PREDICTABLE AND DEFINED MICRO-GRADIENTS IN LIQUID ENVIRONMENT FOR CHEMICAL SINGLE CELL STIMULATION

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ABSTRACT

We present a new method for the precise and predictable generation of defined concentration spots in space and time within liquid environments *via* a discrete substance release in the sub pL-range (V = 10 pL) with a high spatial (< 50 µm) resolution to stimulate individual cells within densely populated cell cultures. A modular diffusion barrier (phase-gap) placed on the tip of the pL-dispenser (Pico-Injector) prevents of any diffusion based leakage. This allows for the positioning of the dispensing system at any position in a target liquid (e.g. next to an individual cell) to realize sharp and controlled concentration profiles predicted by analytical diffusion model. Dispensing individual droplets enables concentration profiles locally ($c < 1/5 \cdot c_{max}$ outside a radius of r = 50 µm) and temporally ($c < 1/5 \cdot c_{max}$ after 8 s) well defined. This setup was proven by labeling a single L 929 cell [1].

KEYWORDS: micro gradients, single cell stimulation, Pico-Injector, rhodamine, L 929 cell, discrete agent release

INTRODUCTION

A distinct chemical stimulation of single cells requires 1) well defined impact positions of 2) small agent droplets ($d_{droplet} < d_{cell} \sim 45\mu m$), 3) within a liquid cell environment, 4) controllable concentration profiles and 5) preventing of diffusion based agent leakage as essential criterions. In 2008 we presented the Pico Injector stimulating in a gel matrix immobilized single cells in an air environment by releasing small droplets (V = 10 pL; $d_{droplet}$ = 25 µm) impinging on a sharp spot (< 50 µm) [2] as well as the phase gap at a dispenser tip preventing diffusion based leakage of agent into target medium by forming a stable phase separation [3]. Now, for the first time we combined these approaches for the generation and investigation of defined concentration spots in the sub pL-range within liquid environments.

EXPERIMENTAL SETUP

A single L 929 cell (mouse fibroblast) [1] was labeled by ejecting a discrete volume (10 pL) of a fluorescent marker (lipopeptide coupled to Rhodamine [4]) inside the cell medium after positioning the Pico-Injector at a distance of approximately 200 microns next to a selected cell. Using an inverted microscope the distribution of the concentration profile was investigated online (Fig. 1, Fig. 2a).

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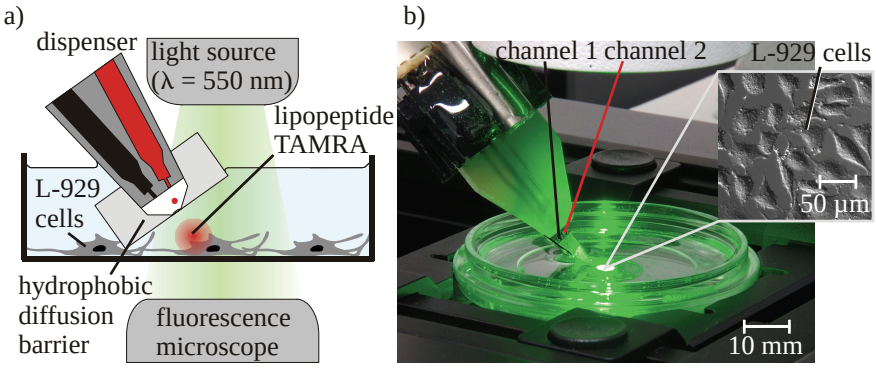

Figure 1. a) Experimental setup for the defined generation of micro gradients for single cell stimulation b) setup photography

DEFINED MICROGRADIENTS FOR SINGLE CELL STIMULATION

The 3-dimensional diffusion in combination with the released pL volumes (10 pL) leads to sharp and predictable profiles and negligible fluorescent background signals (Fig. 2). Lipopeptide becomes inserted into the cell membrane and thus the labeled cell emits an obvious fluorescent signal after 60 seconds (Fig. 2b).

