MICROFLUIDIC DEVICES FOR INTEGRATED SYNCHRONIZATION AND ANALYSIS OF BACTERIA

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ABSTRACT

We report microfluidic devices for automated synchronization and analysis of bacteria. To generate cells with similar ages, cells were seeded on chip, flushed with fresh media at regular intervals, and released on demand into an analysis channel. We evaluated device performance over six consecutive collections at 10-min intervals and any time over four days. Flow cytometry and fluorescence cell tracking were used to determine synchrony quality, and our devices compared favorably to the conventional plate-release method, but without the need for manual operations. In addition, we studied individual cells from these synchronized cell populations and monitored the increase in their stalk length in phosphate-deprived media.

KEYWORDS

Automated cell culture, microfluidics, synchronization, Caulobacter crescentus, biofilm

INTRODUCTION

Microfluidic platforms are advantageous over traditional techniques because the local environment can be precisely controlled, and individual cells can be tracked to determine their response to changes in the environment. Moreover, cells can be cultured in one area on the device, and newborn cells can be transferred to an uncontaminated region of the same device for downstream analysis. To better understand how biofilms initiate and form, our group has worked with the non-pathogenic freshwater bacterium *Caulobacter crescentus*. *C. crescentus* undergoes asymmetric binary fission that results in two genetically similar but morphologically different progeny, which age over time [1]. Therefore, synchronization of *C. crescentus* cells to the same age prior to analysis is critical to prevent convolution of the cellular response with changes due to aging.

EXPERIMENTAL

We have developed two devices to culture and synchronize *C. crescentus* cells (**Figure 1**). Device 1 is fabricated from three layers (fluid, membrane and control) of PDMS (poly(dimethylsiloxane)) [2]. The control layer contains 40-µm deep channels and vacuum/pressure access ports. The control layer is irreversibly bonded to a 100-µm thick PDMS membrane and aligned with the fluid layer which contains microchannels that are 300 µm wide and 20 µm deep. The assembled device is autoclaved prior to use. Device 2 incorporated the fluid layer in the membrane to produce a lifting gate valve [3]. The PDMS part of the device was irreversibly bonded to a thin coverslip to permit microscopy with high numerical aperture objectives. Both devices contain valves that are actuated by applying vacuum and pressure to open and close the valves, respectively. For both devices, we inoculate the incubation region with a mixed culture and allow the biofilm to grow overnight.

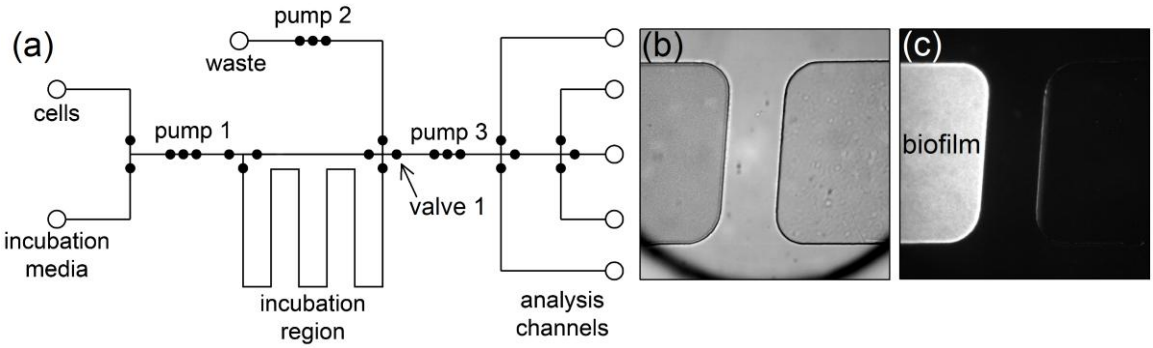

Figure 1. (a) Schematic of the microfluidic device for integrated cell synchronization and analysis. Open circles represent reservoirs, and closed circles represent valves. The valves labeled pumps 1, 2, and 3 are used as peristaltic pumps. The biofilm is seeded and grown in the incubation region, and the synchronized population collected from the biofilm is transferred to one or more of the analysis channels for downstream experiments. To confirm that the biofilm is confined to the incubation region, panel (b) is a bright field image of valve 1 at the exit of the incubation region, and panel (c) is a fluorescence image of the biofilm (bright region) confined by the valve.

Device 1 was used to culture and synchronize wild-type *C. crescentus* cells that express gfp (green fluorescent protein). The quality of the cell synchrony and cell density for device 1 were assessed with conventional flow cytometry and fluorescence microscopy (**Figure 2**). Device 2 was used to monitor the response of *C. crescentus* to phosphate deprivation. Fluorescence microscopy of *C. crescentus* cells that express cytoplasmic red fluorescent protein (DsRed) and stalk specific StpX-gfp was used to monitor stalk elongation. A synchronized population from the biofilm was transferred into an analysis channel and permitted to adhere in phosphate rich media. The media in the channel was then replaced with a low phosphate media, and the rate of stalk growth due to this stress was observed over a 20-h period. After 20-h, the media was switched back to media that contained phosphate to observe the time required to halt stalk elongation.

RESULTS AND DISCUSSION

Figure 2a summarizes the overall quality of the synchronized cells collected from device 1 compared to the mixed culture and plate-release method, and results from our device are comparable to or exceed those from the traditional plate-release method. Also, synchronization was evaluated over six consecutive collections at 10-min intervals for 1 h and over four days (see **Figure 2b-c**), which is not possible with the plate-release method. The synchrony quality is reduced if the biofilm is cultured with M2G instead of PYE media. This difference is due to changes in the structure of the biofilm and is observed with the plate-release technique as well as the microfluidic device.

