MICROHEATER-MEDIATED MECHANICAL SINGLE-CELL LYSIS

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ABSTRACT

We demonstrate the first mechanical single-cell lysis via on-chip integrated microheaters. The chip combines an electrical layer with embedded microheaters and a fluidic layer, fabricated by soft lithography. Vapor bubbles can be generated above the microheaters resulting in fast fluid displacement and therefore in mechanical cell lysis by pushing the cell against the wall or the ceiling of the microfluidic channel. Single-cell lysis was analyzed with Calcein AM stained mouse fibroblasts via monitoring the exclusion of the live cell stain after bubble formation. 27 of 27 cells were lysed, even in a distance of up to 37 µm away from the heater.

KEYWORDS: Bubble Jet, cell lysis, single-cell analysis

INTRODUCTION

Mechanical cell lysis is advantageous for downstream analyses, since no critical reagents must be added. Single-cell lysis by laser-induced thermal bubble generation was shown before [1], requiring expensive optical equipment and being effective only for cells localized few micrometers distant to the bubble formation area. In another approach, single-cell lysis was performed by on-chip integrated sharp nanoscale barbs which have to be fabricated in a complex process [2]. In contrast, we present cell lysis using on-chip integrated microheaters, widely used in bubble-jet printers, as a robust and established tool with minimal area consumption [3].

THEORY

A schematic of the cell lysis is shown in Figure 1. Cells flow in a microfluidic channel (120 µm width and 45 µm height). For vapor bubble generation 30 x 30 µm² microheaters are placed inside the channel. While a single cell of interest is passing, the microheater is actuated with a series of electrical pulses. The liquid above the bubble generator rapidly evaporates and the cell is pushed against the channel ceiling or side wall by the resulting liquid displacement. This greatly increases the shear stress on the cell membrane, which finally leads to its rupture and the release of the intracellular components such as nucleic acids or proteins.

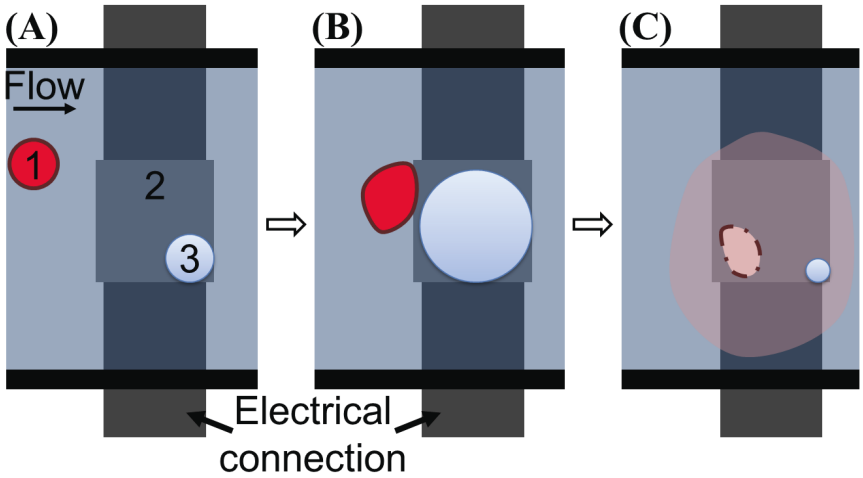

Figure 1: Schematic single-cell lysis. When a cell of interest ① approaches, multipulse actuation of the microheater ② can be initiated, creating a vapor bubble ③ by microboiling (A). Fast bubble volume expansion leads to a flow impulse acting on the cell (B), which is pushed against the channel ceiling or wall (deformation) and lysed as soon as the generated pushing force exceeds the cell membrane´s binding force (C). Exclusion of the intracellular dye (red) indicates membrane integrity loss.

