HIGH-DENSITY ARRAY OF SINGLE CELL TRAPS FOR HIGH-THROUGHPUT IMAGING OF CALCIUM DYNAMICS IN RESPONSE TO OXIDATIVE STRESS

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ABSTRACT

Stochasticity in gene expression, protein or metabolite levels contributes to cell-cell variations. The analysis of these variations could lead to a better understanding of cellular processes and drug responses, but require single cell analysis techniques. To this end, we developed a new microfluidic platform for high-throughput capture (in about a minute) with low shear and imaging of thousands of single cells. This platform is compatible with upstream components for complex cell stimulation patterns. Imaging of calcium dynamics in response to oxidative stress with this platform reveals variable phenotypic responses within clonal populations.

KEYWORDS: Single cell, Array, Cell traps, Imaging, Calcium

INTRODUCTION

Single-cell analysis is important for quantitative systems biology and drug discovery, but current technologies require trade-offs of throughput, resolution (in space, time, and tracking individual cells instead of population average) and the ability to control cellular environment. For instance, flow cytometry is high throughput but cannot track same cells over time and under different conditions. To elucidate intracellular signaling, gene network regulation and cell-cell variation in response to changing environments, one would need to image large number of individual cells over time under different conditions [1].

Microfluidic tools have been developed to trap cells [2,3]. However, in most designs available to date, there seems to be a compromise between loading efficiency and single-cell trapping. We present here a new microfluidic platform for highly efficient and parallel trapping of thousands of cells, interfaced with an upstream gradient generator, for calcium dynamics imaging in Jurkat cells in response to oxidative stress.

EXPERIMENTAL

One layer PDMS devices are fabricated by traditional soft lithography. Briefly, a first layer of 2 μm of SU-8 is spun onto a silicon wafer to create cross flow channel. An additional layer of 15 μm is deposited on top to create the traps. PDMS devices are cast on the SU-8 master and bond to glass slides by oxygen plasma treatment. The devices are primed with solutions of PBS, 2% BSA to prevent cell sticking, before cells are delivered by pressure driven flow to the trapping chambers. A small amount of cell suspension (20 μL) at $5 \cdot 10^5$ cells/mL is sufficient to fill the 8 chambers of the device in a few minutes.

Jurkat E6-1 cells (ATCC) were cultured in complete medium in a humidified 5% CO_2 incubator. Viability and calcium dynamics experiment were performed in a microcontrolled environment on an epifluorescent microscope (Nikon Eclipse Ti) using respectively Live/Dead stain (Invitrogen) following manufacturer's protocol and 6μM Fluo3 (Invitrogen). High resolution imaging for colocalization studies was performed on a 2-photon confocal microscope (Zeiss LSM 510 NLO). Image J and customized Matlab programs were used for image analysis.

DESIGN

We present here a microfluidic tool for highly efficient and parallel trapping of more than 4,000 single cells in a minute, with a loading efficiency of ~ 95%. The one-layer PDMS device consists of eight chambers of highly packed single cell traps, easily integrated with upstream microfluidic components, for environmental manipulation, such as a concentration gradient generator, therefore enabling real-time kinetic studies, or steady-state response to drug treatment (Fig. 1a). Each chamber consists of a wide serpentine cell delivery channel and an array of cross-flow channels that connect each section of the serpentine channel (Fig. 1b). Each cross-flow channel includes a cell pocket connected to a shallow channel (2 μm) (Fig. 1c). The geometry of the channels is optimized so that cells experience identical trapping condition throughout the entire array, to significantly increase trapping efficiency. In addition, high bulk stream flowing perpendicular to the loading flow flushes out extra cells to ensure single cell trapping in each trap. The ratio of the cross flow to the bulk stream determines the loading efficiency as well as the number of cells in each trap.

