LETTERS

Escherichia coli swim on the right-hand side

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The motion of peritrichously flagellated bacteria close to surfaces is relevant to understanding the early stages of biofilm formation and of pathogenic infection[1–4]. This motion differs from the random-walk trajectories[5] of cells in free solution. Individual *Escherichia coli* cells swim in clockwise, circular trajectories near planar glass surfaces[6,7]. On a semi-solid agar substrate, cells differentiate into an elongated, hyperflagellated phenotype and migrate cooperatively over the surface[8], a phenomenon called swarming. We have developed a technique for observing isolated *E. coli* swarmer cells[9] moving on an agar substrate and confined in shallow, oxidized poly(dimethylsiloxane) (PDMS) microchannels. Here we show that cells in these microchannels preferentially 'drive on the right', swimming preferentially along the right wall of the microchannel (viewed from behind the moving cell, with the agar on the bottom). We propose that when cells are confined between two interfaces—one an agar gel and the second PDMS—they swim closer to the agar surface than to the PDMS surface (and for much longer periods of time), leading to the preferential movement on the right of the microchannel. Thus, the choice of materials guides the motion of cells in microchannels.

Peritrichously flagellated bacteria are propelled by long (about 10 μm), thin, helical filaments distributed randomly over the surface of the cell body. A reversible rotary motor embedded in the cell wall drives each filament at its base[10,11]. If all motors are rotating anticlockwise, the flagella bundle together and propel the cell forward in a 'run'. When one or more of the motors switches to clockwise rotation, the corresponding flagella unbundle and reorient the cell in a 'tumble'[12]. During a run, the forward thrust generated by the flagellar bundle is balanced by the viscous drag on the cell body, and the torque produced by the rotating flagellar bundle is balanced by the torque due to the counter-rotation of the cell body[13]. If a cell swims close to a planar surface, these rotations and the resistance from the surface affect the direction of movement. The flagellar bundle rolls to the left near the surface, and the cell body rolls to the right near the surface. These two motions cause the cell to swim in a clockwise, circular trajectory[4,6,7,14].

Cells swim in circles at surfaces for seconds to minutes, although one might expect them to drift from the surface quickly because of the effects of rotational brownian motion and bundle fluctuation (wobble) on their trajectories[5,15]. Hydrodynamic interactions cause the extended interaction of cells with surfaces[4,14]. Figure 1 shows a schematic representation of *E. coli* cells swimming near two horizontal surfaces. The cells swim in clockwise, circular trajectories at each surface: the trajectories of cells close to the bottom surface seem to follow clockwise paths, and the trajectories of cells close to the top surface seem to follow anticlockwise paths[6,7,15]. (A clockwise trajectory appears anticlockwise when viewed from the opposite side.) The direction of curvature of the trajectory of the cell therefore indicates whether the cells are swimming closer to the top surface or to the bottom surface.

We have developed a new technique for examining the movement of individual bacteria on nutrient agar by confining the cells in shallow microchannels to constrain their motion to two dimensions. Using soft lithography[16], we fabricated thin (150 μm thick), flexible, gas-permeable[17] films of PDMS embossed with grooves. The surface of the film was rendered hydrophilic by treatment with an air plasma (the advancing contact angle of water on the PDMS film after treatment was 10–20°). We placed the oxidized PDMS (ox-PDMS) film on the agar a few millimetres from the edge of a swarm of *E. coli*[9]. The film sealed conformally to the agar substrate and formed microchannels in which the bottom agar surface formed the floor of the channel, and the ox-PDMS film formed the sidewalls and ceiling (Fig. 2a). An aqueous solution of nutrients from the agar substrate wetted and filled the hydrophilic microchannels. Observation of small, suspended polystyrene beads in the channel showed that no net flow of fluid occurred in the microchannels once they had filled. Individual cells from the swarm migrated into the microchannels. Once they entered the liquid-filled microchannels, they separated from other swarmer cells and swam independently.

In rectangular agar/ox-PDMS microchannels that were only slightly taller than the width of one cell (1.3–1.5 μm tall and 7–10 μm wide), most *E. coli* cells moving in either direction swam preferentially along the channel wall to their right (when viewed from above). The swimming of cells in a clockwise direction (movement to the right) implies that cells are swimming closer to the bottom surface (agar) than to the top surface (ox-PDMS) (Fig. 1). This ordered movement to the right in opposite directions along the two walls resembles that of cars driving along a two-way street. Figure 2b–d

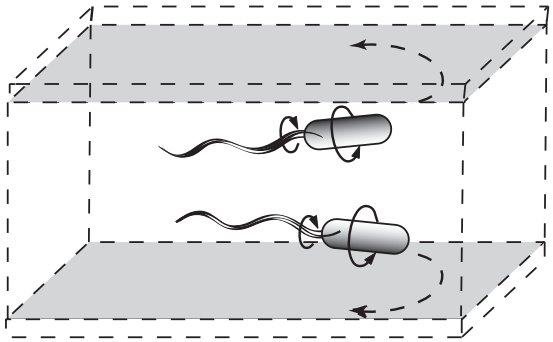

Figure 1 | Cells swim in clockwise, circular trajectories at solid, planar surfaces. When a cell executes a run, all flagella in the bundle rotate anticlockwise (when viewed from behind) and the cell body counter-rotates in a clockwise direction. When viewed from above, the cell trajectories at the bottom surface appear clockwise and the cell trajectories at the top surface appear anticlockwise.

