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Sorting and lysis of single cells by BubbleJet technology

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ABSTRACT

We demonstrate that chip-integrated BubbleJet technology can be used for cell sorting as well as for single-cell lysis. Vapor bubbles on the millisecond timescale are generated by titanium microheaters manufactured on transparent glass wafers. A PDMS layer is bonded on top of the glass wafer and defines microfluidic channels. Cell sorting is demonstrated with mouse fibroblasts which are hydrodynamically focused and travel at velocities of about 500 μm/s. For cell sorting the bubble generators are placed outside the microfluidic channel but are connected to it via a 20 μm nozzle. Randomly selected fibroblasts have been deflected laterally up to 60 μm from their original streamline to a collector outlet by actuation. Single-cell lysis is performed by bubble generators placed inside a flow channel directly below the cells' path. Bubble generation pushes the cells towards the channel ceiling or the channel wall and cells are lysed due to the large shear force at an efficiency of 100%. Cycle times for sorting and lysis have experimentally been determined to be 5 ms and 20 ms, respectively.

1. Introduction

Molecular analysis of single cells is needed for understanding cell development in physiological and pathological states as well as for analyzing the effect of drugs on cell metabolism. Microfluidics enables the integration of all unit operations needed for that kind of analysis in a high-throughput manner, but so far only a few systems have been demonstrated that include the entire process chain [1–3]. The main challenge is the integration of cell separation, cell lysis and molecular analysis on one chip [4]. To achieve continuous sorting and separation different forces and effects such as optical forces, magnetic and electric fields, acoustics, hydrodynamics and flow switching have been utilized [5]. Among different approaches for cell lysis in microfluidic chips [6] mechanical lysis is the most favorable since usage of critical reagents or protein denaturing heating can be avoided. Mechanical lysis has for example been shown using laser-induced thermal bubble creation [7] or by nanoscale barbs [8].

Here we demonstrate that two of the unit operations, cell sorting and mechanical cell lysis, can be realized by the same underlying BubbleJet technology. For cell sorting vapor bubbles are generated outside the microchannel in an actuation chamber that is connected with this main channel via a 20 μm nozzle. So the bubble actuation generates a gentle lateral fluid stream deflecting the cells from the focused streamline in the main channel. For cell lysis the bubbles are generated inside the main channel. Bubbles are initiated while cells are passing above the bubble generator. This leads to a very fast acceleration and consequently to a mechanical lysis of the cells. The proposed technology provides several advantages compared to the State of the Art: The BubbleJet actuators are completely integrated on chip thus enabling a higher integration density than off-chip valves [9] or piezo actuators [10]. In comparison to a BubbleJet sorting chip fabricated on opaque silicon [11] we demonstrate also cell lysis and our setup is realized on a transparent glass wafer which enables observation of experiments via transmission light microscopy on a standard microscopy slide format of 76 mm × 26 mm. In contrast to laser-induced cell lysis [7] BubbleJet mediated lysis does not need expensive optical equipment. Compared to the fabrication of nanostructures for lysis [8] the BubbleJet actuators are much easier to manufacture. Last but not least we demonstrate that two basic processes, cell sorting and cell lysis can be performed by the same actuation technology which allows the implementation on the same chip at no additional costs. Although not demonstrated here, downstream integration of Real-Time-PCR for gene expression studies after single-cell lysis on the same chip could be performed, e.g. by downstream integration of a suitable segmented flow module [12].

