DYNAMIC TRACKING OF SINGLE CELL SYNTHESIS OF CdSe QUANTUM DOTS WITH A MICROFLUFIDIC DEVICE

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ABSTRACT

By using microfluidic devices coupled with a time-lapse and multi-location image acquiring confocal microscope, the live yeast cell biosynthesis of fluorescent CdSe quantum dots could be dynamically monitored at a single cell level. Whereas seleniumized yeast cells were prepared before loading, the biosynthesis could be studied by examining the incubation time and $CdCl_2$ concentration in the culture medium. Compared to the batch experiments, the on-chip biosynthesis required a lower concentration of $CdCl_2$ (0.5 mM) and a short incubation time (4-5 h) for an optimal quantum dot synthesis.

KEYWORDS: Live cell biosynthesis, Quantum dots, Dynamic tracking

INTRODUCTION

Intracellular vital movement involves thousands of biochemical reactions, which can be regulated in pre-designed ways by controlling both extracellular and intracellular environments. In particular, some of the cellular processes can be used for material synthesis under laboratory conditions. We have recently demonstrated that fluorescent CdSe quantum dots(QDs) can be obtained using yeast cells cultured in a flask by a new strategy of 'Space-Time Coupling' [1] of intracellular irrelated biochemical reactions. While more systematic investigations are being carried out in parallel, we have tested the feasibility of high throughput screening of biosynthesis conditions and single yeast cell tracking based on microfluidic technologies. Microfluidics is known to have the potential of micro-environmental regulation with a spatial and temporal resolution. Our work consists of a first step towards a dynamic tracking of single cell bio-synthesis under microfluidic conditions.

EXPERIMENTAL

Single layer PDMS devices were fabricated using soft lithography. The designed chip pattern was printed on a plastic film by a high resolution laser printer and then used as mask for photolithography with a 30 μm thick AZ 50XT resist spin coated on a silicon wafer. After exposure and development, the resist pattern (mould) was exposed to trimethylchlorosilane (TMSC) vapor for 3 min to avoid the adhesion between PDMS and silicon wafer. Commercial PDMS components were mixed at a ratio of 10:1 by weight and cast on the mould with a thickness of about 5 mm and then cured at 80°C for 2 h. Afterward, the PDMS layer was peeled from the mould, and access holes were punched with metal pipes. Finally, the PDMS layer was irreversibly bonded to a glass slide after air plasma treatment.

Before cell culture, the PDMS chip was washed with ultra pure water and sterilized in an autoclave for 20 min in an aluminum canteen. Then, it was baked at 80°C overnight to obtain a good hydrophobicity of the PDMS surfaces. Afterward, the chip was placed in vacuum (100 mTorr) for a half hour just before the cell load experiment.

Figure 1(a) shows the layout of a designed device which consists of micro chambers of different sizes in the range of 100 to 400 μm. As demonstrated previously, such a device can be used for degas-based cell loading and cell culture without shear stress [2]. In this study, yeast cells (*Saccharomyces cerevisiae* BY4742 MATα his3-Δ1, leu2- Δ0, lys2-Δ0, ura3-Δ0) were culture in YPD (1 % yeast extract, 2 % peptone and 2 % glucose). Before loading, cells were cultured in YPD broth for 24 h and then co-incubated with YPD containing 5mM Na_2SeO_3 for 24 h at 30°C, followed by centrifuging, harvesting, and transporting into fresh culture medium. After cell loading into the PDMS micro chambers, culture media of different $CdCl_2$ concentrations were perfused into the main channels at a flow rate of 30 μL/h and the culture was performed in dark for 10 to 40h. Meanwhile images were acquired with a laser confocal microscope equipped with an automated scanning stage in a time-lapse and multi-location mode (Andor Revolution, UK).

RESULTS AND DISCUSSION

First, we checked the growth behavior of normal yeast cells in micro chambers. Figure 1(c) shows a logistic growth deduced from a series of images as shown in Figure 1(b). The growth curve of yeast cells cultured in a flask has also been plotted in Figure 1(b), showing a similar behavior but at a doubled growth rate compared with that under batch culture conditions.

