TOWARDS SINGLE CELL FINGERPRINTING IN MICROFLUIDIC DEVICE FORMAT: SINGLE CELL MANIPULATION, PROTEIN SEPARATION AND DETECTION

W. Hellmich[1], K. Leffhalm[1], A. Sischka[1], T. Duong[1], N. Jensen[2], K. Niehaus[2], K. Tönsing[1], A. Ros[1], and D. Anselmetti[1]

[1]*Experimental BioPhysics & Applied NanoSciences, Bielefeld University, Germany*
[2]*Molecular Phytopathology and Genetics, Bielefeld University, Germany*

ABSTRACT

Our single cell analytical experiments for future protein fingerprinting combines microfluidic on-chip protein electrophoresis with latest laser technology for single cell manipulation and (label-free) protein detection, and is presented by the following issues: 1) Single cells were trapped and navigated by optical tweezers (OT) in a PDMS microfluidic device, and lysed at a predefined position. 2) Separation and detection of proteins was achieved with laser induced fluorescence (LIF) detection in the visible (488 nm) as well as in the UV (266 nm) spectral range for label-free protein detection. 3) First successful experiments with single GFP-transfected Sf9 insect cells exhibit distinct single component electropherograms.

Keywords: Single cell analysis, microfluidics, protein separation, label-free detection

1. INTRODUCTION

Single cell analytics for proteomic analysis is considered as a key method in the framework of systems nanobiology [2] which allows a novel proteomics without being subjected to ensemble-averaging, cell-cycle or cell-population effects, in contrast to conventional protein fingerprinting which is at the level of 10^5-10^6 cells.

The present work is based on the pioneering developments in micro total analysis (μTAS) or lab-on-a-chip systems [3,4], LIF detection of proteins [5] and single cell fingerprinting with capillary sieving electrophoresis [6-8].

In this manuscript, we focus on three main issues: Firstly, trapping and navigating of single target cells by means of OT in a PDMS microfluidic device. Secondly, separation and (label-free) protein detection with LIF detection in the VIS (488 nm) as well as in the UV (266 nm) spectral range. Thirdly, monitoring of first single cell electropherograms with single GFP-mutated Sf9 insect cells in this microfluidic device.

2. EXPERIMENTAL

Details on chemicals and reagents [1], fabrication and operation of the microfluidic device [9], optical tweezers and liquid handling [10], VIS/UV-LIF detection [11,1], have already been published elsewhere. Sf9 insect cells (*Spodoptera frugiperda)* from Novagen (USA) were transfected with pIEx4-vector (Novagen, USA) containing the gene for the fusion protein. The Sf9 cells expressed a 49.5 kDa GFP-labelled ´loss-of-function´ mutant (T31N-GFP) of the cytoplasmic G-protein ArF1 of *Medicago truncatula.*

3. RESULTS AND DISCUSSION

a) Single Cell Manipulation and Lysis: Single cells were optically selected, trapped and injected into the microfluidic channel with OT. The manipulation of the cell and its transfer to the crossing of our microfluidic device was realized with the dedicated x/y-stage. Once the cell was navigated into this position the optical trap was switched off and the cell was allowed to adhere to the microchannel wall. Cell lysis was performed either by flushing 0.5% SDS in PBS by hydrostatic pressure from channel 4 to 2 or by applying an electric field pulse of 1250 V/cm for 50 ms between channels 4 and 2. Both was controlled by optical bright-field microscopy (Fig. 1 d-g). Complete cell lysis was typically achieved within 6 seconds. Subsequent protein separation and detection was performed in channel 2.

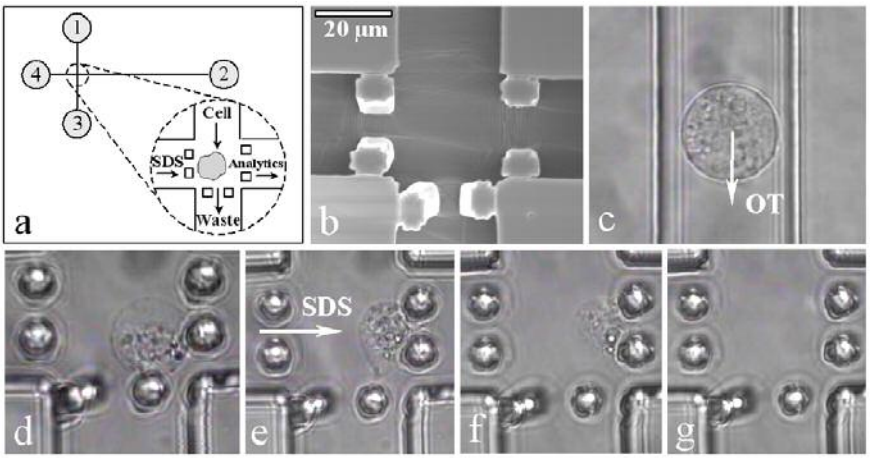

Figure 1. Microfluidic device with crossing and cell trap (b) SEM of the cell trap, (c) single cell navigated by OT (d-g) optical micrographs of a single cell at the injection position during SDS lysis.