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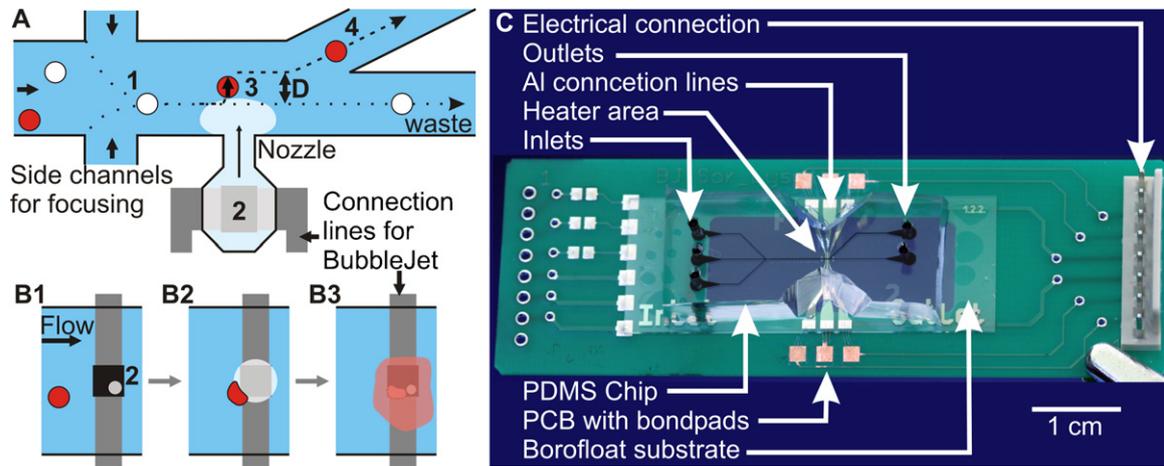
Fig. 1. Principle of sorting and lysis and assembled chip: for sorting (A) cells are hydrodynamically focused (1). The microheater (2) creates a vapor bubble on the millisecond time scale which leads to fluid displacement out of a nozzle and thus a cell is gently deflected (3) by a lateral distance (D) towards a collector outlet. For cell lysis (B) a microheater (2) is actuated inside the main-channel while the cell is passing (B1). The fast volume expansion of the created vapor bubble leads to a large shear force on the cell (B2) which results in deformation and rupture of the cell membrane. After cell membrane integrity loss intracellular components are released (B3). C shows a completely assembled chip for cell sorting. A PDMS layer defining microfluidic channels is plasma bonded to the heater substrate and then assembled onto the PCB.

2. Methods and fabrication

A schematic of the cell sorting is shown in Fig. 1 A. L929 mouse fibroblasts are hydrodynamically focused to a streamline about 30 μm away from the channel side wall with the integrated bubble jet nozzle. The main channel dimensions are 120 μm width and 45 μm height. Sample and side channel flows are controlled by high precision syringe pumps (neMESYS, Cetoni GmbH, Germany). Electrical actuation is done with a pulse generator (Pico-Injector Signal Box, Biofluidix GmbH, Germany). The application of a series of typically 50 electrical pulses of 2.5 μs duration to the BubbleJet heater results in formation of a rapidly expanding vapor bubble that exists for about 5 ms. Bubble expansion leads to a fluid displacement out of the nozzle into the main channel. This deflects a cell passing the

nozzle to a new streamline that leads towards the collector outlet. Cell viability after sorting is compared to a reference population by the administration of Trypan Blue and the count of the blue stained (=lysed) cells.