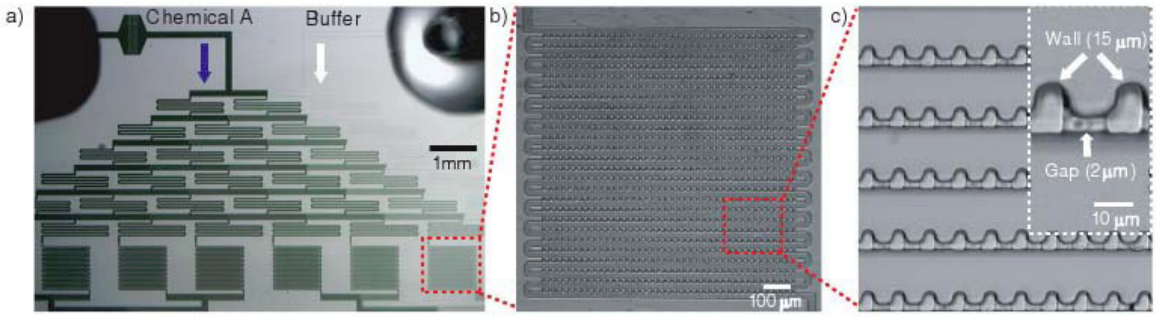

*Figure 1: a) Optical micrograph showing the device consisting of a gradient generator and eight chambers of highly packed single cell traps. Stable chemical gradients (not disturbed by cell presence) can be generated and delivered to individual chambers, each including 500 cell traps. b) Zoom-in image of the boxed region in **a** showing a single chamber. c) Zoom-in image of the boxed region in (b) showing the structure of a cell trap.*

RESULTS AND DISCUSSION

Cell capture occurs with minimal shear on the cells, as demonstrated with a 94% viability of Jurkat cells after 24h of culture. The high-density packing of single cell traps enables to simultaneously image up to 500 cells at 10x, and 8 cells at 100x, for high resolution microscopy, e.g. for colocalization studies (Fig. 2).

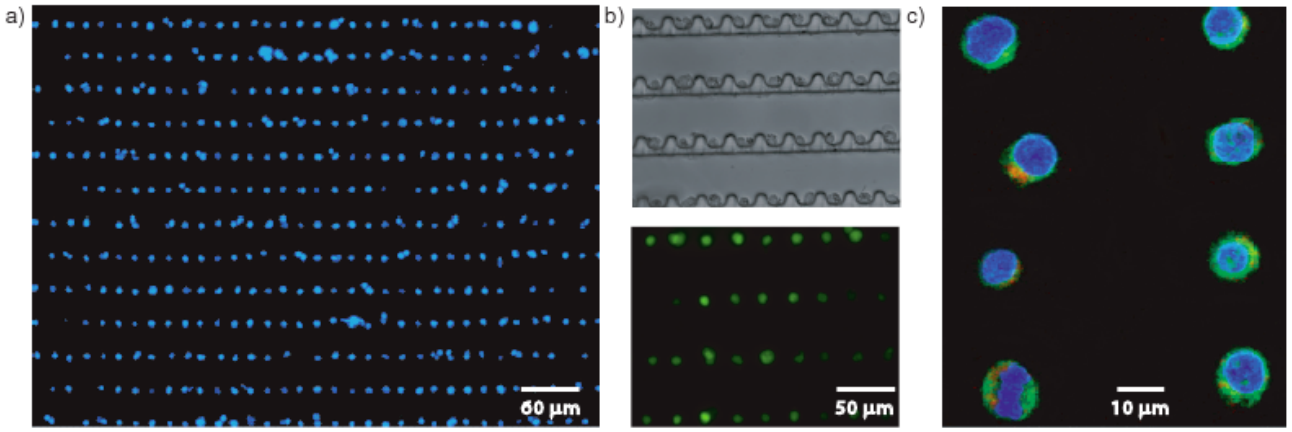

Figure 2: Densely packed single cells at various magnifications. a) At 10x up to 480 cells can be simultaneously imaged. B) 36 cells at 40x. Top, bright field; bottom, fluorescence mode. c) 8 cells at 100x. Cells have been immunostained for DNA (Hoechst - blue), Calnexin for the Endoplasmic Reticulum (Alexa 488 - green), and profilin-1 for the cytoplasm (Alexa 532 - red).

Using this platform, we studied calcium dynamics in response to hydrogen peroxide stimulation in Jurkat cells. Calcium is a ubiquitous signaling molecule exhibiting oscillating patterns in lymphocytes. A tight control over the frequency of these oscillations enables control of gene expression in the case of antigenic stimulation [4]. It is also known that oxidative stress induces calcium oscillations in some cells [5]. To simulate lymphocytes' response to higher levels of reactive oxygen species at the site of inflammation, we stimulate Jurkat cells with hydrogen peroxide in our cell trap array and record calcium responses. Increasing concentrations of hydrogen peroxide (0-1mM) led to increased release of calcium (Fig. 3a-c). In addition, preliminary data (Fig. 3d) suggests the existence of variable phenotypic responses: ~50% of the cell population exhibits calcium oscillations for 15 minutes following stimulation and sustained elevated calcium levels for an additional 15 minutes; other cells have a linear increase in their cytosolic calcium concentration, and a few cells, start exhibiting calcium oscillations after 15 minutes of stimulation.

