CELLJET: LABEL-FREE CELL PRINTING VIA REAL-TIME IMPEDANCE FLOW CYTOMETRY FOR SINGLE CELL ANALYSIS

Jonas Schoendube[1], Daniel Wright[2], Azmi Yusof[1], Roland Zengerle[1,3], Peter Koltay[1,4]
[1]Laboratory for MEMS Applications, Department of Microsystems Engineering - IMTEK, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, GERMANY
[2]Zurich Instruments AG, Technoparkstrasse 1, 8005 Zurich, SWITZERLAND
[3]HSG-IMIT, Georges-Koehler-Allee 103, 79110 Freiburg, GERMANY
[4]BioFluidiX GmbH, Georges-Koehler-Allee 103, 79110 Freiburg, GERMANY

ABSTRACT

The CellJet microfluidic dispenser chip prints single living cells encapsulated in free-flying droplets. Two sets of parallel facing electrodes in a 50 x 55 µm channel are applied to measure the presence and velocity of a single cell in real-time. Typically a 500 pl droplet is printed on demand, when a cell is at the nozzle. Feeding 20 µm polystyrene beads, a cell model, resulted in a peak-to-peak voltage of 70±16 µV and velocity of 7.5±0.8 mm/s. Single bead printing efficiency was 26% (N=124) with 64% void droplets. Moreover, viable HeLa cells and fibroblasts have been printed successfully.

KEYWORDS

Single cell analysis, cell printing, impedance spectroscopy

INTRODUCTION

Single cell analysis is an emerging method of the life sciences [3]. Conventionally, cell populations are analyzed as an integral ensemble. The averaged results obtained this way can possibly produce misleading results due to cell heterogeneity. Single cell printing enables to isolate and position cells individually on any open substrate. Our approach (Fig. 1) uses drop-on-demand printing and flow-through impedance-based cell detection [4]. It differs from our previous work [1, 2] by impedance-based chip-integrated real-time detection and from other's work [5, 6] by an improved electrode configuration (parallel facing vs. planar) and smaller droplet sizes.

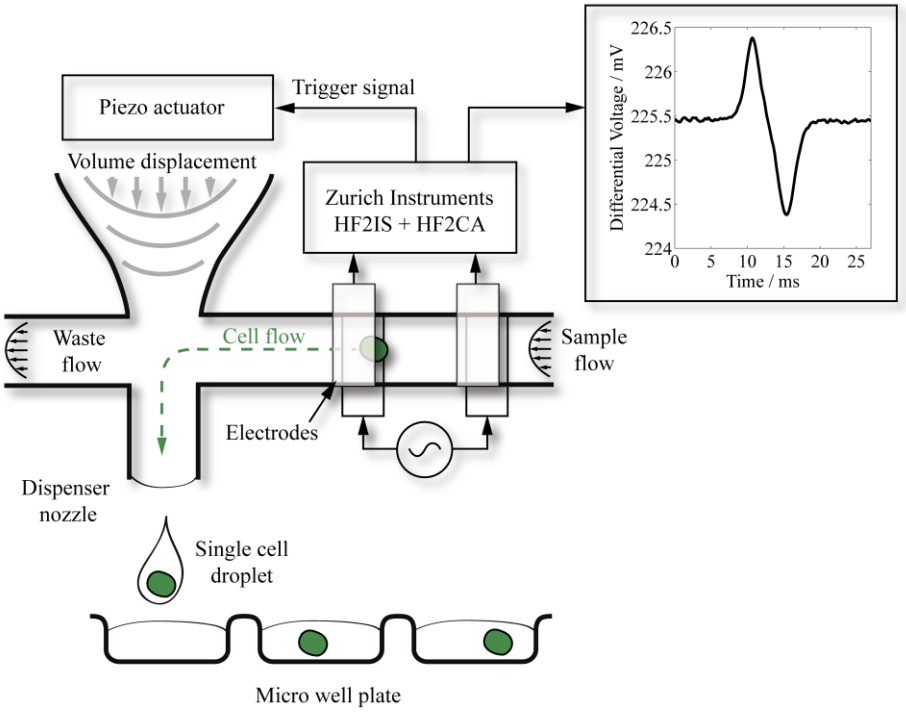

Figure 1. Schematic of single cell printing with the CellJet. Cells enter with the sample flow. Due to differential measurement of the channel impedance, they create a positive peak at the first electrode pair and a negative peak at the second electrode pair. The signal is analyzed in real-time and a piezo actuator deflects a silicon membrane to produce a droplet containing the single cell.