Cell lysis is illustrated in Fig. 1B. Bubble generators are placed inside the main-channel. Actuation is done with a series of typically 200 electrical pulses of 2.5 μs duration while a single cell is passing. The cell of interest is pushed against the ceiling or channel side wall. Lysis of trypsinated L929 mouse fibroblasts stained with Calcein AM live cell stain was observed via fluorescence microscopy (Axio Observer Z1, Carl Zeiss AG, Germany). Heater geometries were 35 μm × 35 μm for cell sorting and 30 μm × 30 μm for cell lysis. Heaters were made of 100 nm titanium and electrically connected by 600 nm aluminum lines. All metal layers are fabricated on
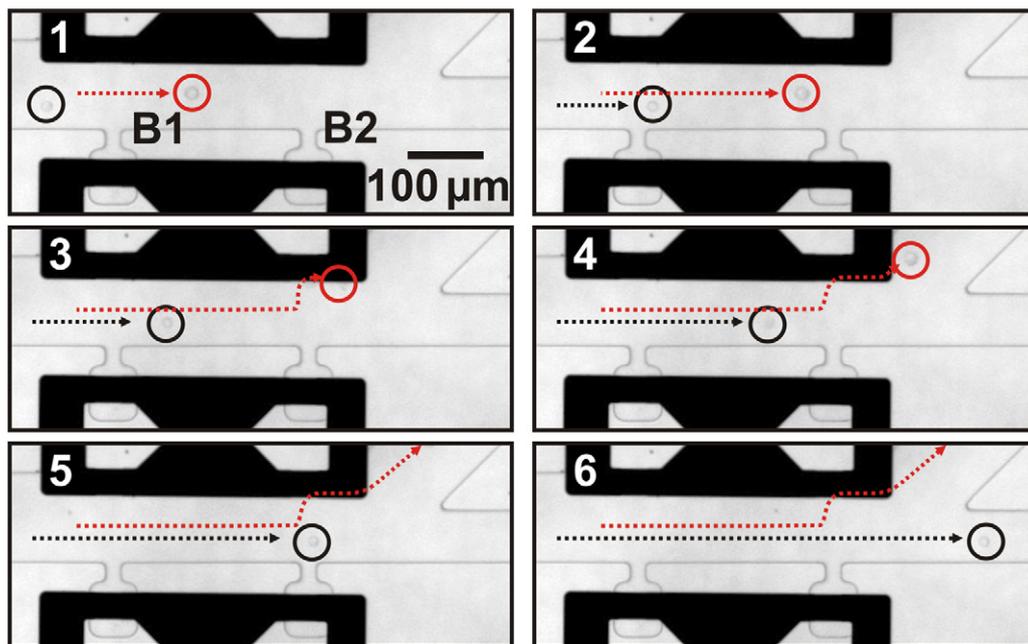
Fig. 2. Cell sorting: the photograph series shows the sorting area of the chip with two cells passing the nozzles of two BubbleJet actuation chambers (1, B1 and B2). The dark area on the chip corresponds to the metallization layers. The first cell (red) is deflected at the second nozzle (3) and enters the collector channel (4) while the second cell (black) is not displaced and is moving straight to the waste. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of the article.)
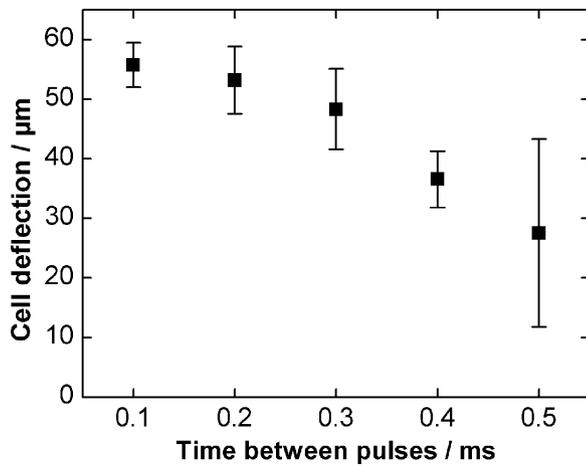
Fig. 3. Cell deflection: the diagram shows the lateral displacement of cells from their original streamlines. With growing time intervals between the individual heating pulses heat dissipation increases and the deflection efficiency is reduced.

Borofloat®33 wafer substrates by photolithography as described in [13]. The microchannels are fabricated in PDMS by common PDMS replica molding [14]. Bonding to the glass chip is done after oxygen plasma treatment. The chips are assembled onto customized printed circuit boards (PCBs) in standard microscopy slide format (76 mm × 26 mm) as depicted in Fig. 1C.

