BACTERIA IN SUBMICRON CHANNELS AND MICROVALVES

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ABSTRACT

For rational design of lab-on-chip devices for bacterial cell cultures, one has to understand how bacteria can be effectively confined in designated reservoirs without contaminating fluidic lines. Here we investigate how *Escherichia coli* and *Bacillus subtilis* bacteria grow and propagate in PDMS-based fluidic structures consisting of slit-like shallow channels and pressure-actuated valves. Both types of bacteria can grow in flexible PDMS based channels which are much narrower than their diameters. We also show that *E. coli* bacteria can survive pressure cycles of closing and opening of pressure-actuated PDMS valves without lysing.

KEYWORDS: microfluidic cell culture, nanofluidics, bacteria, uniaxial stress

INTRODUCTION

There has been much recent interest in cultivating bacteria on a lab-on-a-chip platform for biochemical analysis and for studying their cellular organization, collective behavior, and even evolution [1]. Lab-on-a-chip based structures typically consist of various chambers, channels and valves. For a successful operation of these devices, it is critical to understand how bacteria can be effectively contained in designated cell-culture chambers without undesired contamination of fluidic lines needed for the delivery of nutrients and reagents.

In previous study we have shown that Gram-negative *Escherichia coli* bacteria can penetrate rigid slit-like channels in silicon which are twice narrower than typical bacterial diameter (limiting channel width 0.4 μm). At the same time we have found that Gram-positive bacterium *Bacillus subtilis* can only penetrate these channels when the width of the channel exceeds the bacterial diameter (the limiting channel width was 0.8 μm) [2]. While our earlier study focused on the bacterial penetration of mechanically rigid channels, we here investigate how bacteria grow and propagate in PDMS-based fluidic structures that are flexible to some degree. Our aim is to understand what structural elements constitute effective barriers for bacterial propagation and how bacteria respond to the mechanical forces that confine them in small microstructures.

EXPERIMENTAL

Examples of the device layout are shown in Fig. 1. Bacteria are studied in two types of structures: narrow slit-like channels and pressure-actuated microvalves. Narrow slit-like channels are defined using either e-beam or photolithography and etched into silicon/silicon nitride using reactive ion etching. The height, which is the smallest dimension of the channel, ranges from 150 nm to 300 nm here. Note that the heights of the channels are significantly smaller than the typical diameters of about 0.7-1.0 μm of *E. coli* and *B. subtilis* bacteria [2]. The channels are closed using a ≈30 μm thick PDMS layer on a glass coverslip. The PDMS layer forms the ceiling for the channel and is in contact with bacteria. The mechanical properties of these hybrid silicon/PDMS channels are essentially determined by the PDMS ceiling and comparable to typical channels in PDMS. An image of finished chip with slit-like channels is shown in Fig. 1A.

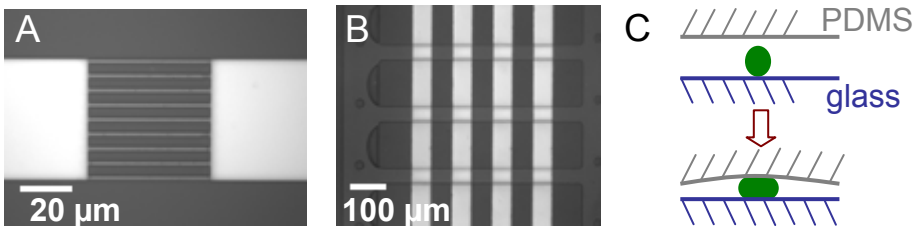

Figure 1: Two types of microfluidic structures where bacteria are studied. A) A chip with narrow fluidic channels. 50 μm long, 2 μm wide and 250 nm deep channels connect between two square-shaped cell culture patches. The channels are made into silicon nitride using e-beam lithography and reactive ion etching. The channels and patches are covered with 30 μm thick PDMS layer and a glass coverslip. B) Top-view image of a chip with pressure-driven PDMS valves. White vertical lines (control lines) are used to apply pressure on bacteria which are located in grey horizontal channels (flow lines). C) Side-view sketch of the effect of uniaxial mechanical stress on bacteria in pressure-actuated PDMS valves. Top panel depicts a side-view cross-section of an open valve and bacterium. Bottom panel shows the pressurized valve and a squeezed bacterium.