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shows time-lapse images of the traffic-like behaviour of cells in microchannels (see Supplementary Movies).

We wanted to confirm that the motion of cells in microchannels was analogous to the motion of cells confined between two glass surfaces; that is, that cells moving clockwise (or to the right) were at the bottom surface, and that cells moving anticlockwise (or to the left) were at the top surface. We could not easily observe the surface to which the cells were closest in agar/ox-PDMS channels 1.3–1.5 μm tall; we then examined taller channels. Using fluorescence microscopy, we observed HCB437 cells, expressing enhanced green fluorescent protein, in rectangular (5 μm tall and 10 μm wide) ox-PDMS channels sealed to glass. Imaging with a 100 ×, 1.4 numerical aperture, oil objective, we could easily focus on either the floor or the ceiling of the microchannel and directly observe the surface to which cells were closest. Cells swimming near the floor of the channel were moving along the channel wall on their right (with respect to the direction of movement of the cells); cells swimming near the ceiling of the channel were moving along the channel wall on their left.

Although most cells swam on the right in the 1.3–1.5 μm tall agar/ox-PDMS microchannels, occasionally some cells travelled in the 'wrong' direction, swimming on the left. To quantify the preference of cells to swim on the right (or to swim closer to the agar surface than the ox-PDMS surface), we fabricated ox-PDMS films embossed with microchannels containing three-way junctions (Fig. 3). Swarmer cells travelling along the right channel wall entered the right side of the junction; cells travelling along the left channel wall entered the left side of the junction. We counted those cells that passed through the junction and entered each curving side-channel. We defined the percentage of cells swimming on the right as the number of cells that entered the right channel divided by the total number of cells that entered the left or right channels. We did not count the small number of cells that continued straight and did not enter either side channel. Table 1 shows the resulting preference of cells to swim on the right for different bacterial strains and materials comprising the channels.

Smooth-swimming *E. coli* cells, which do not tumble, exhibited a stronger preference to travel on the right side of the channel than did wild-type cells of *E. coli*. Smooth-swimming cells stayed aligned with the channel wall to their right over distances of several millimetres; when wild-type cells tumbled in the rectangular channels, they briefly lost their preference for the right-hand wall. One of the trajectories in Fig. 2e shows a wild-type cell that tumbled and temporarily moved away from the microchannel wall. Even smooth-swimming cells did not swim along the right wall indefinitely. Wobbling or rotational brownian motion eventually caused cells to separate from the wall and then to reassociate (apparently randomly) with either the left or right channel wall. The preference to the right in shallow agar/ox-PDMS channels for all strains examined indicates that tumbling, wobbling and/or rotational brownian motion is suppressed more when cells swim near the agar surface than when cells swim near the PDMS surface.

Cells continued to swim close to the agar surface even when we inverted the experimental system. In an inverted system, nutrient agar formed the channel ceiling and ox-PDMS formed the walls and floor; here cells preferred to move to the left (when viewed from above); that is, they swam closer to the top agar surface. This observation shows that the preferential movement of cells for the right when agar is the floor of the microchannel is not a result of the influence of gravity. We observed preferential movement of the cells only when agar was used as either the floor or ceiling of a composite microchannel; when the floor of the channel was composed of oxidized glass or ox-PDMS, and the channel sidewalls and ceiling