CHIP FABRICATION AND PRINTING SETUP

The CellJet dispenser chip comprises a cross-flow configuration of a droplet dispenser and a supply channel with two sets of parallel facing electrodes (Fig. 2). The electrodes are patterned on silicon and Pyrex wafers that are bonded via an intermediate TMMF layer defining the microchannels [7]. A droplet is ejected when a piezo stack actuator deflects the silicon membrane of the dosage chamber. A driving signal of 1 mV at 800 kHz is supplied to the electrodes. The channel impedance is measured differentially and converted to a voltage. A real-time algorithm running within an impedance spectroscope (HF2IS, Zurich Instruments, Switzerland) calculates the mean and standard deviation of the signal and thresholds for cell detection events (Fig. 4). Upon detecting an event, the algorithm determines both extrema of the double peak created by the cell and calculates the cell's velocity from transit time and electrode distance. This enables to identify the time delay for triggering the dispensing.

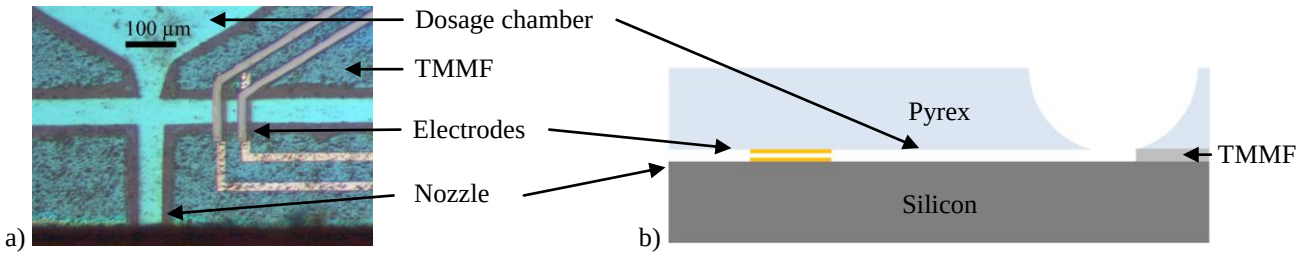

Figure 2. a) Optical micrograph of the microfabricated CellJet dispenser chip fabricated as a silicon / TMMF / Pyrex chip. b) Schematic cross-section of the dispenser chip.

EXPERIMENTAL RESULTS

To evaluate the dispensing performance the chip was filled with PBS and the piezo extension has been varied. Droplet volumes in the range of 500 pl – 800 pl depend linearly on the piezo extension (Fig. 3). Subsequently, polystyrene beads (20 µm, GKisker), suspended in PBS, have been fed to the dispenser chip. Fig. 4 b) shows eleven impedance signals of beads passing the electrodes without dispensing. One bead was not detected (see missing cross in 4 b)), because the detection algorithm was inactive during dispensing mode. Real-time analysis of the sensor signals of the detected beads resulted in peak-to-peak voltages of 70±16 µV and velocities of 7.5±0.8 mm/s (Fig. 4).

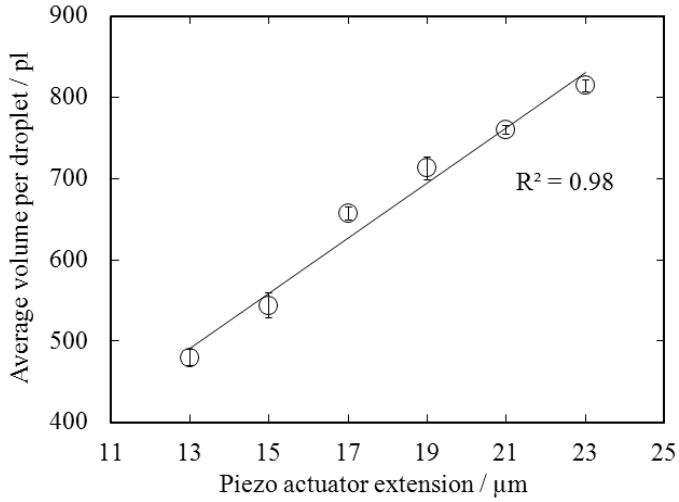

Figure 3. Variation of dosage volume with actuator extension for PBS. The dosage volume depends linearly on the piezo actuator extension, which deflects the silicon membrane of the chip. Droplet volume has been measured gravimetrically using a precision scale (XP2U, Mettler Toledo). To avoid evaporation, droplets have dispensed into oil (Dow Corning, 200 Fluid). Each measurement point represents the average of 50 dispensed droplets and its error bar represents the standard deviation of ten measurements.