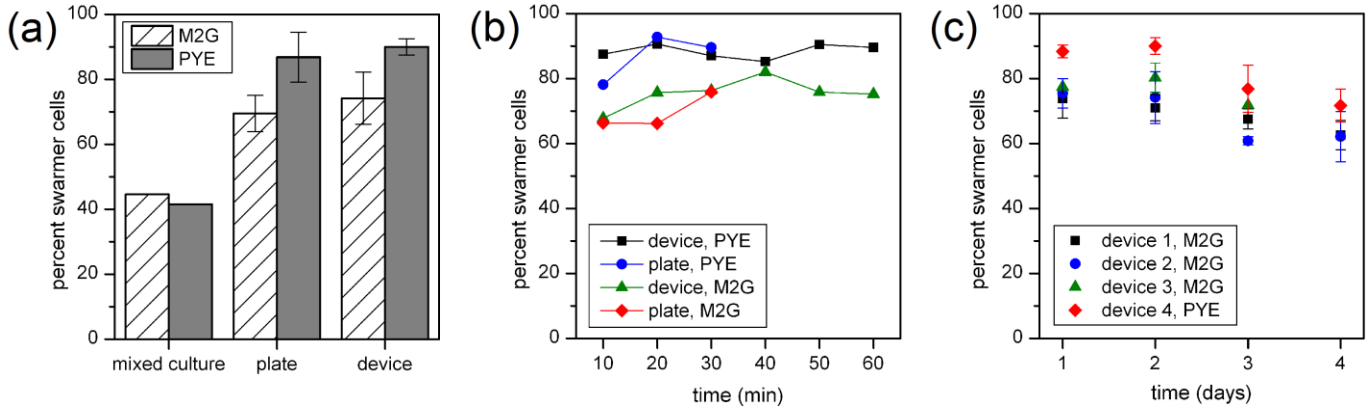

Figure 2. (a) Comparison of percent swarmer cells for a mixed culture, plate-release method, and microfluidic device with M2G and PYE media. (b) Variation of percent swarmer cells for up to six consecutive collections at 10-min intervals from the plate-release method and microfluidic device. (c) Variation of percent swarmer cells collected from four devices over four days in M2G and PYE media.

Figure 3a-b shows fluorescence images after initial adhesion (0 h) and after 20 h. After 20 h in a phosphate-deprived microenvironment, average stalk length for 72 single-cell measurements was 2.9 μm. To probe the response of cells to changes in the microenvironment, balanced-phosphate media was introduced after 20 h, and elongation of the stalks was observed to halt immediately (**Figure 3c**). Individual cells were tracked over the entire experiment to determine heterogeneity within the cell population.

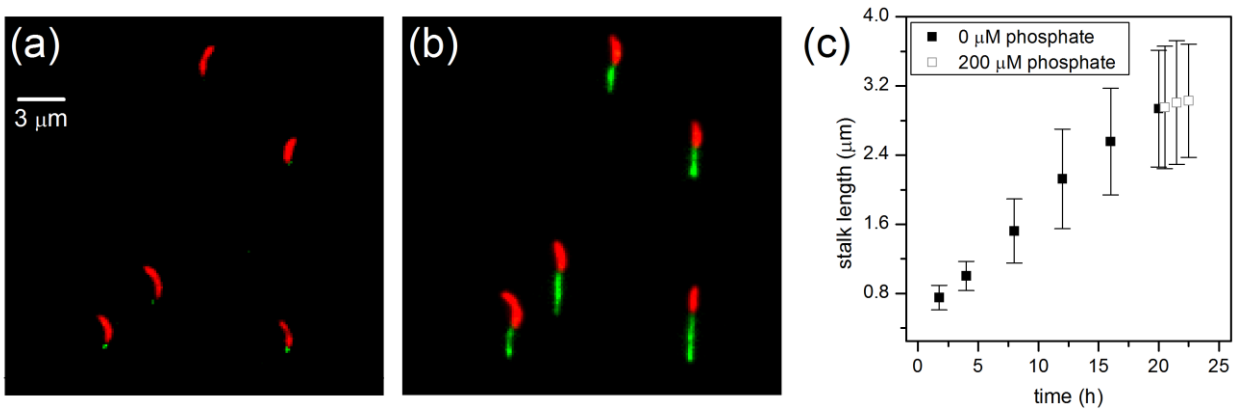

Figure 3. Fluorescence images of *C. crescentus* cells in phosphate-deprived media after (a) initial adhesion (0 h) and (b) 20 h. Both images include the same cells, for which the cell body is false-colored red, and the cell stalk is false-colored green. (a) Immediately after adhesion, the cell stalk is short and barely visible. (b) After growing in the low phosphate media for 20 h, the stalks have elongated. (c) Average stal k length over time calculated from 72 single cell measurements. Measurements were taken through 20 h in 0 μM phosphate HIGG media (filled symbols). At 20 h, the media was switched to 200 μM phosphate HIGG media to halt stalk elongation (open symbols).

CONCLUSIONS

We have developed the first microfluidic bacterial baby machine. Specific advantages of our device over traditional laboratory-scale techniques include continuous cell collection over minutes, hours, and days, high-quality swarmer cell enrichment that can be confirmed on-chip by fluorescence cell tracking and off-chip by flow cytometry, significantly lower reagent consumption, and automated device operation with integrated pumps and valves. The microfluidic device was modified to incorporate a thin coverslip to allow high-resolution imaging over extended periods of time. With our device, we have measured the rate of stalk elongation under low phosphate conditions and observed that the cells rapidly respond to an increase in phosphate concentration and halt stalk elongation in less than 30 min.

References

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