration dynamics is similar to our previous work. In short, a pulsed and focused laser beam (Nd:YAG with a wavelength of 532 nm and a pulse duration of 6 ns, Orion, New Wave Research, Fremont, CA) creates the cavitation bubbles. The energy of the laser pulse is 100 μJ. The laser beam is expanded and directed into the back aperture of a microscope objective (20 ×, NA = 0.75, Olympus) housed in an inverted microscope (Olympus 71X). This allows a tight focusing of the laser pulse into the liquid within the microfluidic chip. Illumination for the high-speed photography is provided from a laser mirror of the microscope and onto the sensor of a high-speed CMOS-based camera (FASTCAM SA-1.1, Photron). A notch filter at 532 nm (NF02-532S-25, Semrock) is used to protect the sensor of the camera from the intense laser light. The timing of the image acquisition and the pulsed laser is controlled by a digital delay generator (575-8C, BNC). Videos of the bubble dynamics without and with boundaries are recorded at 300 000 frames per second with an exposure time of 370 ns. The frames span an area of 64 μm × 32 μm and the movie is available as M1 in the ESI. Selected images are shown in Fig. 4(a), Fig. 6(a) and Fig. 7(a).

Experimental results and discussions

Bubble-boundary interaction

The growth and collapse of a single bubble without/with the boundary effects from the trapping structure are first studied in the absence of a cell with a high-speed camera. Selected images in Fig. 3 reveal that the bubble dynamics is strongly affected by the trapping structure. Here, time t = 0 is the time when a bubble is first seen in the recordings. In the absence of a boundary, the bubble expands radially to a diameter of approximately 100 μm within 3.3 μs and collapses completely at 13.3 μs as shown in Fig. 3(a). Both the expansion and the shrinkage do not show translational motion of the bubble center. Yet, when the bubble expands in the vicinity of the trapping structure as shown in Fig. 3(b), the bubble flattens at the side closer to the structure. During the collapse starting at t = 6.7 μs, the bubble takes a triangular shape and develops a fast and thin jetting flow at t = 10 μs. The flow is directed towards the gap of the trapping structure. This jetting flow is evident from the thin black stripe passing through the bubble center. Similar jetting has been observed for much larger bubbles close to a rigid boundary.

Cell membrane deformation and poration

Selected high speed images from the movie of the dynamic process of membrane deformation are shown in Fig. 4(a). A suspension myeloma cell is trapped by a converging structure and a bubble is created at a 25 μm stand-off distance at t = 0 μs. The maximum diameter of the bubble is 42 μm. The cell is pushed by the expanding bubble towards the trapping structure at t = 0 μs, i.e. it flattens horizontally. When the bubble begins to shrink and collapse at t = 2 μs, the cell elongates vertically towards the collapsing bubble. Then, a high speed jetting flow is created between t = 4 μs and t = 6 μs due to the asymmetric growth and collapse of the cavitation bubble. It causes a pronounced indentation at the top of the

![Fig. 3](image-url) Bubble growth and collapse dynamics (a) without and (b) with a boundary/trapping structure. No cell is trapped in the structure. The scale bar is 100 μm. In (a) the bubble oscillates spherically. In (b) a jet develops towards the structure.

![Fig. 4](image-url) (a) Experimental and (b) Simulation results of the deformation of a single trapped cell from a cavitation bubble. The scale bar is 10 μm. Note, the change of the time interval from the 5th frame to the 6th frame.
cell as shown in the frame \( t = 6 \) ms in Fig. 4(a). After the bubble collapses, the cell regains approximately its original shape, which takes several milliseconds as shown in frame \( t = 8 \) ms in Fig. 4(a). More details on the cell recovery are given in the ESI.

The bubble-cell interaction process is simulated numerically using a solver for potential flow. The boundary element solver\(^ {33-38} \) has been adapted to simulate the bubble-cell interaction process with the assumption of axisymmetric and inviscid flow. The cell boundary is modeled as free fluid particles, while the trapping structure is modeled as fixed in space. Detailed information of the implementation of the boundary element solver is provided in the ESI.\(^ {33} \)

The experimental and simulation results are compared in Fig. 4(a) and (b), respectively. The cell trapped in the converging structure is stretched both in the simulations and the experiments at \( t = 0 \) ms. The upper part of the cell is not pushed as much in the experiments as predicted in the simulations. This discrepancy is due to the fact that the simulation neglects the membrane tension and viscoelasticity of the cytoplasm. The simulations are able to provide a closer look on the dynamics during the collapse. This event is too fast to be captured with the camera, yet can be revealed in the simulations. At \( t = 4.5 \) ms in Fig. 4(b), the bubble is shrinking with its major axis perpendicular to the cell. During the collapse, the bubble upper part of the cell accelerates towards the structure, which causes the formation of a fast microjet at \( t = 5.2 \) ms in Fig. 4(b). This rapid jetting flow is the likely cause of the pronounced indentation seen in the experiment at \( t = 6 \) ms.