3. Experimental results and discussion

While for InkJet printing a single electrical pulse of typically 2–6 µs duration is enough to generate a bubble and to displace a picoliter droplet [15,16], this does not work for single cell displacement. The vapor bubble has to live long enough so that the cell can pass the nozzle before liquid is sucked back into the actuation chamber by the collapsing bubble. Therefore multi-pulse patterns of 50 pulses (5.5 V, 2.5 µs single pulse width) and different time intervals from 0.1 to 0.5 ms between the pulses were investigated (Fig. 3). The configuration leads to a permanent vapor bubble that lasts for 5–25 ms. Cells were laterally displaced up to 60 µm, sufficient to switch them from a waste streamline to a collector outlet streamline. In our experiments heater activation has been triggered manually while monitoring the incoming cells under a microscope. Therefore, the flow velocity of the cells has been reduced to about 500 µm/s. Successful sorting of polystyrene beads (∅ 26 µm) and trypsinated L929 mouse fibroblasts (∅ 15–25 µm) was demonstrated within an overall cycle time of 5 ms (Fig. 2). It can be extrapolated that future automation applying integrated image analysis should enable sorting frequencies of up to 200 cells/s for an optimum cell density in the sample. In a first cell viability study on 77 sorted cells only 2 cells were found lysed being stained with Trypan Blue about one hour after sorting. A reference population of 100 cells was driven through the same fluidic system without sorting. In this population one cell was found lysed which was examined by the application of the death stain. Within the cell stock outside the system the ratio of dead cells was also about 1%. Thus a significant
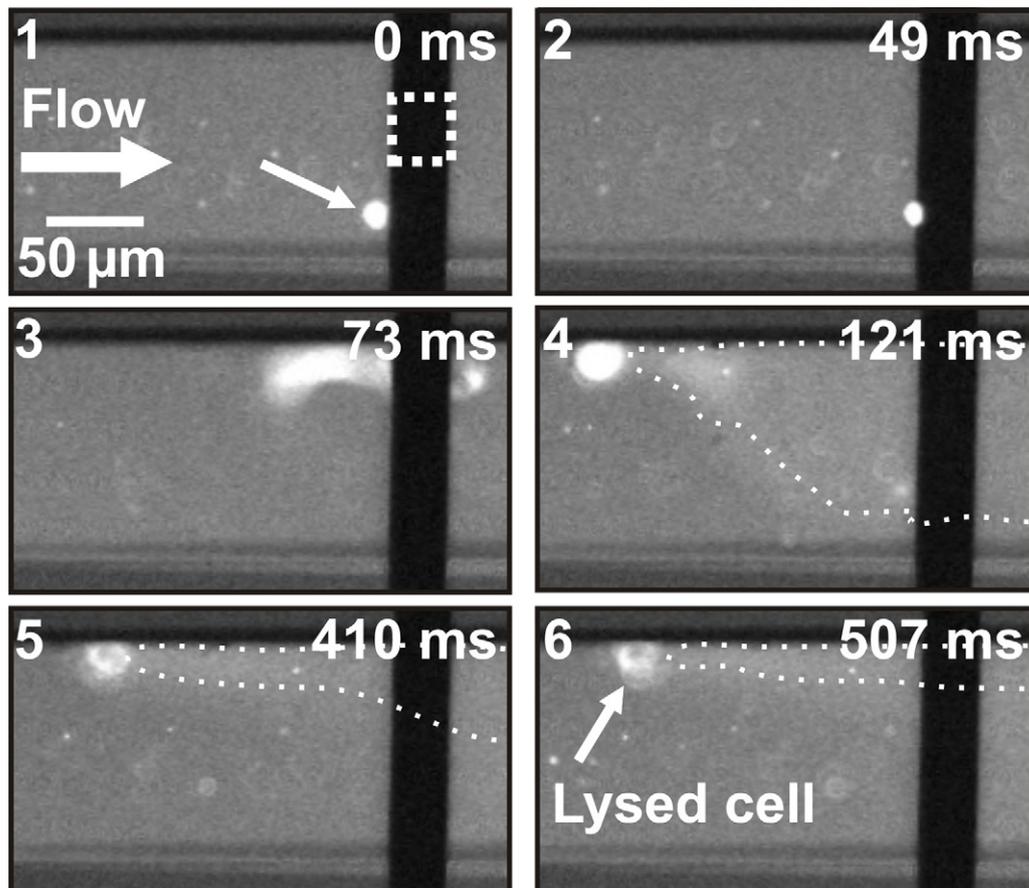
Fig. 4. Single-cell lysis: a fluorescently stained cell (1, arrow) approaches the microheater (1, dashed line box, see also Fig. 1B for orientation). The heater is actuated creating a rapidly expanding vapor bubble above the heater (not visible) which heavily deforms and displaces the cell (3). Release of the intracellular dye can be observed during and after displacement (3–6, dotted line indicates released Calcein dye). Excitation filter: 470 nm, emission filter: 525 nm, exposure time: 20 ms.

negative effect on cell viability by BubbleJet actuated sorting could not be detected.