EXPERIMENTAL

The single-cell lysis chip consists of two layers (Figure 2): 1) Titanium heater pads (30 µm x 30 µm) and aluminum electrical connections are deposited on Borofloat®33 wafers and comprise the electrical chip layer, which is fabricated by a process adapted from [4]. 2) A PDMS fluidic layer is fabricated by soft lithography. Both layers are irreversibly bonded after oxygen-plasma treatment and assembled onto customized printed circuit boards (PCBs) in a standard microscope slide format (26 x 76 mm^2). For triggering of electrical pulses on the heater, the PCB is connected to a voltage generator (Pico-Injector Signal Box, Biofluidix GmbH, Germany). Filling, fluidic operation of the chip and cell injection is done by syringe pumps (neMESYS, Cetoni GmbH, Germany). To perform on-demand lysis of single cells the heaters are actuated during observation of the cell flow in the transparent chip with a microscope (Axio Observer Z1, Carl Zeiss AG, Germany). Therefore, a low flow velocity in the order of about 500 µm/s is required. Actuation is done in a multipulse mode with 200 pulses (5 V, 2.0 µs single pulse width) and a pulse-to-pulse time interval of 0.1 ms.

To test the functionality of lysis, trypsinated L929 mouse fibroblasts (∅ 15-25 µm) were stained with Calcein AM live cell stain (0.4 nM). Fluorescence observation was performed on the same microscope, operated in fluorescence mode. An intact cell membrane spatially resolves the fluorescence of the intracellular dye in the shape of the round cell. Membrane rupture results in dye exclusion and therefore fluorescence increases in the cell´s surrounding.

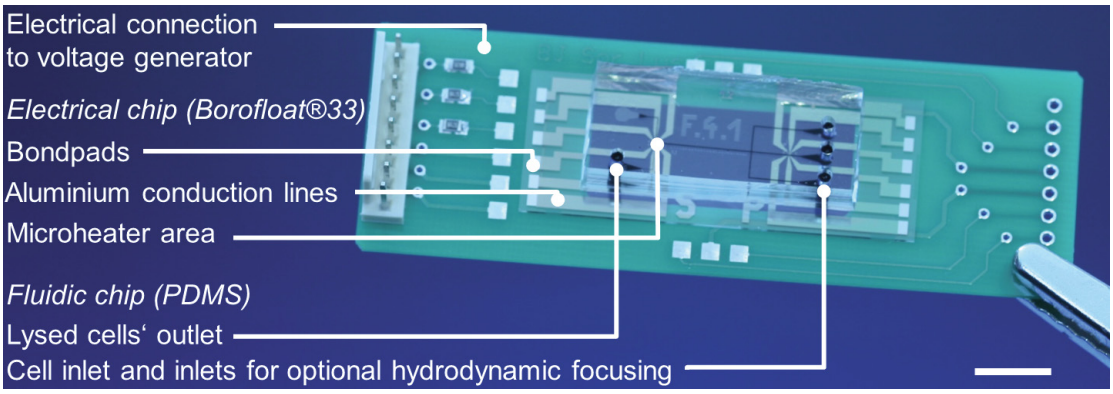

Figure 2: Single-cell lysis chip. The chip combines an electrical layer and a fluidic layer. Irreversible assembly is done by O_2 plasma activation of both chip materials. Cell filling with syringe pumps and heater actuation after connection to a voltage generator allow on-demand single cell lysis. Scale bar: 1 cm.

RESULTS AND DISCUSSION

Actuation of the microheater with a series of electrical pulses results in the formation of a permanent vapor bubble, which lasts for about 20 ms. The bubble expansion time to reach a diameter in the size of the heater was 18.3 (+/- 2.5) ms (Figure 3). This relatively long period in comparison to a ~ 2 µs bubble-formation period in Ink-Jet printing increases the time frame in which a passing cell can be lysed and thus reduces the influence of the cell-to-heater distance prior to actuation.