Pressure-actuated valves are fabricated using a multilayer soft-lithography process which involves bonding two PDMS layers to a glass coverslip [3]. The pressure-actuated valve consists of perpendicular flow and control lines which are separated by a thin (~10 μm) PDMS membrane (Fig. 1B). Parameters used in present study are: flow line

width 40 μm and height 12 μm, control line width 60 μm and height 25 μm. Pressure in control lines is determined by an argon tank and calibrated pressure regulator.

RESULTS AND DISCUSSION

First, we describe bacterial growth and movement in microfabricated channels. Fig. 2 show fluorescent images of *E. coli* RP 437 and *B. subtlis* 168 strains in shallow slit-like channels which height is only 250 nm. Remarkably, both bacterial species are able to grow and penetrate such shallow channels. We have observed *E. coli* to enter and grow even in channels as small height as 150 nm. During bacterial entrance and growth in the channels their shapes undergo noticeable changes. These changes are particularly striking for *E. coli* (top panel of Fig. 2): the bacteria widen considerably while growing compressed between walls of the channel. Besides broadening, the bacteria frequently obtain irregular shapes which deviate strongly from a common rod-shaped morphology of *E. coli*. In contrast to observations in *E. coli*, we do not observe significant broadening in *B. subtilis* bacteria (bottom panel of Fig. 2). However, shapes of *B. subtilis* also sometimes differ from normal morphology in that we observe significant filamentous growth. We speculate that the filamentous growth helps these bacteria to overcome frictional force which opposes bacterial growth in the channel. While still in shallow channels, *B. subtilis* are observed to be able to return to their normal short rod-shape morphology after initial filamentous growth.

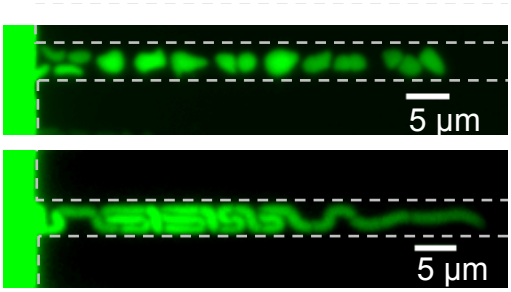

Figure 2: E. coli (top panel) and B. subtilis (bottom panel) in 250 nm high channel. Contours of channels are shown by grey dashed lines. Length of the channel is 50 μm and width is 5 μm. Note that E. coli bacteria are considerably broadened and have obtained irregular shapes compared to normal rod-shaped phenotype. A filamentous B. subtilis bacterium can be seen on the right while short rod-shaped bacteria are seen on the left of the bottom panel.

To understand the dramatic changes in shape of *E. coli* as they enter and grow in shallow channels, we plot the cell diameter as a function of time for a representative bacterium (Fig. 3). At time 0 minutes the bacterium is pressed into the shallow channel of 280 nm height by an externally applied flow. Entrance to the channel results in a broadening of the bacterial diameter by ~30% due to the uniaxial stress to the bacterium by the walls of the channel. As the bacterium starts to grow in the channel, its diameter increases further but now at a much slower rate.

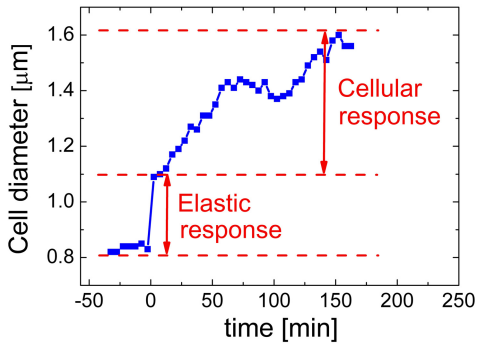

Figure 3: Upon entry in shallow channel, an immediate elastic deformation of bacteria is followed by a slow response to the prolonged uniaxial mechanical stress. The diameter of bacterium as a function of time is plotted before (t<0) and after (t>0) the bacterium is pushed into a 280 nm high slit-like channel. The first sharp increase in bacterial diameter reflects the elastic response at t=0 min. The dip in the curve at t=120 min coincides with the cell division into two daughters.

