COMPARATIVE MICROFLUIDIC CULTURING OF IMMOBILIZED SINGLE CELLS WITH ON-SITE FLUORESCENT-PROTEIN INDUCTION
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ABSTRACT
This work presents a microfluidic chip that enables the comparative culturing of single cells, immobilized along both walls of a channel, and exposed to different media under a laminar-flow regime. The key feature is that single cells can be reliably trapped using hydrodynamic forces and selectively released on-demand by using negative dielectrophoretic forces. This combined system allows for controlled population of trapping sites before a certain experiment and for release of individual cells showing interesting behavior during or after specific compound exposure.

KEYWORDS: Microfluidics, Single-Cell Analysis, Cell Trapping, Cell Culturing

INTRODUCTION
Parallel cell culturing studies play an important role in biological research, as many experiments are comparative in nature and require control experiments. Direct comparison of cells during culturing gains more and more importance in microfluidics [1-3]. In this work, two different media are delivered into a single channel side by side. By operating the device in the laminar-flow regime, the two media hardly mix (only minor inter-diffusion) and provide different conditions for cells immobilized along both walls of the channel.

Based on the approach in previous work [4], single particles and cells can be reliably immobilized at defined sites in a microfluidic chip. Here, the chip design has been slightly modified to suit the new application. An engineered yeast strain (S. cerevisiae), which can be induced to express a yellow fluorescent protein (YFP) upon addition of β-estradiol in the cell-culturing medium, has been utilized to verify the chip functions. Long-term culturing of immobilized cells under exposure to different media has been performed and assessed using time-lapse fluorescence imaging to monitor YFP expression.

EXPERIMENTAL
Figure 1 shows the schematic of the microfluidic chip for comparative single-cell culturing, which is similar to our previous microfluidic device for single-cell manipulation and cultivation [4]. This chip consists of a cell-culturing channel with three inlets and one outlet. Two suction channels are connected to the central channel via several orifice-type cell-trapping sites (4 µm wide). A large common electrode placed along the central channel and individual tip electrodes, situated at the respective cell-trapping sites, are used for selective cell release by use of negative-dielectrophoretic (n-DEP) forces. The suction channels here have been patterned as multilevel Y-shaped connections to the cell-trapping sites to ensure uniform pressure at each cell-trapping site.

The chip has been fabricated by using a simple hybrid glass-SU-8-PDMS process. First, platinum microelectrodes have been patterned on the Pyrex glass substrate using a lift-off process. The microfluidic channels (30 µm high), have then been formed in SU-8 photosist using a lithographic process. After chip dicing, the SU-8 surface has been modified with (3-aminopropyl)triethoxysilane (APTES) using a vapor phase silanization to irreversibly bond a PDMS cover on top and seal the microfluidic network. Access holes have been punched into the PDMS for fluidic interfacing prior to bonding.

Biological validation of this microfluidic system was performed by using an engineered diploid S. cerevisiae strain, derived from BY4743. This strain contains an estrogen-dependent transcriptional activator that triggers the expression of YFP upon the addition of the inducer (β-estradiol) to the cell-culturing medium. In a typical experiment, the suspension of cells was introduced into the cell-culturing channel using a conventional syringe pump through inlet 3 (Fig. 1a & 2). Low pressure was applied to both suction channels via pressure controllers, to achieve reliable cell immobilization at the cell-trapping sites along both sidewalls of the cell-culturing channel. During cell loading, syringe 3 filled with cells, was set to a flow rate of 1.2 µl/min, while the other two syringes were switched off. Microelectrodes situated at each cell-trapping site were used to generate n-DEP forces to release potentially unwanted cells and select the optimal cells for the experiment. Afterwards (Fig. 1b & 2), syringe 1 (containing cell-culturing medium plus β-estradiol at a concentration of 0.1 mM) and syringe 2 (with cell-culturing medium only), connected to inlet 1 and inlet 2, were set to flow rates of 0.5 µl/min, and parallel perfusion in the laminar flow regime was established. Syringe 3 was set to 0.2 µl/min so that the total flow rate remained the same as for cell loading and trapping. This way, any abrupt change in hydrodynamic forces, which may affect the immobilization stability of the cells, was avoided. During the experiment, automated multi-site time-lapse imaging was performed on an inverted fluorescence microscope in order to monitor cell proliferation and YFP expression selectively induced by β-estradiol.
RESULTS AND DISCUSSION

Figure 3a shows the experimental results of a long-term comparative culturing of immobilized yeast cells with overlaid bright field and fluorescence images taken during the culturing. Fluorescence intensities of each cell have been extracted and are shown in Fig. 3b. As can be seen in Fig. 3a, immobilized cells at the upper trapping sites (from S1 to S5) have been exposed to cell-culturing medium with inducer for 11 hr, while the cells at the lower trapping sites (from S11 to S15) were simultaneously cultured without any inducer in the medium. Time-lapse images and correlated...
fluorescence intensity measurements in Fig. 3 illustrate that cells at the upper sites started to express YFP after 2-3 hours, whereas the cells at the lower sites (no inducer exposure), did not develop any fluorescence during the whole culturing period. The significant difference in fluorescence intensity between the upper and lower set of cells proves that there is no crosstalk between the perfusion media applied in a laminar flow regime.

CONCLUSION

We present a microfluidic device that includes the functions of hydrodynamic trapping, selective releasing by n-DEP forces, and comparative culturing of single yeast cells for an extended time period. An engineered yeast strain, which expresses YFP under the exposure to a specific inducer, has been employed in the experiment to test and validate the device. The experimental results demonstrate that this microfluidic comparative culturing system can be used to cultivate sets of immobilized cells in parallel, to expose them to different media and to conduct time-lapse fluorescence imaging at single-cell resolution.

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REFERENCES


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