Figure 2 shows a microphotograph and a fluorescence image of seleniumized yeast cells in a typical microfluidic cavity after 20 h culture with 1mM $CdCl_2$ stimulation. As can be observed, most of yeast cells became fluorescent because of the presence of biosynthetic CdSe quantum dots.

Figure 3 shows dynamic tracking of single yeast cell biosynthesis during a long period, allowing a viability survey of the cells as well as the change in the fluorescence intensity as a function of time.

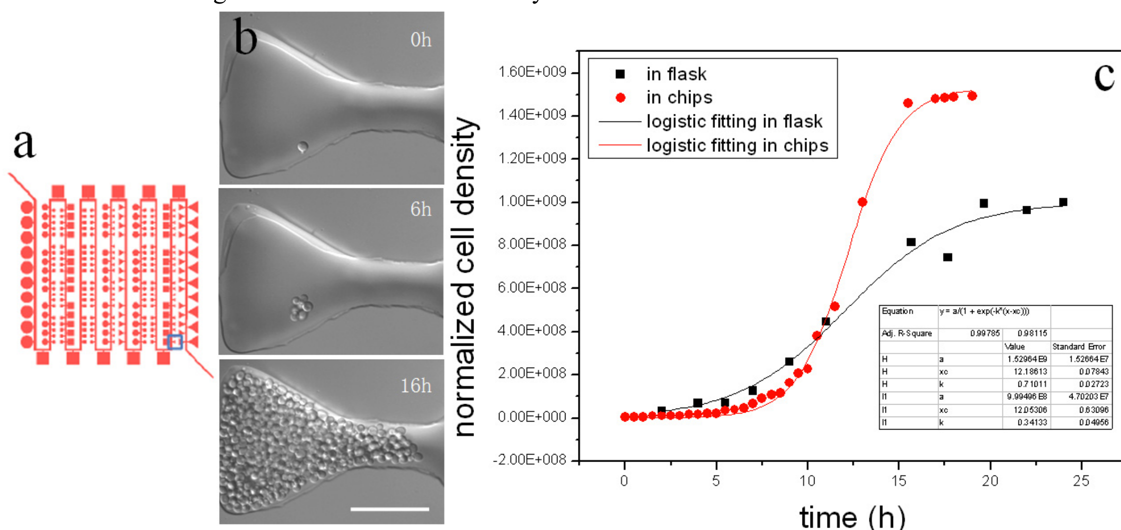

Figure 1. (a) Layout of a microfluidic device used in this work, (b) DIC imaging of yeast cells after loading and culturing in a triangle cavity. (c) Growth curves of yeast cells cultured on a chip (red) and in a flask (black), both fitted using a logistical model. Scar bar=50 µm.

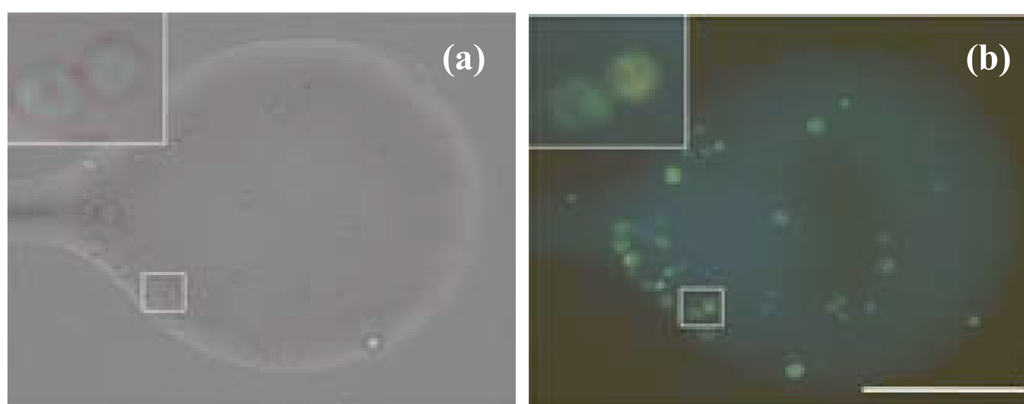

Figure 2. Microphotograph (a) and fluorescence image (b) of yeast cells after 20 h culture in a microfluidic chamber with 1mM $CdCl_2$ stimulation. Scar bar=50 µm.

