A MULTI-PURPOSE MICROFLUIDIC PIPETTE FOR SINGLE-CELL ANALYSIS

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ABSTRACT
We report a multi-purpose microfluidic pipette, with a recirculating liquid tip. This device, made in poly(dimethylsiloxane), enables contamination-free manipulation and chemical stimulation of selected single cells in cell collectives or tissue slices. The pipette is capable of carrying out a variety of complex fluid processing functionalities, such as mixing, multiplexing, or gradient generation. The concept is flexible and scalable as the geometry and the size of the recirculation zone is defined by pressure, channel number, and geometry. We have applied the pipette in a fluorescence uptake assay, electrophysiology studies and for chemical induction of membrane protrusion from biological cells.

KEYWORDS: Microfluidics, Single-cell analysis, Single-cell manipulation, Solution exchange, Superfusion

INTRODUCTION

To manipulate solution environments on the micro-scale is of a great interest for molecular and cellular biologists and biophysicists, who aim to chemically stimulate single cells, small cell groups, or parts of a selected single cell. Recently, several new concepts to deliver small amounts of liquid to microscopic objects of interest have been reported. Examples include the chemistrode [1], which uses a microfluidic flow cell to transport aqueous droplets in oil onto an area of interest. We have extended that methodology and developed a multifunctional microfluidic pipette, featuring a circulating liquid tip that generates a self-confining volume in front of the outlet channels (Figure 1) [2]. This pipette is conveniently applied to a large variety of microscopy based experiments, using micromanipulation tools for positioning.

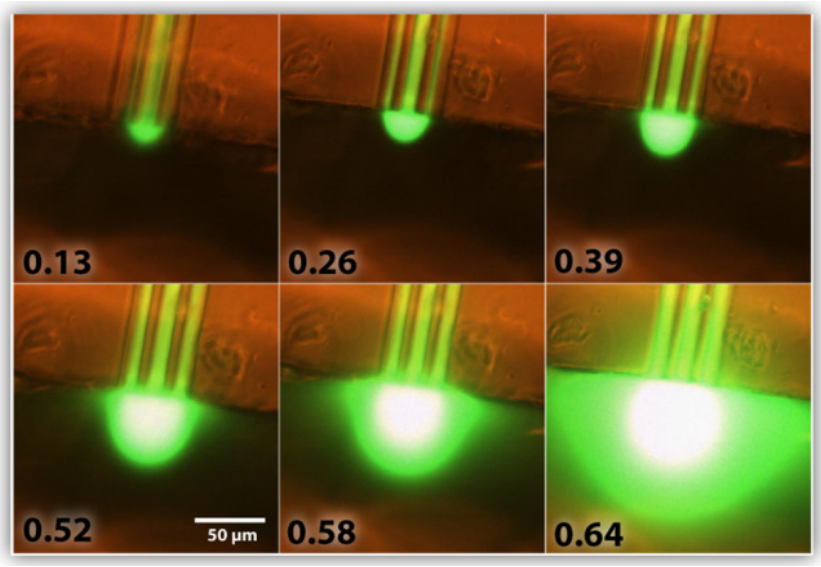

Figure 1: Flow recirculation generated by microfluidic pipette, visualized by fluorescence microscopy. The outflow contains 1mM fluorescein solution. The volume of the recirculation zone is depending on the ratio of outflow over inflow.

THEORY

The confined recirculation zone is formed by forcing all injected liquid back into the pipette, which allows exchanging solution inside the zone, while leaving the outside volume unaffected and contamination-free. The geometry of the recirculating solution is defined by the channel geometry and the ratio of the outflow over inflow (Figure 1). The major purpose of the recirculating flow is compensation of diffusion, which would otherwise not allow to maintain one solution composition inside another over an extended period of time. The ratio of convection and diffusion is described by the Péclet number

$$Pé \equiv \frac{diffusion\ time}{convection\ time} = \frac{vL}{D} \qquad (1)$$

where *v, L* and *D* are flow velocity, characteristic size and diffusion coefficient, respectively. The higher the Pé number the stronger is the confinement and the thinner is the boundary separating recirculation zone and open volume (Figure 2).

