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Integration of single cell traps, chemical gradient generator and photosensors in a microfluidic platform for the study of alpha-synuclein toxicity in yeast

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Abstract

Alpha-synuclein (aSyn) is a key player in Parkinson's disease. Genetically engineered yeast cells producing aSyn fused with GFP (aSyn-GFP) have been used to study this protein. In this work, we present a microfluidic platform with integrated photosensors that captures single yeast cells in arrays of hydrodynamic traps and exposes them to a chemical gradient of precise composition. This platform enables the study of the effects of aSyn expression level and aggregation in genetically modified yeast cells by chemical stimulation. The photosensors allow the detection of cells in the traps by measuring the variations in light transmission or of the fluorescence produced by aSyn-GFP for real-time signal acquisition.

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Keywords: cell chip; microfluidics; gradient generator; cell trapping; yeast; alpha-synuclein; Parkinson's disease; photodiodes

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1. Introduction

Alpha-synuclein (aSyn) is the main component of Lewy bodies, protein aggregates that are the hallmark of Parkinson's disease (PD). Although aSyn has an important role in PD, the mechanisms behind its aggregation and toxicity are not yet well understood and yeast cell models have been used to study the biological effects of normal and misfolded aSyn [1]. *S. cerevisiae* (Sc) was chosen since it is a well known organism, and it is easily manipulated into producing and correctly folding human proteins. To study the effects of aSyn expression and aggregation, Sc cells were genetically engineered to express aSyn fused with GFP (aSyn-GFP) under the control of a galactose-induced promotor [1].

Classical cell culture offers tools that study yeast cell populations by exposing them to chemical stimuli, however it is almost impossible to achieve single-cell resolution, real time monitoring and dynamic microenvironment control in these classical systems. Microfluidic technology, which has been widely used for biological applications [2], can potentially address these challenges through the use of single-cell capturing techniques [3] and gradient generators [4]. The combination of these two strategies allows the precise control of a cell's microenvironment and the tracking of its behaviour.

2. Device description

2.1. Microfluidic device

To be able to track hundreds of single cells and study their behaviour when exposed to chemical stimuli, we developed a PDMS-based microfluidic device that combines a chemical gradient generator (Fig. 1) **[4]** with hydrodynamic single cell traps (Fig. 2). The chemical gradient generator is composed of a network of microfluidic channels that allow the diffusive mixing of three initial solutions with different chemical compositions. This network is coupled to 9 chambers, each of which have an array of hundreds of hydrodynamic traps with dimensions designed to hold Sc cells. These traps are distributed along the streamlines of cells in suspension inserted into the device, and are able to capture individual cells. The cell traps are semi-circular structures with a gap in the middle: fluid is able to flow through the trap when it is empty, but the gap becomes blocked when the trap is occupied, rerouting incoming cells to other traps.

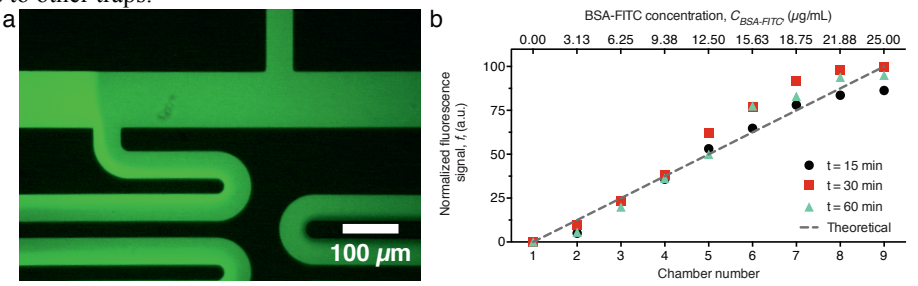

Fig. 1. (a) Gradient generator. Fluorescence micrograph of two fluids with different concentrations of FITC splitting and mixing by diffusion at the start of a new level of the network.; (b) Linearity and stability of the chemical gradient generator. Normalized fluorescence signals of BSA-FITC gradients generated by the microfluidic device with three initial solutions with concentrations of 0, 12.5 and 25 μg.mL^{-1}.

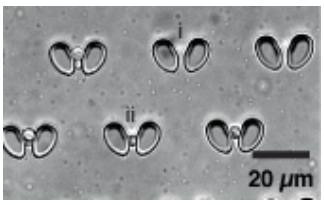

Fig. 2. Hydrodynamic cell traps. Bright field optical microscopy image of hydrodynamic traps in a chessboard configuration. The traps have a circular cell receptacle with 8 μm in diameter and a 4 μm gap. They can be empty (i) or occupied with cells (ii).

The above described system can expose populations of cells to precisely controlled environments of different chemical compositions. These chemical stimuli are used to modulate aSyn expression and aggregation and, since the cells are trapped, we were able to analyse its effects on several hundreds of single Sc cells over time.

2.2. Integrated photosensors

To be able to detect fluorescence signal in real time, we fabricated and integrated a-Si:H p-i-n photodiodes (Fig. 3) with the microfluidic platform. Since we aimed at detecting the signal emitted by the aSyn-GFP produced by the genetically modified yeast cells, a 2 µm thick a-SiC:H filter was deposited on top of the sensors. This allows to cut most of the excitation wavelength (480 nm) while allowing most of the emission wavelength (509 nm) to reach the sensor.