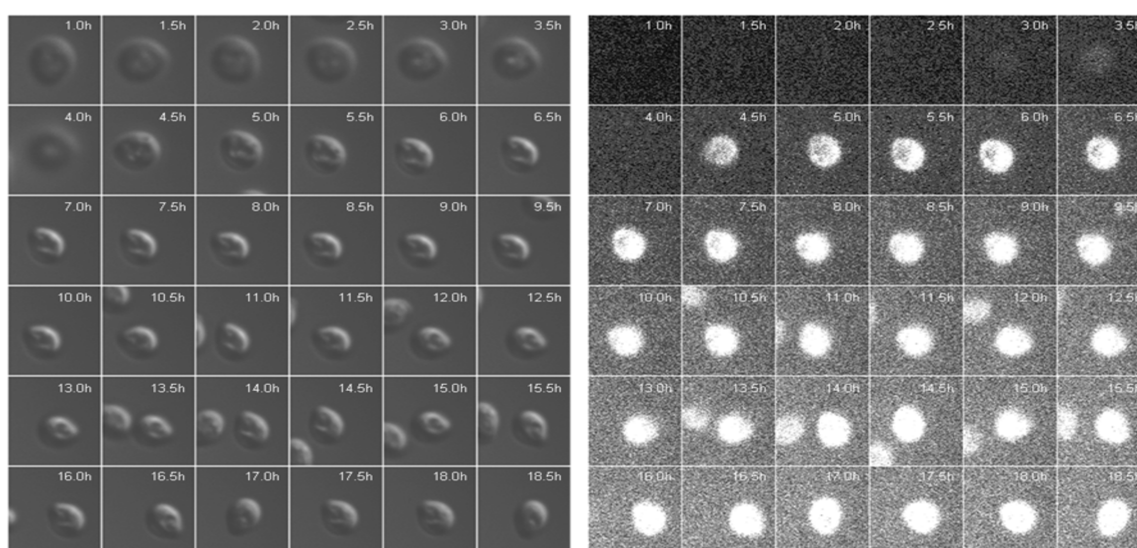

Figure 3. Longtime tracking (DIC and fluorescence images) of single seleniumized yeast cells in 1mM $CdCl_2$ culture medium in a microfluidic chamber.

Multichannel devices were also fabricated for more efficient parameter screening (Figure 4a). Single yeast cells could also be dynamically tracked by taking the fluorescence image of targeted objects. Figure 4(b-d) shows the mean fluorescence intensity as a function of time for three excitation wavelengths. As expected, a strong $CdCl_2$ concentration dependence was observed. While the cells grow more efficiently at low $CdCl_2$ concentrations (Figure 3e), the optimal concentration for the quantum dot synthesis is about 0.5 mM, which is lower than the batch culture value as the concentration is kept constant in microfluidic devices.