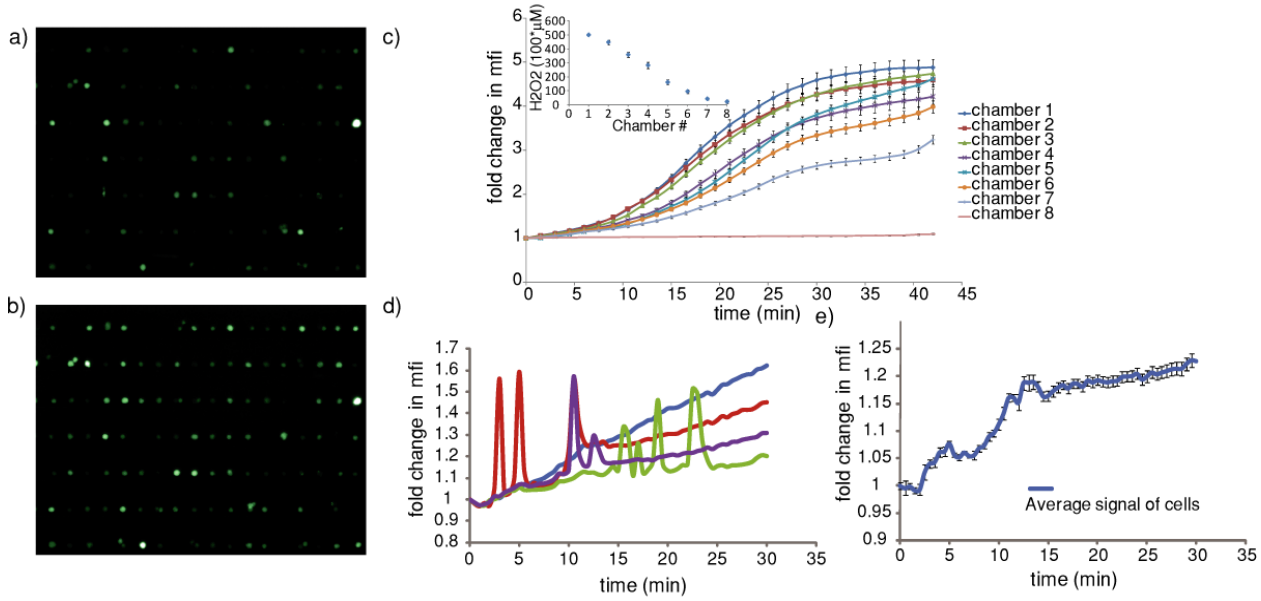

Figure 3: Jurkat cells calcium kinetics upon oxidative stress, imaged in the microfluidic cell trap array. a) and b) 20x micrographs of Jurkat cells loaded with Fluo3 (6 µM) before (a) and after (b) the addition of 500 µM hydrogen peroxide. c) Calcium kinetics (fold change in mean fluorescence intensity (mfi) normalized to non stimulated value) of about 80 cells subjected to different concentrations of hydrogen peroxide, ranging linearly from 0 to 500 µM .The inset represents hydrogen peroxide concentration in each of the 8 chambers (average of 4 stable gradients) d) Representative single cell responses from (a) and (b). Oscillation patterns are very specific to individual cells. e) Calcium kinetics of 112 cells trapped in the chamber represented in (a) and (b). Note that this set of data have higher time resolution (compared to those shown in (c)).

CONCLUSION

We present here a new trapping mechanism for non adherent cell capture in dense arrays. This trapping mechanism is completely passive, does not induce undesirable shear stress and can be maintained for at least 24 hours in the presence of a low pressure driven flow. The trapping mechanism allows for sequential capture of arriving cells, thus limiting the amount of cells going to waste and enabling the capture of rare or precious cells (e.g. transfected cells). This trapping mechanism also enable to densely pack the traps without fear of clogging, therefore allowing for higher throughput higher resolution imaging. We also showed that these cell trap chambers can be easily integrated with upstream components able to manipulate the environment the trapped cells are subjected to. The measure of calcium dynamics in response to oxidative stress revealed heterogeneity in cell response. We envision this imaging platform to enable high throughput single cell response to complex temporal or spatial stimuli, therefore facilitating a broad range of studies requiring single-cell quantitative analysis.

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