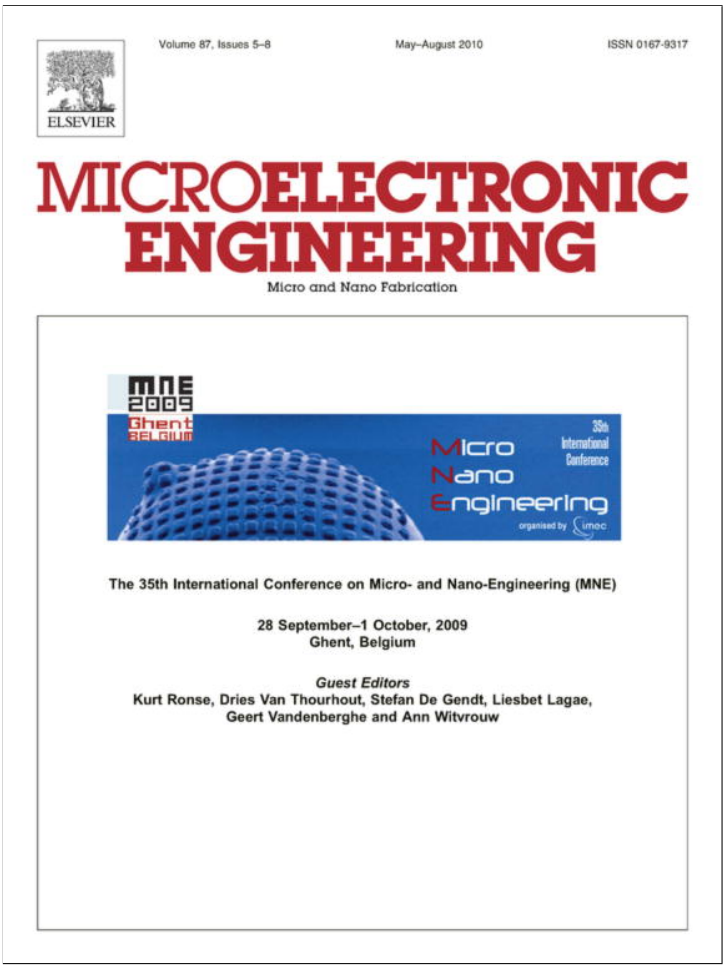

Microelectronic Engineering 87 (2010) 810–813

Contents lists available at ScienceDirect

Microelectronic Engineering

journal homepage: www.elsevier.com/locate/mee

Motility of bacteria in microfluidic structures

Marie Binz [a], Abraham P. Lee [b], Clive Edwards [c], Dan V. Nicolau [a,*]

[a] Department of Electrical Engineering and Electronics, University of Liverpool, Liverpool L69 3GJ, United Kingdom
[b] Henry Samueli School of Engineering, University of California, Irvine, CA 92697-2715, USA
[c] School of Biological Sciences, University of Liverpool, Liverpool L69 7ZB, United Kingdom

ARTICLE INFO

Article history:
Received 14 September 2009
Accepted 16 November 2009
Available online 22 November 2009

Keywords:
Bacterial motility
Microfluidics
Serratia marcescens

ABSTRACT

Traditionally bacteria are cultivated on the surface of agar, an environment that is experimentally convenient, but it does not resemble the diverse micro-geometries of their natural habitat, such as abiotic (e.g., soil) and biotic (e.g., plant and other organisms) surfaces. In this work, microstructured environments were manufactured from a biocompatible polymer, polydimethylsiloxane, to test the motility behaviour of bacteria moving into complex, channel-like, closed geometries with different shapes and dimensions. The motile bacteria were imaged by light microscopy and observed in replicate trials. It was found that bacteria were capable of swimming through even narrow microchannels and that their swimming paths, velocity and modes were influenced by a combined effect of channel width and its complexity. These results show that the structured microfluidic environment can be used as a simple means to observe and quantify the movement and navigation behaviour of bacteria through geometrically heterogeneous environments.

1. Introduction

Bacteria, microorganisms that are critical to so many areas of human life and activities with both beneficial and deleterious effects, live in a wide range of environments presenting complex surfaces. The process of colonisation of these environments usually involves the motility of individual bacteria, either continuously or as an initial stage of the formation of biofilms [1]. In order to predict the movement of bacterial cells through various substrates, e.g., soil or living tissues, one must know the factors controlling bacterial movement in these environments.