The concentration at the release site decreases drastically within 8 s ($c < 1/5 \cdot c_{max}$). Furthermore, the concentration profile never exceeds $1/5 \cdot c_{max}$ outside a radius $r = 50$ µm. The distribution of the concentration profile can be approximated using 3 dimensional diffusion (Fick´s laws). With Dirac´s delta and a temporal offset ($t_{Offset} = 0.7$ s) to mimic the initial droplet dimension the analytic model fits reality very well. A diffusion coefficient of $D_{lipopeptide+Rhodamine} = 6.4 \cdot 10^{-11}$ m²/s ($D_{Rhodamine\ B} = 3.6 \cdot 10^{-10}$ m²/s [5]; $Ø_{lipopeptide+Rhodamine} >> Ø_{RhodamineB}$) is derived (Fig. 3). This was double checked with Computational Fluid Dynamics (CFD) simulation.

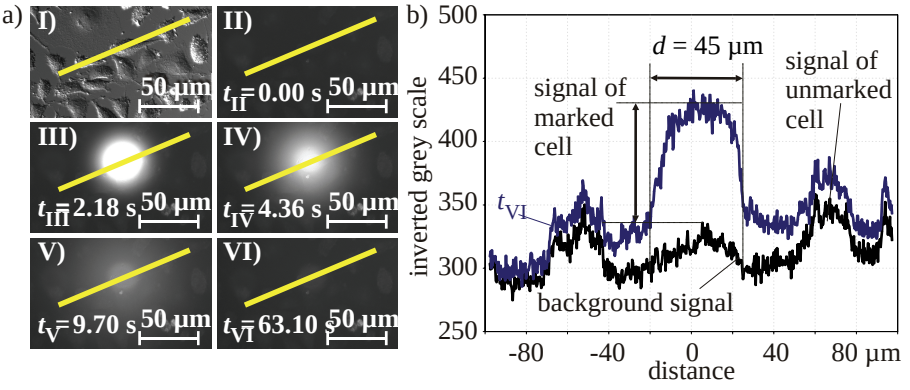

Figure 2. a) DIC (I) and fluorescence (II) image of L-929 cells before agent release. After 1 s the agent is released with an initial fluorescence concentration (III) and starts to diffuse (IV,V) while a single cell is marked precisely (VI). b) Fluorescence signal of three cells including a marked single cell.

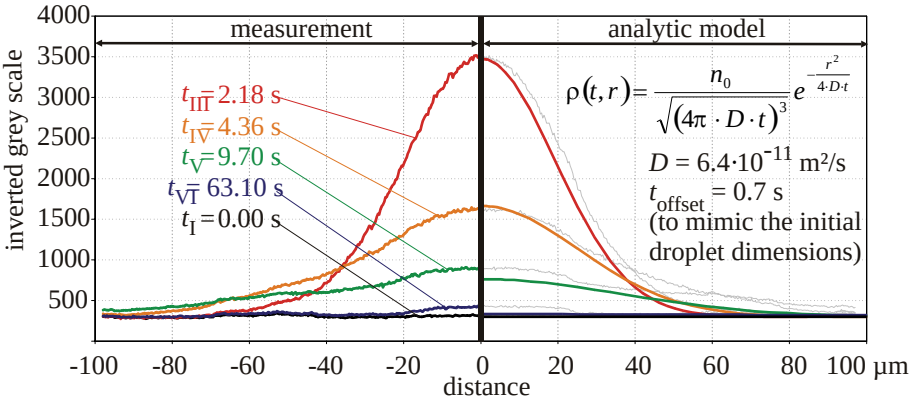

Figure 3. Greyscale level of the concentration gradient (line in Figure 2a)) after releasing a single droplet lipopeptide (V = 10 pL; $d_{droplet}$ = 25 µm) on a cell. Due to the small volume and 3-dimensional diffusion the maximum concentration is negligible in the ambiance ($c < 1/5 \cdot c_{max}$ outside a radius of r = 50 µm) whereas a high concentration in the center can be observed marking the cell. The right side shows the accordant analytic model using 3 dimensional diffusion with Fick´s laws.

CONCLUSIONS

This new method for defined generation of controlled concentration profiles within liquid environments allows for chemical stimulation of individual cells within densely populated cell cultures. Furthermore, the Pico-Injector features two individually addressable channels leading to various customized delivery protocols. This way, complex multi-drug gradients could be generated to pave the way for future applications such as specific ion channel screening, biological analysis, drug screening and many more.

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