Microfluidic platform for studies of self-organizing processes in a bacterial cell

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ABSTRACT

Lab-on-a-chip platform presents many new opportunities to study bacterial cells and cellular assemblies. Here we describe fabrication and characterize two promising platforms that enable the study of cellular organization in *Escherichia coli* bacteria. One of the platforms allows for rapid delivery of chemical agents, and the other, mechanical perturbations to *E. coli* cells while observing sub-cellular structures in a high resolution optical microscope in real time. The first platform follows the so called mother machine design where bacteria grow in dead-end pockets. While enabling the observation of bacteria by a much larger number of doublings than by conventional means we find that the cells in the pockets experience small but significant growth limitation. The second platform makes use of micron-sized pressure actuated valves to apply uniaxial stress to bacterial cells. By placing bacterium under a miniature valve and closing the valve by externally applied pressure deforms the cell. We show at the proof-of-principle level that this approach can be used to study mechanical properties of bacterial cells and their internal organization.

Keywords: Lab-on-a-chip, cell growth, pressure actuation, mechanical stimuli, *Escherichia coli*

1 INTRODUCTION

The challenges to study bacteria stem from their rapid growth, small dimensions and often motile lifestyle which all hinder observations at a single cell level. Lab-on-a-chip (LOC) technology presents a natural choice to overcome these challenges offering new ways to grow, manipulate, and observe bacterial cells and cellular assemblies [1,2]. In microchips, a controlled environment for cells can be defined and maintained for extended periods of time during which high throughput measurements can be performed. Typical measurements are carried out using optical microscopy but progress has been also made in on-chip genomic studies [3,4] and electrical measurements [5,6] of bacterial cells. Unlike conventional approaches, the microchip technology enables one to perform these measurements in real time and to observe cellular responses to various physical and chemical stimuli as they unfold.

LOC technology offers a particular promise in studies of bacterial cellular organization. Although the bacterial cell was long considered to be a simple enclosure, whose only function was to carry the genome from one generation to the next, more recent research has demonstrated exquisite spatial organization within these micro-organisms [7]. The ordered placement of proteins and DNA within the cell arises due to many self-organizing processes. The details of these processes are still poorly understood. The most common approach to understand how cellular organization rises and is maintained is to perturb this organization and then observe a response from the cell. Essentially all approaches used by cell biologists in these studies enable the assessment of only ensemble-averaged responses, overlooking significant cell-to-cell variations that exist in a population. The current methods are also not suitable to study processes in cells with fast response times. Here, we describe the development of two microfluidic platforms that allow mechanical and chemical perturbation of cellular organization while following the cellular response at a single cell level in real time using a high resolution fluorescence microscope.

2 METHODS

2.1 Microchips Fabrication

Microchip fabrication is based on soft-lithography techniques using PDMS (polydimethylsiloxane, Sylgard 184 by Dow Corning) as molding material [8]. Molds for PDMS are defined by a combination of e-beam and photolithography, and fabricated using RIE etching of silicon and SU-8 patterning. Details of our mold fabrication process can be found in [9]. Silicon molds are subsequently silanized in a desiccator using (tridecafluor0-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (UCT Specialties, CA) for 15 minutes or longer. For mother machine chips, a 1:5 mass ratio PDMS curing agent to base is poured on the Si mold making about a 5 mm thick layer. The layer is cross-linked at 90 °C for 15 minutes. The hardened PDMS is then peeled off from the template, access holes are manually punched to it and after a short treatment in O_2 plasma the PDMS and a clean #1.5 glass coverslip (Fisher Scientific) are bonded together.

The chips with microfluidic valves consists of two different layers of PDMS. For both layers a 1:10 mass ratio PDMS curing agent to base is used. One of the layers, where channels for cells are located, is about 20 μm thick. The other layer, which houses pressure lines, is about 5 mm thick. The thin layer is formed by spinning PDMS at 4000 rpm for 1 minute. The thick layer is formed by pouring PDMS to a 5

mm thick mold. The two layers are first cured at 90 °C for 15 minutes. After treatment of both layers for 15 seconds in O_2 plasma the layers are aligned using a home-made micropositioner and bonded together. After initial bonding the layers are baked for 15 minutes at 90 °C. The access holes are then punched to the layers. Subsequently the composite PDMS piece and #1.5 coverglass are O_2 plasma treated and joined. Finally, a 15 minute bake at 90 °C is used to strengthen the bonding between the glass and PDMS.

2.2 Cell Cultivation and Imaging

In these studies *Escherichia coli* strain BW25113 is used. The strain carries pKen plasmid that encodes for cytosolic eGFP label [10]. The strains are grown and imaged at 28 °C in LB medium (Fisher Scientific). During growth and imaging 100 µg/ml ampicillin is used to maintain the plasmid. In demonstrations of pressure actuated microvalves *E. coli* strain RH3 is used [11], which is grown in M9 minimal medium supplemented with 0.2% glycerol as a carbon source.