b) VIS/UV-LIF Detection of Proteins: Electropherograms of fluorescein (100fM) and proteins (avidin 84 nM) my means of VIS-LIF (488 nm, 25 mW) (Fig. 2a and b) and label-free (native) UV-LIF (266 nm, 6 mW) (Fig. 2c) are shown in figure 2. With respect to the detection volume 100fM fluorescein solution corresponds roughly to 50-100 molecules, which is well below the anticipated number of low abundant protein copies in a single cell (~10^4 [1]). Furthermore, this is to our knowledge, the smallest dye concentration electrokinetically injected and detected in a microfluidic device so far.

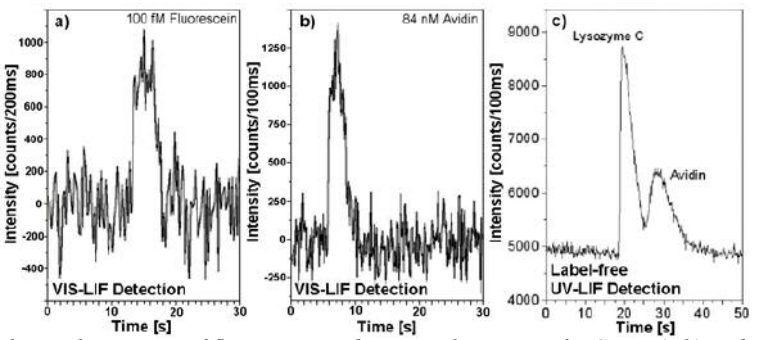

Figure 2. Electropherograms of fluorescein and proteins by means of VIS-LIF (a,b) and UV-LIF (c).

c) Single Cell Electropherograms: Figure 3 shows two single cell electropherograms (A: SDS-lysis, B: electric field pulse lysis (EF)) recorded by VIS-LIF detection (488 nm, 2 mW, Ar^+). The separation buffer contains 100mM Tris, 100mM CHES, and pullulan (4-8%) (pH 8.6). As expected from a single component analyte one distinct peak can be identified in both electropherograms which is attributed to the expressed GFP-construct protein. These are to our knowledge the first electropherograms of a single protein from a single cell in a microfluidic device.

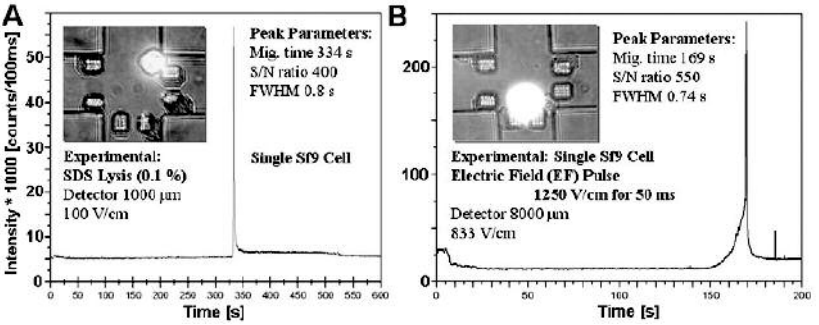

Figure 3. Single cell electropherograms with single component peak from the expressed GFP variant (A. SDS Lysis, B. EF-Lysis) with micrograph insets of fluorescent cells at the injection position.

4. SUMMARY & CONCLUSIONS

We developed a novel microfluidic single cell protein fingerprinting setup for systems nanobiology, and successfully tested for 1) trapping and navigating of single biological target cells by OT in a PDMS microfluidic device with consecutive lysis at a predefined position with SDS or an electric field pulse, 2) electrophoretic separation and detection of fluorescent dyes and proteins via VIS-LIF and label-free UV-LIF detection, and 3) recording of first single cell electropherograms of GFP-mutated Sf9 insect cells. These experiments proved the validity of the single cell analytical concept, and will allow novel and fascinating single cell experiments for systems nanobiology in the future.

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