For cell lysis multi-pulse patterns of 200 pulses (5.0 V, 2.0 µs single pulse width) and a pulse-to-pulse time interval of 0.1 ms have shown best results to create rapidly growing vapor bubbles above the microheater. The bubbles reach heater-sized diameter within 18.3 ($\pm$2.5) ms leading to a fluidic impulse on the cells. The 30 µm $\times$ 30 µm heaters were actuated with cells being in a distance of 16–37 µm from the center of a heater before lysis. 100% out of 27 analyzed cells were successfully lysed. 70% of them were directly lysed above the heater without being displaced and 30% were lysed but additionally pushed away (Fig. 4). Successful cell membrane disruption is indicated by fluorescence dye diffusion out of the cells after actuation. The fluorescence signal was clearly increased in the neighborhood of the cell debris. Thermal effects generated by the heater elements and the vapor bubble on the cell lysis process seem to be negligible because of the short time the cells might get into contact with the surface of the expanding bubble. If actuation of the heater is done with the cell directly above its center the cell is pushed aside, still being separated by a liquid film from the vapor. The maximum time the bubble and thus a heating effect exist is limited to about 20 ms following the actuation pattern. Thermal lysis of comparable cells takes place at much higher heating times as about 20 s at a temperature of 93 °C for NIH 3T3 fibroblasts [17] or 25 s of heating for HeLa cells [18]. The possibility of accumulated heating is excluded due to heat dissipation through the solid materials and the continuous replacement of liquid by the microfluidic flow itself in the region of a heater.

4. Conclusion and outlook

We successfully demonstrated single-cell sorting and single-cell lysis by using the same vapor bubble generation technology in just two different arrangements. The technology enables the fabrication of transparent chips and enables observation of experiments via transmission light microscopy on a standard microscopy slide format. The presented technology enables further integration of upstream and downstream processes such as single-cell stimulation [19,20] and analysis of molecular content of the cells [12,21]. The experiments presented here were done by manually triggering the bubble generation while observing the cells under the microscope. A sorting time of about 5 ms per cell was achieved with one individual bubble generator this way and a first cell viability study on the sorted cells showed no significant effect of the BubbleJet actuated sorting procedure. Single-cell lysis has been demonstrated with 100% efficiency within cycle times of 20 ms. Future work will focus on integration of several bubble generators in a row along the main-channel and on implementation of automated image analysis for cell detection. This will enable to analyze and optimize reproducibility and to determine the maximum throughput of cell sorting and lysis.

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Biographies

Henning Hoefemann was born in 1981. He studied physics at the University of Bielefeld, Germany, and received his diploma in 2007. Since 2007 he works in the Microfluidic R&D group at HSG-IMIT and on his PhD in the field of microfluidic systems for blood and cell handling.

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Natalia Bakhtina was born in 1987. She received her bachelor degree as translator in the field of professional communication (information technologies) and her diploma in electrical engineering at the Department of Advanced Technologies of Radioelectronics, Russian State Technological University named after K.E. Tsiolkovsky (MATI), Moscow, Russia in 2009. In 2011 she received her MSc degree in microsystems engineering from Hochschule Furtwangen University of Applied Sciences, Germany.

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Nicolai Wangler was born in 1981. He studied micro systems technology at the University of Freiburg, Germany, from 2001 to 2006 and received his diploma in 2006. Afterwards, he worked for his PhD there at the chair of MEMS Applications from 2007 to 2010. His field of activity comprised the research and development of different tools for the specific and individual chemical stimulation of single cells and united cell structures. Since 2011 Nicolai Wangler works at the chair of process technology at the technical faculty of the University of Freiburg.

Roland Zengerle was born in 1965. He received his diploma in physics from the Technical University of Munich in 1990, the PhD from the "Universität der Bundeswehr München" based on the development of an electrostatically driven micropump in 1994. Since 1999 he is full professor at the Department of Microsystems Engineering (IMTEK) at the University of Freiburg, Germany. Today Prof. Zengerle in addition is a director at the Institut für Mikro- und Informationstechnik of the Hahn-Schickard-Gesellschaft (HSG-IMIT) and vice director on the center for Biological Signalling Studies (bioss). The research of Prof. Zengerle is focused on microfluidics and nanofluidics.