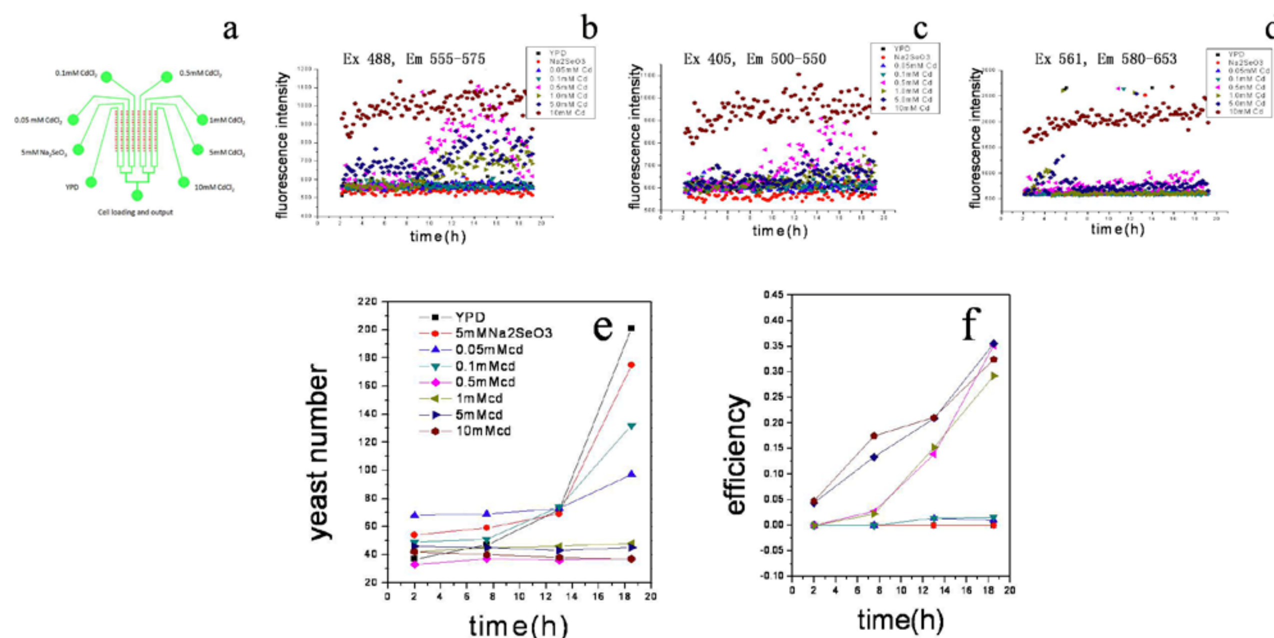

Figure 4. (a) Layout of a designed microfluidic device for parameter screening; (b,c,d) Time dependent fluorescence intensity of one single yeast cell in microfluidic chambers connected to a zero shear-stress perfusion channel; (e) Seleniumized yeast cell growth behavior, and (f) CdSe quantum dot biosynthesis efficiency as a function of time, which is the ratio of fluorescent yeast cell to total yeast cell number, at different $CdCl_2$ concentrations.

CONCLUSION

We used a time-lapse and multi-location image acquiring system to dynamically track single yeast cell synthesis of CdSe quantum dots in microfluidic devices. It is known that although thousands of biochemical reactions can be involved in their cellular processes, yeast cells can quickly and precisely respond to the environmental changes through their regulation networks [3,4]. The microfluidic culture platform we demonstrated in this work should be useful for parameter screening and mechanism elucidation in biosynthesis as well as for more general purposes in bioengineering and life sciences.

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REFERENCES

[1]. Ran Cui, Hui-Hui Liu, Hai-Yan Xie, Zhi-Ling Zhang, Yi-Ran Yang, Dai-Wen Pang, Zhi-Xiong Xie, Bei-Bei Chen, Bin Hu, and Ping Shen, *Living yeast cells as a controllable biosynthesizer for fluorescent quantum dots*, Adv. Funct. Mater. 19, 2359–2364 (2009)

[2]. L. Wang, X.F. Ni, C.X. Luo and Y. Chen, *Self-loading and cell culture in one layer microfluidic devices,* Biomed. Microdev. 11, 679 (2009)

[3]. J. V. Gray, G. A. Petsko, G. C. Johnston, D. Ringe, R. A. Singer and M. Werner-Washburne, *"Sleeping beauty": quiescence in Saccharomyces cerevisiae.*, Microbiol Mol Biol Rev 68 (2004): 187-206

[4]. Jochen Förster, Iman Famili, Patrick Fu, Bernhard Ø. Palsson and Jens Nielsen, *Genome-Scale Reconstruction of the Saccharomyces cerevisiae Metabolic Network* , Genome Res 13 (2003): 244-253.

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