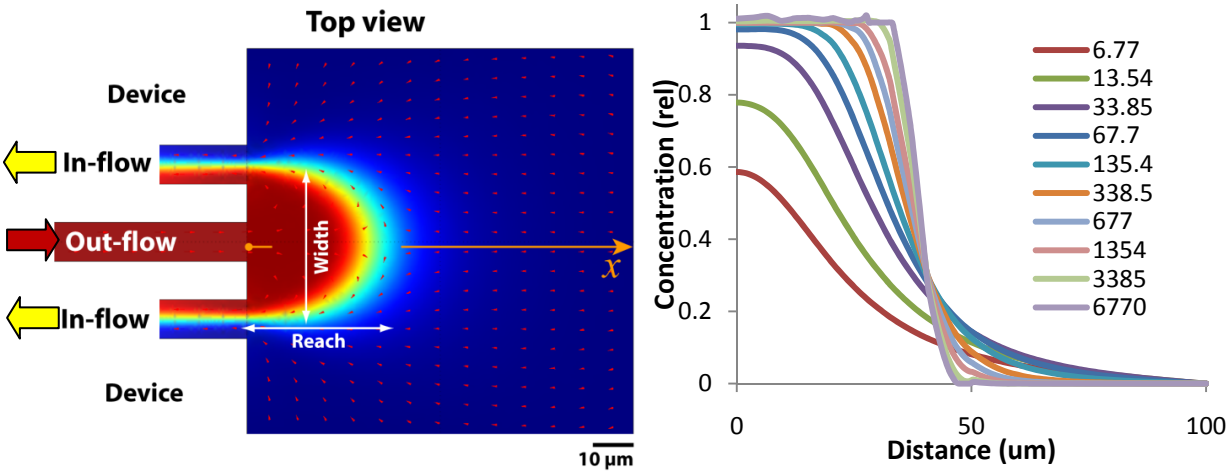

Figure 2: Left panel: Finite-element model (COMSOL) of a recirculating pipette tip. The color gradient denotes the concentration of recirculating solution (red) and open volume solution (blue). Right panel: Concentration distribution along the channel axis depending on the Pé number (in the range from 6.77 to 6770). A critical Pé number (~70) is needed to maintain the outflow solution concentration. In case of higher Pé numbers the diffusive boundary becomes sharper (left panel).

EXPERIMENTAL

We demonstrated the use of the pipette for single-cell manipulation in three application examples. Using a fluorescence uptake assay, we have show that it is possible to generate dose-response curves *in situ* from adherent cells expressing proton activated human transient receptor potential vanilloid (hTRPV1) receptors (Figure 3). For this purpose we used a microfluidic pipette featuring internal solution switching and diluting circuitry in order to create concentration series. In the second example, cells from the same cell line were studied by means of electrophysiology, where the microfluidic pipette was used for localized superfusion. In the third example, selected cells were exposed to a formaldehyde/dithiothreitol-containing solution in order to induce formation of bilayer membrane protrusions (blebs).

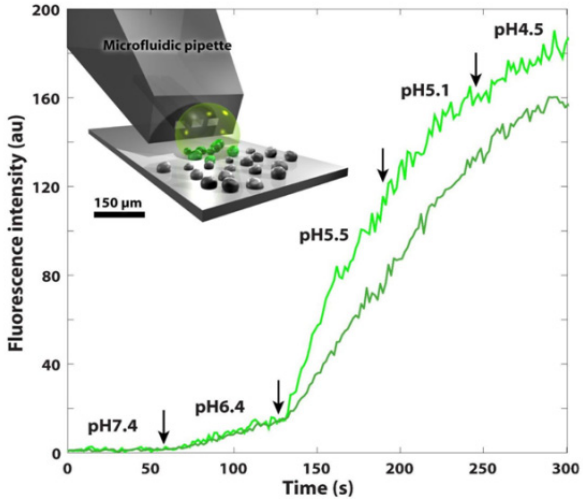

Figure 3.Example application of the microfluidic pipette, acting as localized superfusion device. Only the cells within reach of the recirculation zone are affected. Dose-responses were recorded from adherent single-cells. The cell is exposed to series of solutions with different pH. If a critical proton concentration is exceeded, hTRPV1 *channels are opening for the DNA binding YO-PRO-1 dye. The concentration of YO-PRO-1 is measured as change in fluorescence intensity by means of a confocal microscope.*

RESULTS AND DISCUSSION

This pipette demonstrates the combination of the functional richness of microfluidics, such as solution switching and dilution, with the facile positioning of conventional pipettes attached to micromanipulators. It can be applied to adherent cells and tissue samples, where it circumvents disruption of the cell structure, which can occur when cells are detached to bring them to a probing device. The microfluidic pipette can also be used advantageously in combination with other probes such as patch-pipettes, microelectrodes, optical fiber etc. The flow velocity of the recirculation can be adjusted by pressure control such that the desired steepness of a concentration gradient is obtained or to suit fragile structures such as membrane blebs.

CONCLUSION

Our microfluidic pipette is novel multipurpose superfusion tool compatible with a large variety of experimental setups and conditions, which can redefine the methods for chemical manipulation and analysis of single-cells. Practical benefits are contamination free-superfusion, on-chip microfluidic functionalities and a small enough footprint for direct application to cell cultures and tissue slices.

ACKNOWLEDGEMENTS

This research was funded by the European Research Council (ERC grant), the Knut & Alice Wallenberg Foundation, and the Swedish Strategic Research Foundation. We thank AstraZeneca R&D CNS&P Södertälje for the cell line stably expressing the *h*TRPV1 ion channel.

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