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Single Cell Immobilization at High Flow Rates Using 2PP-Traps in a Microfluidic Channel

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Summary:
This paper presents robust single cell traps fabricated in a microfluidic channel by two-photon polymerization (2PP) that withstand a very high average flow velocity of 1 m/s. The traps consist of a direct laser written thin grid structure allowing a good flow around the captured cells as well as optical measurements. Therefore, they enable the characterization of binding kinetics between active substances and cells. The traps have the shape of half a circular cylinder and capture the cells hydrodynamically. Jurkat cells with a diameter of 15 µm are captured in every trap within the first seconds of sample injection.

Keywords: 2PP, two-photon polymerization, additive manufacturing, cell trap, cell capturing

Background, Motivation an Objective

To characterize the binding behavior and cell specificity of active substances in an early stage of development, for instance in novel cancer drugs, the immobilization of cells in a flow with a flow rate between 40 µl/min and 3 ml/min is crucial. The high flow rate around the cell ensures the immediate removal of e.g. antibodies as soon as an unbinding event is taking place. The current concentration of antibodies on the cell surface can be determined by measuring a fluorescent label. Therefore, in order to realize the cell immobilization, traps are required that withstand high flow rates and enable a sufficient flow around the cell. For this reason, grid structures realized using 2PP are advantageous, because they allow the liquid to flow through the structure. With soft lithography, by which most single cell traps have been fabricated, thin grid structures are not possible. Consequently, 2.5D polydimethyl-siloxane (PDMS) traps [1][2] block the fluid more, resulting in maximum flow velocities of only 50 µm/s [1]. 3D grids have been used in multi cell sorting structures [3] reducing the flow around each cell as well as in cell cages for droplet deposition from the top applying no flow [4].

Description of the New Method or System

The novel 3D grid cell traps, shown in Fig. 1, are designed for cells that have a diameter of about 15 µm, e.g. Jurkat cells, and have a height of 35 µm. The diameter of each strut of the grid is set to 1.8 µm, which is as thin as possible avoiding deformations. The meshes have a size of 6 µm by 8 µm preventing the cells from passing through the trap. In order to observe the capturing success with an optical microscope, there is no polymer covering the top. The cell traps are laser written in IP-Dip resin by 2PP (Photonic Professional GT, Nanoscribe GmbH) on top of a gold electrode, which is structured on a glass chip. The gold improves the adhesion of the 2PP structure and simplifies the interface finding in the Photonic Professional GT.

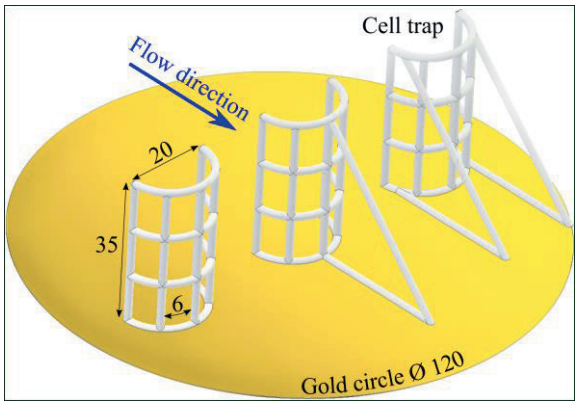

Fig. 1. Sketch of the cell traps with different support structures. The unit of all numbers is µm.

In order to test the stability of the traps with different support structures in a water flow, the chips are sealed with an 800 µm wide and 75 µm high PDMS channel on top of a second glass chip. Thus, the highest flow velocity in the middle of the channel hits the top of the trap. Two chips with 24 traps each are tested increasing the flow rate in steps of 100 µl/min until the traps break. The trapping success is tested with Jurkat cells in a DRX Biosensors system. For this, the channel height is set to 30 µm clamping the traps between the two chips to avoid cells flowing over the traps.

Results

The SEM image shows the 2PP fabricated traps protruding from the chip as designed (see Fig. 2). A slight shrinkage can be noticed at the upper part of the left and middle trap.