In the last decade microfabrication and microfluidics have been found to be very useful in the study of the behaviour of microorganisms, as some fundamental questions can be addressed especially in the field of quantitative microbiology [2]. Microfabrication can be used to create complex environments at the micrometer-scale and therefore enable the study of small populations of cells. For example, it is now possible to analyze a single bacterium or small group of cells under identical conditions with control of the microenvironment and the age of the cells. Among the materials used for microfluidics, polydimethylsiloxane (PDMS) has been the most common because of its suitability for studies involving living cells, as this material is transparent, non-toxic and oxygen permeable [3]. The PDMS-based microfluidics systems therefore offer a critical advantage over agar surfaces, with respect to a more realistic resemblance of the natural environment of bacteria.

The model organism used here is *Serratia marcescens*, a rod-shaped, peritrichously-flagellated, Gram-negative bacterium, about 1 μm in diameter by 2 μm long. The species is commonly found in soil, water and on plant surfaces. In addition, *S. marcescens* is an opportunistic human pathogen that causes nosocomial infections, particularly in immuno-compromised patients [4]. In liquid media bacteria typically undergo a "run-and-tumble" random walk biased by extracellular signals including chemo-attractants and chemo-repellents [5–7]. Briefly, the rotation of the flagellar motor in the counter-clockwise direction propels the cell forward and is referred to as a "run" (long intervals, a mean of 1.0 s, during which the cell swims smoothly). When one or more motors rotate in the clockwise direction, the cells "tumble" (short intervals, a mean of 0.1 s, during which the cell changes course) with little or no movement [8].

Here we describe a methodology that makes it possible to analyze the behaviour of bacterial cells in a wide range of restricted geometries of different shape and complexity. The availability of micro-channelled systems makes the study of how bacteria sense their environment a straightforward exercise. The aim of this study was to investigate quantitatively the influence of confinement on the growth of *S. marcescens* cells in microchannels filled with a nutrient rich medium.

* Corresponding author. Tel.: +44 151 794 4537; fax: +44 151 794 4540.
E-mail address: dnicolau@liverpool.ac.uk (D.V. Nicolau).

0167-9317/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.mee.2009.11.080

2. Methodology

2.1. Design of the test networks

The test networks comprised two parallel 2 mm-wide reservoirs connected through six 1 × 1 mm patterned areas. The six patterned areas consist of a 100 μm-wide, 1 mm-long channel that is actually a concatenation of five 100 × 100 μm patterned areas, intercalated with 100 × 100 μm open, 'plaza'-like spaces (Fig. 1). The microstructures used in this work comprise (a) stripe structures and (b) comb structures.

2.2. Preparation of the tests networks

A silicon master was generated by standard photolithography techniques followed by deep reactive ion etching (DRIE) using the standard Bosch recipe [9]. The mazes were obtained by replica molding of the master using PDMS. To prepare the PDMS precursor and curing agent (Sylgard 184, Dow Corning, MI, USA) were mixed in a 10:1 ratio. The resulting mixture was degassed under vacuum, poured over the silicon master, heat-cured for 12 h at 65 °C and then peeled away from the master. Before use, the PDMS structure was rendered hydrophilic by treatment with UV. In all cases, the base was an oxidized glass microscope slide and the sidewalls and ceiling were the oxidized PDMS. A 10–20 μl culture of cells (OD_{600} of 0.8) were loaded directly into the PDMS structures through open edges. Prior to inoculation, the structures were filled with Luria–Bertani (LB) liquid broth by placing the samples in a vacuum chamber. To avoid the adhesion of cells to the glass, the addition of a surface-active agent such as bovine serum albumin (BSA) to the broth was necessary.

2.3. Bacterial growth conditions

Cells of *S. marcescens* were grown in LB liquid broth in a round polypropylene tube containing 5 ml of medium with aeration at 30 °C. Difco LB medium (Becton Dickinson) consists of 0.5% yeast extract, 1% tryptone, and 1% NaCl prepared in deionized water and adjusted to pH 7.2.