A Nikon Ti-E inverted fluorescence microscope with a 100X NA 1.40 oil immersion phase contrast objective was used for imaging the bacteria. Fluorescence was excited by a 200W Hg lamp through an ND4 neutral density filter. Chroma 41001 filtercube was used to record eGFP images. Images were captured by an Andor iXon DU897 camera and recorded using NIS-Elements software. Image analysis was carried out using Matlab scripts based on Matlab Image Analysis Toolbox and DipImage Toolbox (http://www.diplib.org/). In addition to Matlab, simpler image processing, such as contrast and brightness adjustments, were done using ImageJ software (v1.41o). The procedures for finding cell lengths and widths have been described previously elsewhere [10].

3 RESULTS

3.1 Mother Machine Platform

The platform designed for studies involving chemical perturbation follows the mother machine design [12]. The PDMS chip consists of an array of 600 small dead-end channels, which connect to a larger flow channel (Fig. 1 A, B). The small channels have a design width of 1.0 µm, height 1.3 µm and length either 20 or 25 µm. The width of these channels only marginally exceeds the diameter of a bacterial cell (0.7-0.9 µm). The large flow channel has width of 200 µm, height of 22 µm and length of 11 mm. The large channel connects to external tubing that allows us to rapidly change (within 10 seconds) its content and thereby to introduce different chemical agents to the cell. The important purpose of the flow in the large channel is also to flush away excess cells that would otherwise rapidly accumulate during exponential growth.

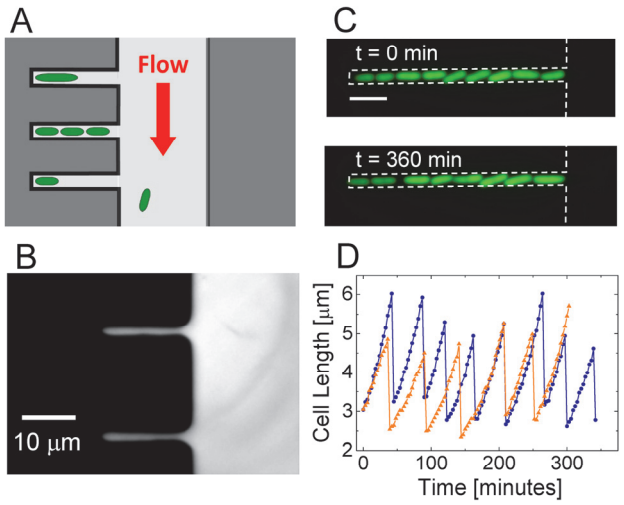

Figure 1: Platform for studies involving chemical perturbations. A) Schematics of the microfluidic system. Cells grow in large numbers of dead-end channels. The flow in the large vertical channel allows for a quick exchange of chemical composition of cell medium and to flush away extra cells. B) Image of a completed chip from an optical microscope. C) *Escherichia coli* cells growing in one of the completed devices. Cells carry a fluorescent GFP label and are imaged in a fluorescence microscope. Dashed lines show approximately channel boundaries. Scale bar is 5 µm. D) Typical growth curves of two cells in the channel.

Escherchia coli BW25113 cells carrying cytosolic eGFP marker were grown in the channels. After about three doubling periods all cells in these channels are direct progeny to the "mother" cell in the end of the pocket (Fig. 1C). It was previously reported that cells can grow in such channels for more than 100 doubling periods [12]. In our experiments we typically image cells for about 6 doublings as this is sufficient to generate statistically large samples (several hundred to thousands of cell cycles). Six doubling periods is also long enough to verify that cells grow in steady state conditions.

The mother machine platfrom enables cells to grow for much longer periods than the conventional methods based on agar pads and dishes, which allow only 3-4 doublings before cell growth becomes significantly altered due to the depletion of nutrients. The platform also enables us to introduce rapidly and in a controlled manner various chemicals to growth medium to the cells. Despite these advantages, our measurements show that cells growing in dead-end channels of the mother-machine chips experience a growth limitation. Cell width (diameter) and length are sensitive indicators of growth rate in *E. coli* as the average size of bacterial cells in steady-state growth condition scales exponentially with their growth rate [13]. Our measurements show that cell width (Fig. 2A) and length (Fig. 2B) increase as the cells move from the dead-end side of the pocket towards the entrance.