By aligning the photodiodes with the cell traps we can also detect when a cell has been trapped through variations in light transmittance.

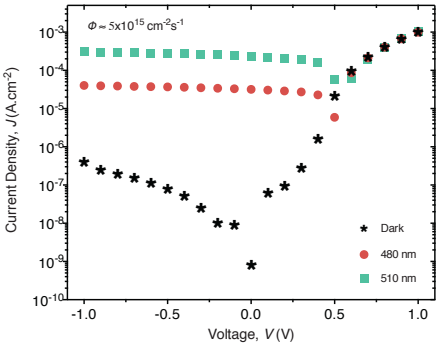

Fig. 3. Photosensor characterisation. Current density for a a-Si:H photodiode measured in the dark (stars) and under illumination at 480 nm (circles) and 510 nm (squares) with $\Phi \sim 5x10^{15}$ cm^2s^{-1} as function of voltage.

3. Results

3.1. Regulation of aSyn expression in the microfluidic device

By using a galactose-induced promoter we are able to regulate the expression of aSyn-GFP in the genetically modified yeast cells. To show that cells behaved normally in microfluidic conditions and to assess the performance of the microfluidic platform, we exposed populations of cells to a linear gradient of medium containing 0% to 1% galactose. We were able to verify that cells in chambers with higher concentrations of galactose emitted a higher fluorescent signal and could pinpoint the time at which aSyn-GFP production peaked (Fig. 4).

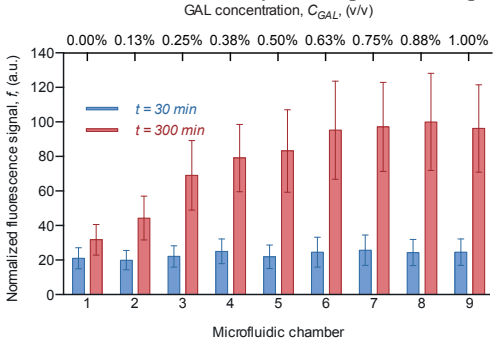

Fig. 4. Yeast response to a linear galactose gradient. Normalized mean fluorescence signal of aSyn-GFP in yeast cells over time when exposed to a linear concentration gradient of galactose from 0 to 1% (v/v). The initial solutions were composed of 0%, 0.5% and 1% of galactose,.

3.2. Assessment of trap occupancy by light transmission variation

The integrated photosensors also allowed the detection of the presence of cells in the traps. Since the photodiodes are aligned with the traps, the presence of one or several cells will absorb and scatter light, which will decrease the amount of light reaching the sensor (Fig. 5).

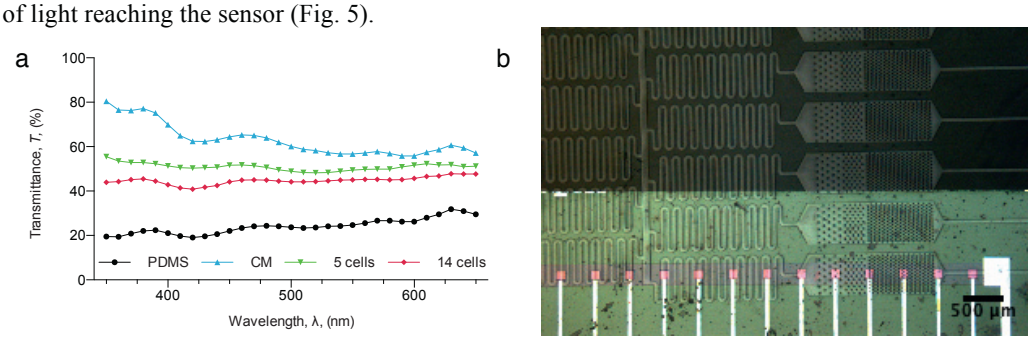

Fig. 5. (a) Cell detection using photodiodes. Transmittance measurements using 100x100 µm photodiodes in 4 configurations: empty PDMS device (PDMS); device filled with culture medium (CM); and presence of 5 (5 cells) and 14 cells (14 cells) immobilized in traps over the photodiode. The transmittance percentage is normalized to the transmittance measured without the device on top of the photodiode and $\Phi \sim 10^{15}$ $cm^{-2}s^{-1}$ (b) Integration of photosensors and microfluidics. Bright field optical microscopy of the photodiodes aligned with the microfluidic device.

4. Conclusion

A novel microfluidic platform was developed that combines a gradient generator, a system of hydrodynamic cell traps and an integrated photosensor aligned to the traps. By studying the production of aSyn-GFP by genetically modified yeast cells as a response to galactose concentration, we were able to demonstrate that the behaviour of these cells in microfluidic conditions are equivalent to what is expected in classical cell culture systems. Furthermore, since cells were trapped we were able to track the evolution of aSyn-GFP production in different cell populations. The integration of photosensors not only allowed to detect the presence of cells in the traps through transmission measurements, but also offers the possibility of real time detection of fluorescence signal without the use of a fluorescence microscope and complex analysis.

Ultimately, this platform will be used to gain new insight in the molecular mechanisms of aSyn in the context of PD and to perform high-throughput assays for potential anti-aggregation and cytoprotective chemicals.

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