2.4. Image acquisition and data analysis

The movement of *S. marcescens* cells in microchannels was observed at room temperature. The microscope slide containing the PDMS structure was placed on the stage of a Leica DM LB2 upright microscope. Video images were acquired using 40× bright field objectives, a 1.5× or 2× magnification changer and a Spot camera (Diagnostic Instruments, Inc., USA) that collected images at 10 frames per second. The tracking of the cells and calculation of velocity were performed using RETRAC 2.10.0.5 [10]. "Statistica 7.1" (Statsoft Inc., OK, USA) was used for statistical analysis and Image Pro Plus 6.1 (Media Cybernetics, Inc., MD, USA) for representation.

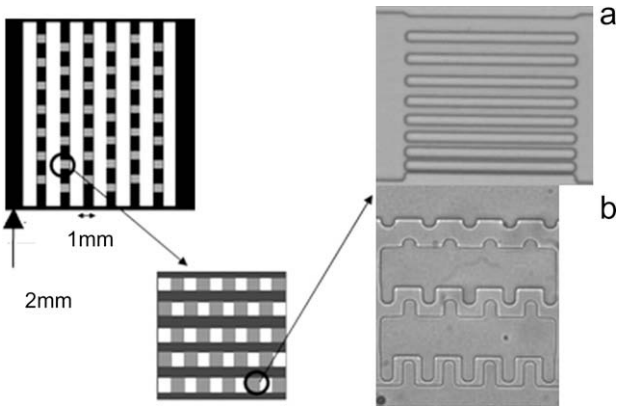

Fig. 1. Scheme of the complete set of structures on the silicon master. Images (a) and (b) represent the two different types of maze structures. The stripe structure shown in (a) has an edge length of 100 μm and channels ranging between 2 and 10 μm in size. The comb structure in (b) has an edge length of 100 μm and channels ranging between 5 and 15 μm. The channel depth for all structures is approximately 10 μm.

3. Results and discussion

3.1. The study of S. marcescens motility in microchannels

The effects of different channel widths (2–10 μm) on motility of *S. marcescens* were investigated (Fig. 2). The cells of *S. marcescens* were loaded into the microchannels constructed of PDMS and glass at midlog phase (OD_{600} of 0.8). Complex mazes were used under the assumption that an enclosed environment with designed dimensions would provide a means to systematically perturb the cell behaviour.

In the 'plaza'-like space (data not shown) the track of a cell appeared as a series of approximately straight segments, during which the cell moves forward, separated by turns at random angles. When a bacterium runs, its flagellar filaments work together in a bundle. When the bundle re-forms after tumbling, the cell goes off in a new direction. The average speed recorded for a straight trajectory was 9.90 ± 4.98 μm s^{-1}. We observed also that *S. marcescens* cells displayed clockwise circular trajectories near the patterned area about 24 μm in diameter. The question of attraction between the swimming bacteria and solid surface has been investigated by many authors. For instance, Vigeant and Ford [11] explained the circular motion observed and proposed that because of their nonspherical shape, the cells constantly swim into the surface and stay near to it for long period of time to enhance the probability of their adhesion to the substrate [11]. This explanation

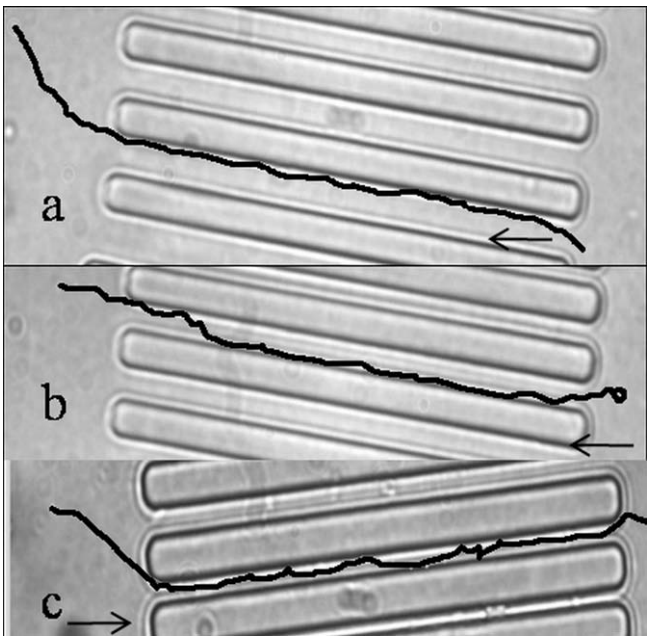

Fig. 2. Represents patterns formed by *Serratia marcescens* in the stripe structure in three different channel widths. Image (a) is a 9 μm wide channel, image (b) is a 6 μm wide channel and image (c) is a 5 μm wide channel. The cells showed a similar directional motion in the three different channels. The black arrows indicate the swimming direction.

however is not applicable in our case, as the bacteria swim in geometrically fully confined microstructures.