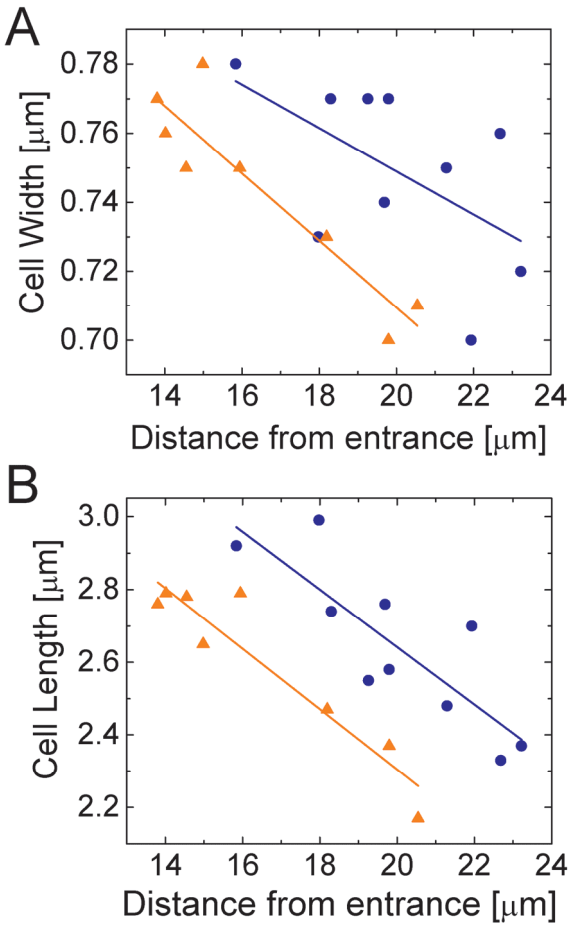

Figure 2: Cells experience growth limitation in the channels. A) Cell width at birth as a function of location of the cell center from the entrance of the channel. B) Cell lengths at birth for the same cells as a function of distance from the pocket entrance. Solid lines in panels A) and B are linear fits. Blue circles correspond to cells growing in 25 μm and orange triangles to cell in 20 μm channel.

The origin of the growth limitation remains yet unknown. Experiments in which flow rate of growth medium in the main channel was increased from 5 μl/min to 10 μl/min did not have an effect on the cells sizes. This finding shows that nutrient levels in the main channel were not depleted. It is likely then that diffusion of nutrients in small channels is not as fast as we expect. A conservative estimate of the diffusion of small molecules in the small channel (D~100 $\mu m^2/s$) shows that the metabolites from the main channel should reach to its furthermost end within about 3 sec. This estimate would imply no growth limitations for the cell in the far ends of the channel. However, this simple estimate neglects the presence of other cells in the channel. It is possible that the other cells in the channel act as effective sinks for small metabolic molecules and hinder their diffusion deeper into the channel.

3.2 Pressure Actuated Valves for Studies of Mechanical Response of Bacterial Cells

In this section, we will discuss the design and demonstrate a proof-of-principle operation of small pressure actuated valves in applying uniaxial stress to *E. coli* cells. In our design the uniaxial stress is applied to the cells by the ceiling of the microvalve (Fig. 3A). By applying external pressure to the control line, the ceiling of the valve deflects and deforms the cell that is placed underneath it (Fig. 3A, bottom).

In a preliminary test, we positioned *E. coli* cells under the valve by fluid flow (Fig. 3B). To capture cells, a valve was partially closed when cells were flown in. Application of external pressure to the control line considerably broadened the cell contours (Fig. 3B) demonstrating feasibility of the approach. In further studies we will investigate the mechanical properties of the bacterial cell wall and also use this approach to alter the organization of the bacterial chromosome.

Our preliminary tests indicate that the convex shape of the valve ceiling tends to push cells away from the valve center. As this happens the measurement becomes useless. The convex shape of the ceiling also means that the cells captured offset from the valve center experience different stress than the ones at the center. In further studies we plan to modify the shape of the ceiling to more effectively capture the cells and to provide that all cells experience the same stress.

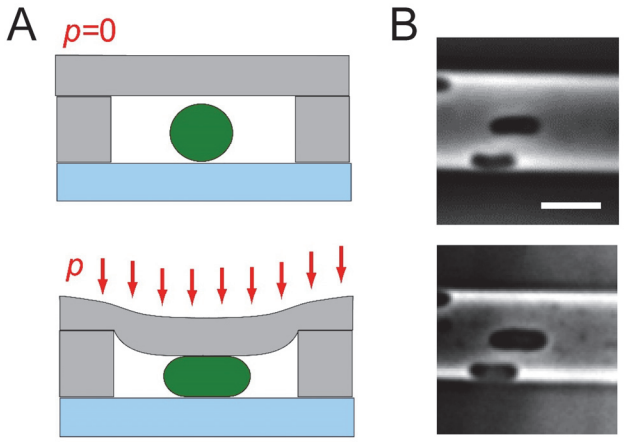

Figure 3: A) Platform for studies involving mechanical perturbations. A) Schematics of the microfluidic system. Shown is a cross-sectional view of a pressure-actuated valve and a bacterial cell. Closing the valve by externally applied pressure (p) deforms bacterial cell. B) Phase contrast images of *E. coli* cells before (top) and during application of mechanical pressure (bottom). Noticeable broadening of cell contours can be seen. Scale bar is 5 μm.

4 CONCLUSIONS

We investigated two promising platforms that can be used to study cellular organization in *Escherchia coli* cells. The mother machine platform allows cells to grow in steady state conditions over a large number of generations and at the same time perform high resolution optical microscopy. However, cells in channels with sub-micron width experience small but statistically significant growth limitation. Further studies need to address this problem. To understand the mechanical responses of bacterial cells we propose a design based on pressure actuated microvalves. We demonstrated on a proof-of-a-principle level that these valves can be used to apply uniaxial stress to bacterial cells. In further studies we plan to alter the shape of the valve to prevent cells from shifting during the squeezing and to provide uniform stress to all cells in the channel.

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