Measuring How Clocks in Single Cells of *Neurospora Crassa* Communicate in Microfluidic Devices

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ABSTRACT

Most eukaryotes and cyanobacterial species have a biological clock that allows adaptation to the daily light/dark cycle of the planet. A central problem in the study of the biological clock is understanding the synchronization of the stochastic oscillators in different cells and tissues, but this problem is largely unstudied, particularly in the context of circadian rhythms. We developed a novel microfluidic platform to make high-throughput and high-precision measurements of biological clocks on a controlled number of *Neurospora crassa (N. crassa)* cells. Single cell measurements in this platform enabled us to test whether clocks of individual cells are able to communicate.

KEYWORDS: Quorum sensing, synchronization, single-cell measurement, biological clock, circadian rhythm

INTRODUCTION

Circadian rhythm is a 24 hour cycle that regulates the processes occurring in our body and mind, also known as our "body clock". Synchronized clocks are important to coordinate rhythmic behavior of individual elements, such as body temperature, immune responses as well as our immune response [1]. Therefore it is crucial for us to gain more insight regarding how cells are able to synchronize with each other to achieve a synchronized clock. *Neurospora crassa (N. crassa)*, a model fungal organism provides us with an unprecedented window into the investigation of clocks in higher organisms. Recent evidence and our preliminary results suggest that biological clocks of *N. crassa* are stochastic on the single cell level [2, 3] and current measurements of the clocks of *N. crassa*, made from tens of millions of cells, indicate that they do have a synchronized behavior [4]. In order to understand this discrepancy and the synchronization of biological clocks between individual cells, we are facing several challenges, including: (1) lack of an ability to measure biological clocks of *N. crassa* on a single cell level; (2) lack of knowledge on how clocks of different *N. crassa* cells communicate to overcome their stochastic asynchrony. Our hypothesis in this study is that single cells are able to communicate through the mechanism of quorum sensing. When the density of cells reaches a certain threshold, the single cells will be able to secrete a signaling molecule to produce a coordinated response. Traditional studies mostly focused on bulk measurements and thus provide us with minimal information due to its low resolution and inability to explain cell uniqueness [5]. Significant challenges in single cell measurements include the ability to scale up for measurements of a large number cells continuously. Furthermore, long-term analysis requires a setup with a stable incubation that retains the physiological function of cells [6]. Hence, there is a critical need for the development of a microfluidic device that has high-throughput, ability for large scale incubation of cell populations for the investigation of the synchronization of cells in the confined area. Here, we are introducing a microwell microfluidic device to capture single cells and track their fluorescence intensity for long-term imaging.

EXPERIMENTAL

Microwell devices are fabricated via soft lithography in two separate layers of polydimethylsiloxane (PDMS). Figure 1(a) details the microwell microfluidic device to trap individual cells that is constructed. The microwell microfluidic device is composed of a microwell array that are 10 μm in diameter and 10 μm deep. MFNC9 strain of *N. crassa* cells with *mCherry* fluorescent recorder are utilized for fluorescence measurements. Cells at a concentration of 6E+7 cells/mL are pipetted into the inlets and allowed time to settle into the microwells. After cells are trapped into their individual wells, the device was flushed with culture medium. This process is repeated until remnant cells are removed. CellProfiler was used to track individual cells over time.

RESULTS AND DISCUSSION

The microwell microfluidic device successfully housed more than ~5000 cells in a chamber that could seed more than ~7500 cells, showing a trapping efficiency around 67%. This device mimics a microtiter plate a

microscale to trap single cells. Single cell fluorescence trajectories were able to be tracked using CellProfiler over a period of 10 days (Figure 2a). We were able to observe oscillations in 10 random trajectories (Figure 2b). After detrending using a 24 hour moving average, the cellular oscillations were more pronounced. (Figure 2c).

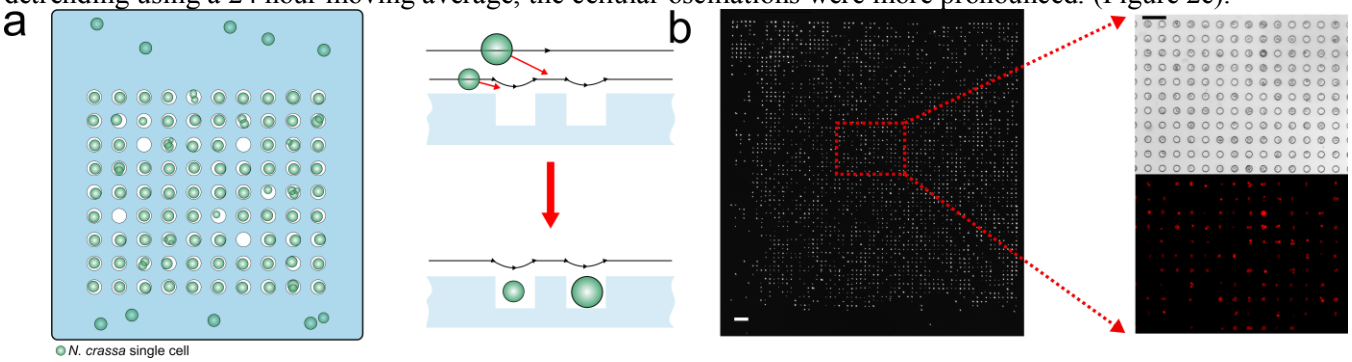

Figure 1: A microwell microfluidic device to trap individual cells is constructed (a) Schematic of cells (in green) seeded in individual microwells at 10μm in diameter. (b) Left: Fluorescence image of N. Crassa (MFNC9) cells in the microwell device. Scale bar : 100μm. Right: Visualization of cells trapped in individual microwells at 20x magnification. (Top: brightfield; bottom: fluorescence). Scale bar: 50μm.

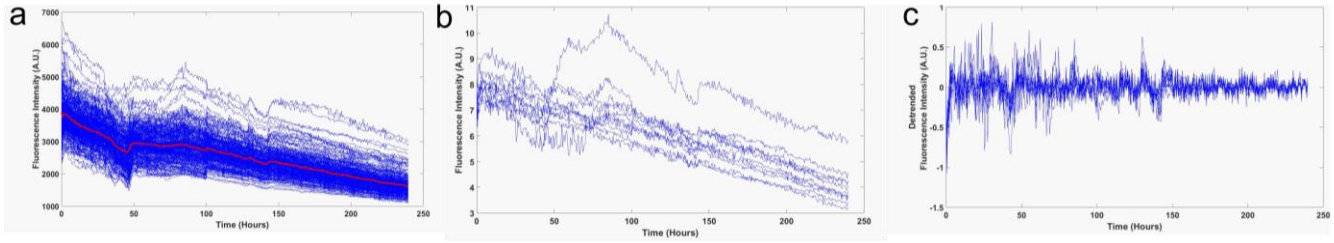

Figure 2: Fluorescence intensity of N. Crassa cells tracked using CellProfiler. (a)Blue curves are individual cell trajectories while the red curve shows the average. (b) 10 random trajectories of the single cell fluorescence intensities. (c) Detrended fluorescence intensities of cells in microwell show oscillations for a period of 10 days.

CONCLUSION

We presented a microfluidic device to trap a large number of single *N. Crassa* cells long term fluorescence imaging in order to understand the communication mechanism of biological clocks between cells.. Future work will involve varying the density of cells in the microwell to obtain a limit for synchronization to occur.

ACKNOWLEDGEMENTS

We acknowledge the financial support from National Science Foundation under Grant No. 2041546.

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