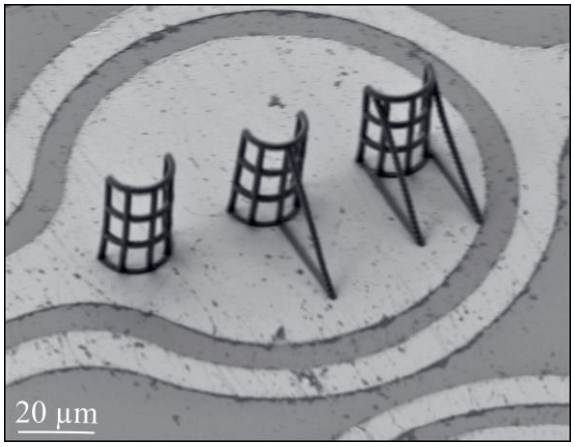

Fig. 2. SEM image of the different traps on a reused chip. Residue on the surface is due to multiple reuse of the chip.

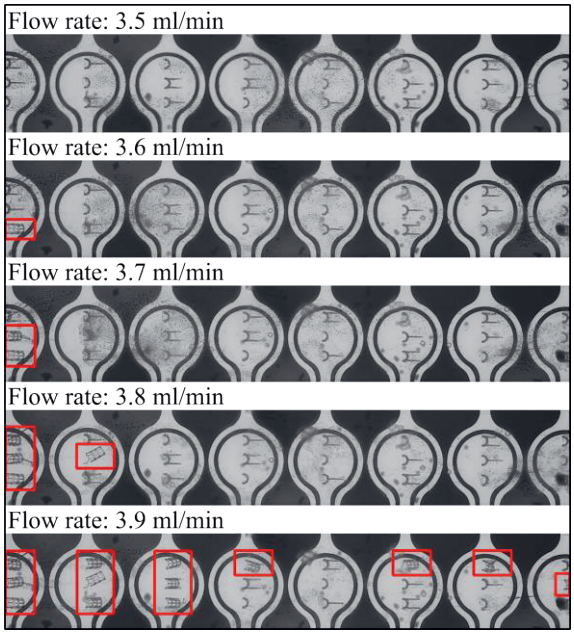

Fig. 3. Photographs of 24 cell traps in one 75 µm high channel from the top at increasing flow rates. The broken traps are marked red. Up to a flow rate of 3.5 ml/min all traps are intact. At higher flow rates the first traps start breaking. At a flow rate of 3.9 ml/min half of the traps are broken.

Fig. 3 illustrates the traps at different flow rates. Up to a flow rate of 3.5 ml/min, which is equivalent to an average flow velocity of 1 m/s, all traps in both chips withstand the flow, proving a very high stability and a very good adhesion between gold and IP-Dip. At higher flow rates the traps start breaking (marked red in the figure). Nearly all traps are broken at a flow rate of 4 ml/min. There is no apparent effect of the different support structures on the stability. Instead, mostly the first traps in the flow (on the left side) break, probably because they are exposed to the highest flow velocity.

Fig. 4 depicts the successful capturing of Jurkat cells 16 s after sample injection. The concentration of Jurkat cells in the sample is 10^6 cells/ml.

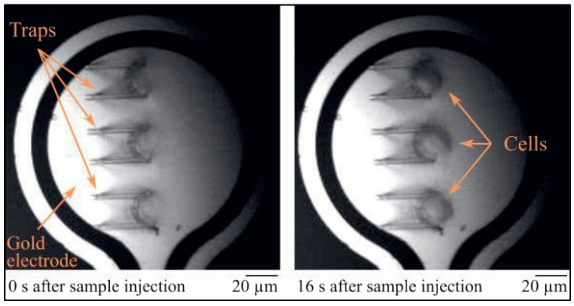

Fig. 4. Photographs of three cell traps before and 16 s after sample injection in a 30 µm high channel. After 16 s all traps are filled with Jurkat cells.

The presented results verify the ability of the novel 2PP traps to capture cells and their robustness at very high flow rates.

References

[1] J. Xu et al, A Microfluidic Chip with Double-Slit Arrays for Enhanced Capture of Single Cells. *Micromachines* 9(4), 157 (2018); doi: 10.3390/mi9040157

[2] D. H. Lee, X. Li, A. Jiang, A. P. Lee, An integrated microfluidic platform for size-selective single-cell trapping of monocytes from blood. *Biomicrofluidics* 12(5), 054104 (2018); doi: 10.1063/1.5049149

[3] B. Xu et al, Arch-like microsorters with multimodal and clogging-improved filtering functions by using femtosecond laser multifocal parallel microfabrication. *Optics express* 25(14), 16739-16753 (2017); doi: 10.1364/OE.25.016739

[4] C. Wang et al, Femtosecond mathieu beams for rapid controllable fabrication of complex microcages and application in trapping microobjects. *ACS nano* 13(4), 4667-4676 (2019); doi: 10.1021/acsnano.9b00893