The cells could swim through every channel regardless of their size, but there were differences in their motility behaviour in relation to the channel width. In large channels, with widths close to 100 μm, the bacterial cells reaching the walls underwent a directional motion following closely the PDMS wall with only small deviations in the swimming trajectory from a straight path within the networks. As the width of the channels decreased, the bacteria maintained nearly constant straight motion (Fig. 2a–c). The flagella of the cells measured between 6 and 10 μm in length. Within the channels with diameters close to this size, the tumbling motion was executed with some difficulty since it is conceivable that the flagella are unable to extend fully, which probably explains the observed linear motion rather than serrated. When cells eventually tumbled, they temporarily moved away from the microchannel wall and then reassociated.

We observed that the motility behaviour was enhanced within the microchannels in comparison to the motility in the 'plaza'-like area. The mean speed of *S. marcescens* cells within the networks varied from 19 to 35 μm s^{-1} (Fig. 3, black curve). In microchannels the bacterial flagella often adhered to the wall during a run, making the motility measurements difficult and thus explaining the speed variability. Several studies have demonstrated that cells are affected by a surface only when they swim within 10 μm of the surface of the wall [12,13], as the hydrodynamic interactions decrease slowly with distance. Ramia et al. modelled the hydrodynamics of flagellated bacteria swimming near a plane wall and concluded that there is a small propulsive advantage to swim very close and parallel to a planar surface [14]. This work confirms our observations that the microchannels increased the swimming speed of *S. marcescens* cells about 3 times in comparison to the 'plaza'-like area.

3.2. S. marcescens motility in complex geometries

The comb structure consists of meandering channels with different width (5–10–15 μm) (Fig. 1b). We could therefore evaluate how complex patterns impacted on the swimming motility of *S. marcescens*. Bacterial cells confined within the comb structures followed closely the shape of the structures over limited distances when swimming within a 5 μm wide channel. In this very narrow channel, the cells seem to be incapable of continuously executing a linear trajectory and instead swam in a zigzag path (Fig. 4c). The 5 and 10 μm channels negatively affected the motility of *S. marcescens* cells. The additional complexity of the network makes motion more difficult. The cells had difficulty following the frequent turn in this structure (Fig. 4b and c). As the channel width increased, the cells kept a directional trajectory and alternated from the right to the left side of the channels over long distances as observed previously in the straight channels (Fig. 2a).

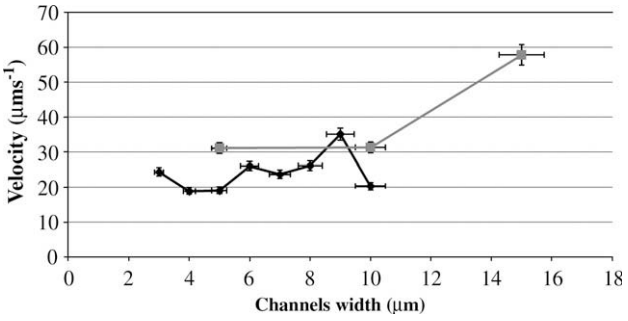

Fig. 3. Represents the variation of bacterial velocity according to the structure and channels width. The results concerning the stripe structure are represented by a black curve and the one concerning the comb structure are represented by a grey curve. The mean velocities were statistically different within a 95% confidence limit.