Next, the extent, location and dynamics of the shear stress imposed on the cell membrane are presented in greater detail in Fig. 5(a) to (c). The radial dynamics of the non-spherical bubble near the structure is obtained as an equivalent radius \( R(t)/R(t = 0) \) as shown in Fig. 5(b). Here, \( R(t) \) is the equivalent radius of the axisymmetric bubble obtained from the volume average and expressed as

\[
R(t) = \left[ \frac{3}{4} \int_0^r r^2 \, dr \right]^{\frac{1}{3}}
\]

where \( r(t, z) \) is the position of the bubble interface in cylindrical coordinates. The quick expansion and collapse of the bubble are observed in Fig. 5(b) with the timescale being enlarged from \( t = 4.5 \) ms.

Within the limits of this simplified cell model, the areal strain on the cell as a function of time and location can be determined. Therefore, the cell surface is discretized through 10 nodes, which are initially uniformly distributed on an
upper quarter of the initial spherical cell as shown in Fig. 5(a). Node 1 is on the axis of symmetry and node 10 is at 9 o'clock position. The normalized areal strain $S'(t)$ of the surface between node $i$ and $i+1$ is defined as

$$S'(t) = (A'(t) - A'(ini))/A'(ini)$$

(2)

where $A'(ini)$ is the area covered between the neighboring nodes $i$ and $i+1$ at the initial time ($t = -5.1$ $\mu$s) and $A'(t)$ is this area at the time $t$. For simplicity, all areas are approximated as plane. If the strain is below zero, the cell surface is compressed locally; while for $S'(t) > 0$, the membrane is expanded relatively to the initial spherical configuration. Four selected areal strains $S'(t)$ with $i = 1, 2, 4$ and 9 are plotted in Fig. 5(c) as a function of time. During bubble expansion and collapse, the upper part of the cell has a positive strain ($i = 1, 2$ and 4), while on the side, e.g. $i = 9$, compression occurs. The strain up to 300% is an over estimation; In the experiment, a milder deformation during this time is observed as shown in Fig. 4. During collapse all strains relax, yet due to the formation of the jet, the strain $S'(t)$ increases sharply ($t = 5.2$ $\mu$s). Unfortunately, the simulation does not capture the later stage of the jet flow. It is likely that the strain may increase considerably above the value during the bubble expansion. All of the expansion, collapse and jetting lead to the largest strains occurring at the node closest to the bubble. Therefore, from the numerical simulation, it can be concluded that if membrane poration occurs, it is most likely near the location of jet impact.

Dye uptake and cell swelling

To prove for the simulation prediction, the uptake of a membrane impermeable dye following the bubble collapse is studied. The uptake of trypan blue is recorded with the camera and selected images of the process are presented in Fig. 6(a). Trypan blue enters only through the upper pole of the cell, from where it slowly darkens the cytoplasm. The change in intensities is tracked and the ratio of the volume to its surface area is plotted in Fig. 7(b). A linear growth is observed when the stand-off distance decreases to 25 $\mu$m, 20 $\mu$m to 15 $\mu$m, respectively. When $\rho_p = 1$ $\mu$m, no uptake is observed. Even 30 $s$ after the bubble generation, no single experiment showed any uptake.

The simulation results indicate that with the decrease of the pore radius, the diffusion distance decreases and the experimental results show that the diffusion distance decrease with the increase of the stand-off distance. Pores of approximately 0.5 $\mu$m in diameter give a reasonable agreement with the experiment. The effective pore size would thus decreases with an increase of the stand-off distance. Bubbles at a distance further away than 30 $\mu$m will not lead to the rupture of the membrane or, may only form sufficiently short lived pores that do not facilitate any noticeable dye uptake. These results also predict that the direction and strength of the microjet is governed by the boundary conditions and the size of the bubble. By modifying the size and position of the bubble, switching between permanent and viable poration of suspensions cells may be feasible.
affected by the toxicity of the dye. In other words, the uptake of a nontoxic drug may not cause osmotic swelling.

In this work, permanent poration leading to cell death due to the toxicity of the trypan blue dye is demonstrated. More works are needed to show viable cell poration, which is essential for drug and gene delivery applications. Another important factor that affects the cells' viability may be a potential heat shock from the laser pulse. Although about 80% of the energy of the laser pulse heats the liquid, the heated spot is limited to the size of the focal area and is quickly equilibrated by adiabatic cooling during expansion. Similar laser based techniques have concluded that a mechanical stress is dominant and that thermal induced stresses are of secondary importance.

Conclusions
A highly controllable contact-free method is developed to porate single suspension cells with fluid mechanical forces. Suspended myeloma cells are trapped individually inside converging structures within a microfluidic chip. A laser-induced cavitation bubble is created at a well-controlled distance from the trapped cell. The strength of the bubble induced strain and the jetting flow is function of the location and the size of the cavitation bubble. The observed strongly deformed cell membranes indicate that the microjet is causing the strongest strain. The poration is probed by the transport of trypan blue molecules through diffusion. The demonstrated cell poration method provides a fast and localized solution to control single suspension cell membrane poration. It seems a feasible approach to integrate the technique into automated microfluidic total analysis systems and high throughput systems.

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