While the first change in diameter happens essentially instantaneously at the time scale of one second, the further broadening is noticeable at the time scale of one hour, i.e., at the time scale of the cell's generation time. The two different time scales of broadening of cell diameter indicate that there are two different types of responses from the bacterium to uniaxial stress. The first response consists of the elastic response of the bacterium to mechanical stress. We have verified that short-term compression leads indeed to elastic behavior (see also below).

Prolonged growth under uniaxial stress, however, leads to irreversible broadening and changes in the bacterial shape. These irreversible changes in the cell shape can be considered a cellular response to the uniaxial stress. The flattened shape of cells after prolonged growth in the channels indicates that bacterial cell wall synthesis is directed towards lowering the elastic energy of the cell wall. The validity of the latter hypothesis and the exact changes taking place at the molecular level within these bacteria remain to be further elucidated.

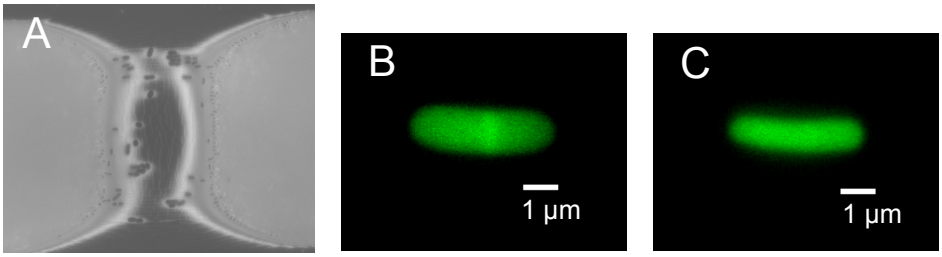

Figure 4: E. coli bacteria in pressure-actuated valves. A) A phase contrast image of a closed PDMS valve with some trapped bacteria, B) Fluorescent image of a bacterium under a closed PDMS valve. A pressure of 4 bar is applied. C) The same bacterium after opening of the valve. The ratio of bacterial diameters before and after opening the valve is 1.37.

As a last topic we report the survivability and shape changes of bacteria in pressure-actuated valves. Remarkably, we see that a large fraction of *E. coli* bacteria can survive pressure cycle of the valves without lysing (Fig. 4). In fully closed valves, corresponding to pressure of 4 bars, we observe the apparent diameters of bacteria to expand by 30-40%. The conditions for the bacteria in closed valves are even more extreme then inside shallow channels. In fully closed valves, the bacteria are pressed down and completely embedded within the surrounding PDMS. The environment of bacteria is then to a large extent depleted from the liquid media. Despite these limiting conditions, the bacteria are able to return to their original shapes after a relatively rapid (tens of seconds) pressure cycle. We do not see any statistically significant change in the bacterial diameter after a few pressure cycles in surviving bacteria (data not shown). The apparent broadening of bacterial diameters by 30-40% thus corresponds to the elastic regime of the deformation of the cell. While elastic regime holds for mechanical deformations, some fraction of the cells undergo lysis and cell death during each pressure cycle. It will require further studies to understand how rupture of the cell wall in these microorganisms can occur while the deformations of the majority of cells appears elastic and reproducible.

CONCLUSION

Our experiments show that two very different types of bacteria – Gram-negative *E. coli* and Gram-positive *B. subtilis* - exhibit the ability to penetrate a variety of on-chip structures that one would expect to act as barriers for bacterial movement. In designing fluidic circuitry for bacterial cell cultures in a lab-on-a-chip setting, one needs to take into account these unexpectedly plastic properties of bacteria and their innate ability to squeeze through narrow constrictions.

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