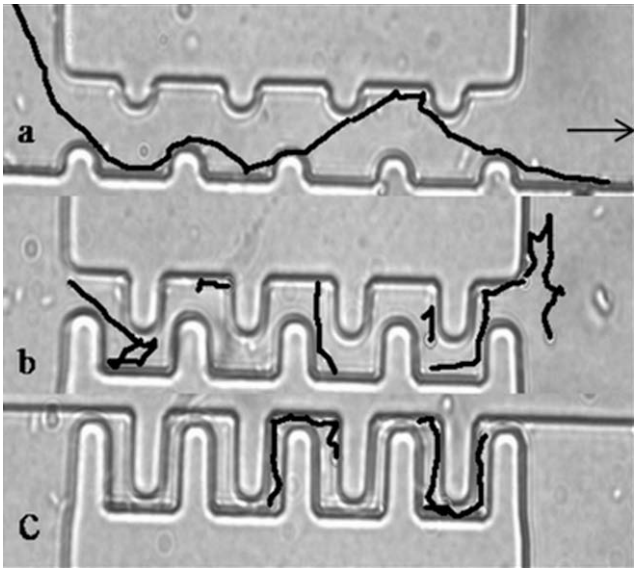

Fig. 4. Represents patterns formed by *Serratia marcescens* swimming in the comb structure in three different channel widths. Image (a) is a 15 μm wide channel, image (b) is a 10 μm wide channel and image (c) is a 5 μm wide channel. The cells showed different motion in the three different channels: from a directional motion in the widest channel to a more random motion within the 10 and 5 μm wide channels. The black arrows indicate the swimming direction.

The fluctuations in distance of the bacterium to the channel wall subsequently led to variation of the swimming speed and swimming trajectory. The motility speed decreased, as the channels width decreased, from 57.78 ± 18.04 μm s^{-1} to 31 ± 7.48 μm s^{-1} as shown in Fig. 3 (grey curve). The bacterial trajectories seem to be altered by the collisions with the walls, thus impacting on the rate at which the cells spread through the microfluidics structures.

While the stripe structures could direct the motion of the cells, the comb structures demonstrated the difficulties for cells to swim through intricate paths. Furthermore, more complicated networks, e.g., mazes, prevent the cells spreading further on the surface and constrained the swimming cells within small areas. Thus microgeometries can be purposefully designed, fabricated and operated to induce the controlled growth at desired positions.

4. Conclusions

An important advantage of the system using microfluidics is that it provides direct visual information on how a constraining environment may affect bacterial motility. The system presented here provided a convenient method to study the hydrodynamics of swimming motility close to surfaces, and to determine the chemical and physical dependence of these interactions. The motility parameters observed in restricting geometries varied with respect to the microchannels size and complexity demonstrating that the bacteria can swim through pores only slightly larger than their own body, but the rate of swimming of cells increased when they negotiated microchannels. Conversely, more complex microstructures restricted the cells movement. The understanding of the motility of single cells will be an important step towards the understanding of their population dynamics, which is relevant to the comprehension of the early stages of biofilm formation and bacterial infection.

Acknowledgements

This project was funded by a Leverhulme Trust Grant. The bacterium *Serratia marcescens* was provided by Liverpool School of Biological Sciences Culture Collection. We wish to thank Dr. Lisen Wang (University of California, Irvine) for the help in the fabrication of the silicon master; and Drs. Jenny Aveyard and Robert Wilson and Marie Held (University of Liverpool) for constant advice regarding experimental procedures.

References

[1] L. Hall-Stoodley, J.W. Costerton, P. Stoodley, Nat. Rev. Microbiol. 2 (2004) 95–108.
[2] D.B. Weibel, W.R. Diluzio, G.M. Whitesides, Nature 5 (2007) 209–218.
[3] S.K. Sia, G.M. Whitesides, Electrophoresis 24 (2003) 3563–3576.
[4] P.A.D. Grimont, F. Grimont, Ann. Rev. Microbiol. 32 (1978) 221–248.
[5] H.C. Berg, D.A. Brown, Nature 239 (1972) 500–504.
[6] D.V. Nicolau Jr., K. Burrage, D.V. Nicolau, P.K. Maini, Biosystems 94 (2008) 47–54.
[7] G.H. Wadhams, J.P. Armitage, Nat. Rev. Mol. Cell. Biol. 5 (2004) 1024–1037.
[8] H.C. Berg, R.A. Anderson, Nature 245 (1973) 380–382.
[9] K.L. Hanson, D.V. Nicolau Jr., L. Filipponi, L. Wang, A.P. Lee, D.V. Nicolau, Small 2 (2006) 1212–1220.
[10] <http://mc11.mcri.ac.uk/Retrac/index.html>.
[11] M.A. Vigeant, R.M. Ford, Appl. Environ. Microbiol. 63 (1997) 3474–3479.
[12] P.D. Frymier, R.M. Ford, AIChE J. 43 (1997) 1341–1347.
[13] S.A. Biondi, J.A. Quinn, H. Goldfine, AIChE J. 44 (1998) 1923–1929.
[14] M. Ramia, D.L. Tullock, N. Phan-Thien, Biophys. J. 65 (1993) 755–778.