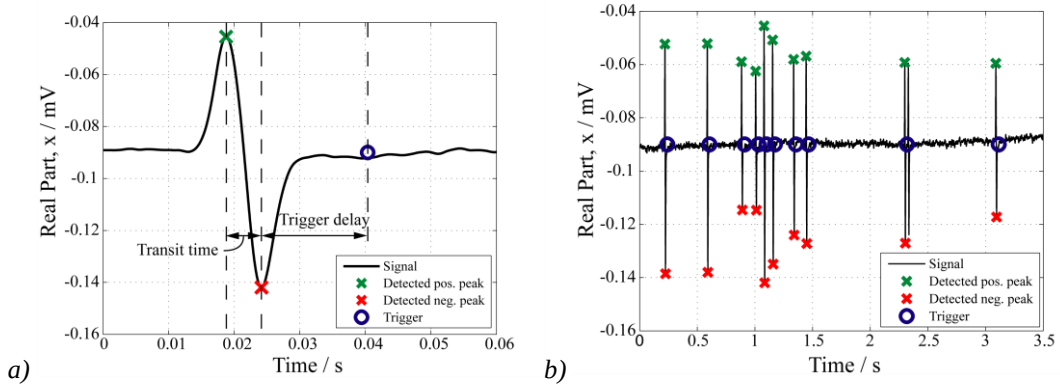

Figure 4. a) Impedance signal created by the two sets of parallel facing electrodes by a single 20 µm bead. The differential measurement creates a positive / negative peak when bead passes 1st / 2nd set of electrodes. Bead velocity is calculated from transit time (40 µm / 5.6 ms = 7.1 mm/s). b) Impedance signals created by eleven 20 µm polystyrene beads. Average peak-to-peak voltage is 70±16 µV. Average velocity is calculated to be 7.5±0.8 mm/s.

Beads have been printed with a single bead efficiency of 26% (N=124) using the described sensor signals as trigger. Fig. 5 shows a 20 µm polystyrene bead printed on a glass slide. To qualitatively evaluate the viability of printed cells, multiple HeLa cells (cervical cancer cell line) and fibroblast (human stem cells) have been printed into micro wells and cultured for eight and ten days, respectively. Fig. 5 b) shows a fibroblast after ten days of culture. Fig. c) and d) show a HeLa cell after dispensing and a grown agglomeration after eight days.

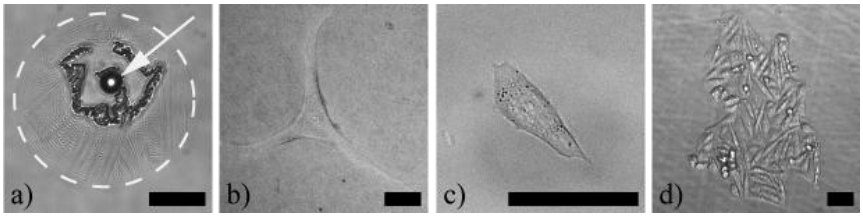

Figure 5. a) Printed 20 µm bead (arrow) in evaporated droplet (dashed line). b) Printed fibroblast after ten days of culture. c) Printed HeLa cell after one day of culture. d) Colony grown from printed HeLa cells after eight days of culture. Scale bars are 50 µm.

CONCLUSION

The presented chip combines impedance-based cell detection, real-time signal processing and drop-on-demand printing. Printing of polystyrene beads and cells has been shown. In future works, trigger parameters still have to be optimized to reduce void droplets and reach a high yield for single bead printing. Once this is achieved, impedance-based cell detection opens up the option for chip-integrated label-free single cell sorting.

ACKNOWLEGMENTS

This work has been financed by the European Commission (Project PASCA, www.pasca.eu, FP7 GA257073).

REFERENCES:

[1] A. Yusof et al., *Towards a Microfluidic Dispenser Chip for Printing of Single Cells*, Proc. of MEMS, 1059 (2011).
[2] A. Yusof et al., *Inkjet-like printing of single-cells*, Lab on a Chip, 11, 2447 (2011).
[3] C. Schubert, *Single-cell analysis: The deepest differences*, Nature, 480, 133 (2011).
[4] K. C. Cheung et al., *Microfluidic impedance-based flow cytometry*, Cytometry. Part A, 77, 648 (2010).
[5] R. Tornay et al., *Electrical Detection and Ejection of Beads in a One-Cell-Per-Drop Microdispenser*, Proc. of TRANSDUCERS, 695 (2007).
[6] T. Kawahara et al., *Air-Flow-Based Single-Cell Dispensing System*, Advanced Robotics, 26, 291 (2012).
[7] A. Yusof et al., *Wafer Level Fabrication of an Integrated Microdispenser with Electrical Impedance Detection for Single-cell Printing*, Proc. of Smart Systems Integration, 99 (2012).

CONTACT

*** Jonas Schoendube, phone: +49 761 203 73260, email: jonas.schoendube@imtek.de**