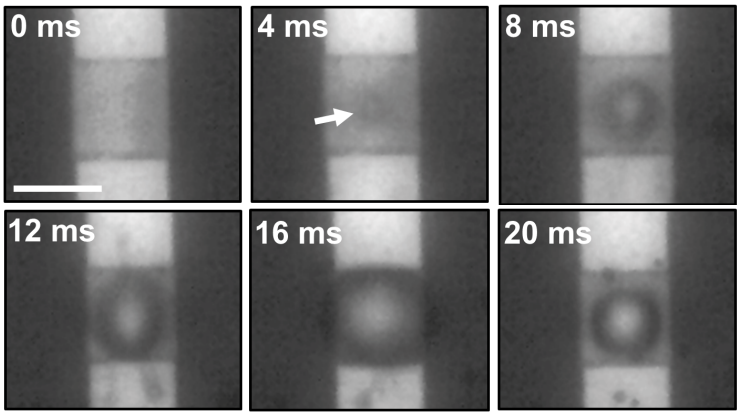

Figure 3: Cavitation bubble expansion and collapse. Bubble expansion is initiated in the center of the microheater (arrow). The thermal bubble expands to the diameter of the microheater within approximately 16 ms. After the last actuation pulse the bubble collapses (20 ms). Scale bar: 30 µm.

27 Cells were observed, 16 - 37 µm distant from the heater´s center at the beginning of actuation. 70% of these cells were lysed above the heater without being displaced and 30% were lysed but additionally pushed away (Figure 4). After actuation, fluorescence stain diffusion out of the cell could be observed and the fluorescence signal clearly increased in the neighborhood of the cell debris in all cases. This indicates efficient cell membrane disruption.

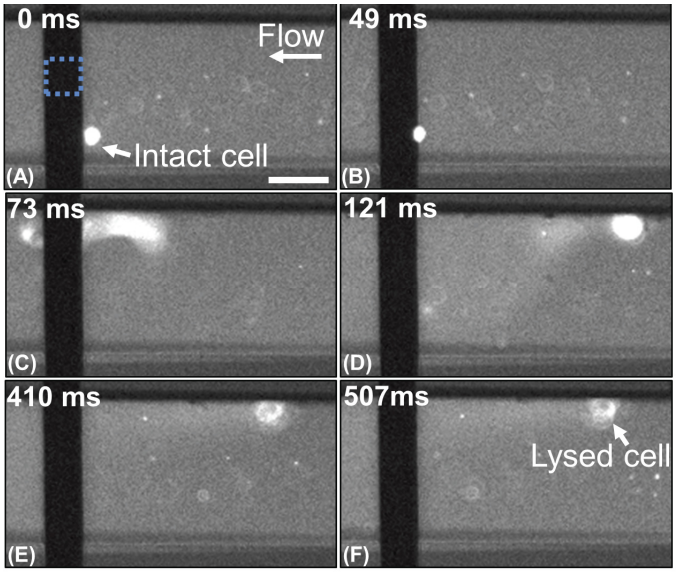

Figure 4: Single-cell lysis. A microheater (blue dashed box) is actuated as a fluorescently stained cell approaches. The cell is heavily displaced due to the fluid impulse generated by bubble expansion and collapse (C). Release of the intracellular dye can be observed during and after displacement. Excitation filter: 470 nm, emission filter: 525 nm, 20 ms exposure time. Scale bar: 50 µm.

CONCLUSION

Microheater-mediated lysis of single cells was demonstrated with 100% efficiency. In the cell-to-heater distance range observed (16-37 µm) lysis was independent of the cell position, which shows a higher flexibility of the presented approach for actuation in comparison to laser-induced cell lysis [1]. According to 20 ms cycle times for lysis we expect cell lysis frequencies of up to 25 cells/s after integration of cell detection to automatically trigger microheater actuation. Small area consumption of microheaters allows for multiple heater integration per chip and thus for parallelization of lysis. More microheaters in the channel and increased lysis frequencies would enable high-throughput screening of single cells by combination with on-chip molecular analysis [5, 6].

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