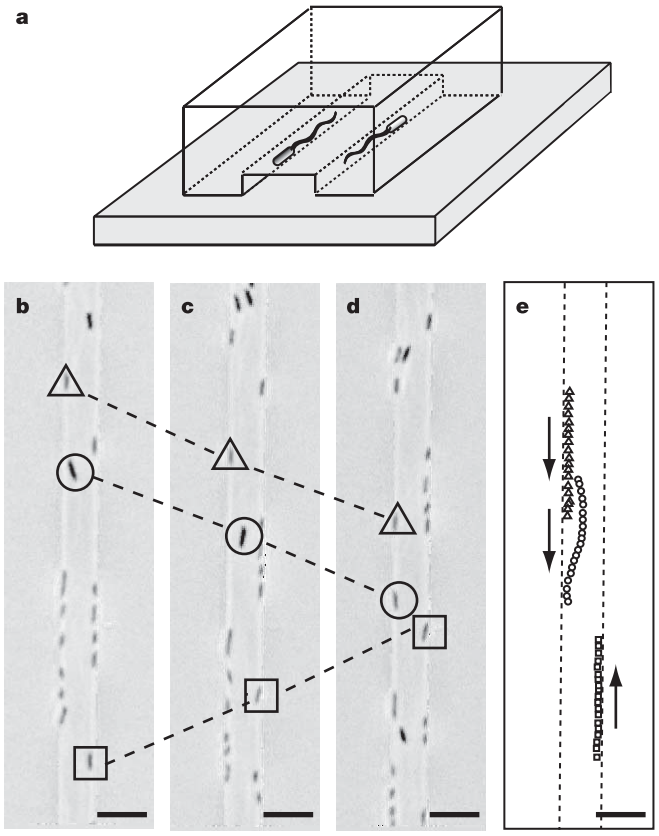

Figure 2 | Images of cells in composite agar/ox-PDMS microchannels. **a**, An oxidized PDMS film with embossed channels seals conformally to an agar substrate and forms liquid-filled channels into which *E. coli* swarmer cells migrate. Within these microchannels (imaged from above), cells that are closer to the floor of the channel swim clockwise or move to the right. **b**–**d**, Three time-lapse images showing *E. coli* swarmer (AW405) cells[26] moving on the right in confining, rectangular microchannels (1.4 μm tall and 7 μm wide) in which nutrient agar formed the floor of the channel and an ox-PDMS film formed the walls and ceiling of the channel. **b**, $t = 0$ s; **c**, $t = 0.33$ s; **d**, $t = 0.67$ s. **e**, The trajectories of three selected cells between images **b** and **d** are shown. Scale bars, 10 μm.

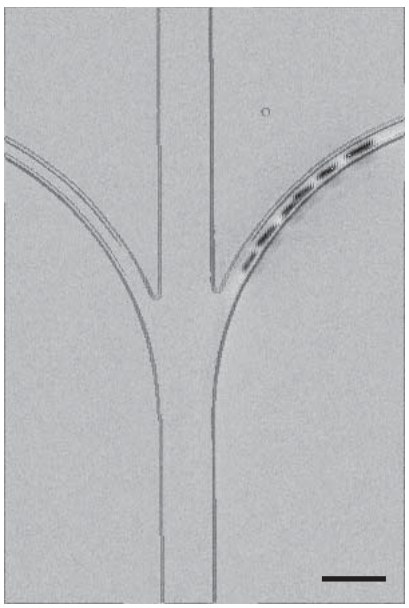

Figure 3 | Quantification of cells displaying a preference to travel to the right by using a microchannel junction. Cells travelling along the right channel wall (closer to the floor of the microchannel) enter the right sidearm; cells travelling along the left channel wall (closer to the ceiling of the microchannel) enter the left sidearm. Six smooth-swimming *E. coli* (HCB437) cells[27] are shown entering the right sidearm. Scale bar, 20 μm.

Table 1 | Determination of preference of cells for swimming on the right

E. coli strain	Floor material	Ceiling material	Cells swimming on the right (%)
AW405	Nutrient agar	Ox-PDMS	75 ± 7
RP437	Nutrient agar	Ox-PDMS	77 ± 2
HCB437	Nutrient agar	Ox-PDMS	88 ± 7
HCB437	Ox-PDMS	Nutrient agar	16 ± 13
HCB437	Ox-PDMS	Ox-PDMS	55 ± 12
HCB437	Ox-glass	Ox-PDMS	53 ± 2

The microchannels used for analysis were 1.3–1.5 μm tall and 7–10 μm wide. Strains AW405 (ref. 26) and RP437 (ref. 28) are wild-type for chemotaxis, and strain HCB437 (ref. 27) is a smooth-swimming strain that is deleted for most chemotaxis genes. The percentage of cells swimming on to the right was determined as 100 times the number of cells that entered the right divided by the sum of the cells that entered the right and the cells that entered the left. For each percentage given, at least 1,000 cells were counted in at least seven separate channels. Errors are s.d. for the individual channel percentages. Nutrient agar contained 3 g l^{-1} beef extract, 10 g l^{-1} peptone, 5 g l^{-1} sodium chloride, 4.5 g l^{-1} Eiken agar and 0.5% glucose.

were made of ox-PDMS, and channels were filled with liquid growth medium, cells showed no preference for one side of the microchannel.

To examine whether short-range interactions between the bacteria and the surfaces of the microchannels contributed to the mechanism underlying the preference for the right, we added surfactants to the solution used to cast the agar that formed the floor of the microchannel; we also changed its ionic strength. For these studies we used non-nutrient motility agar (10 mM potassium phosphate pH 7.0, 0.5% Eiken agar). The addition of a surface-active agent such as bovine serum albumin or surfactin (a lipopeptide biosurfactant produced by *B. subtilis*[18]) to the motility agar was necessary to inhibit the adhesion of cells to the ox-PDMS surfaces, but we continued to observe a preferential movement of cells to the right (more than 80% preference for all the surfactants examined; see Supplementary Information). Each surfactant would be expected to alter the van der Waals and steric interaction of cells with the channel walls in a slightly different way. We also varied the ionic strength of the motility agar, and continued to observe a preference of more than 80% for the right (see Supplementary Information). Taken together, these results indicate that short-range molecular interactions (van der Waals, ionic, hydrogen bond, hydrophobic or steric) are not relevant for the preferential movement of cells.

As the channel height increased, the preference of cells for movement along the right wall slowly diminished (Fig. 4). The simplest rationale for the slowly decaying preference to the right with channel height is that cells must experience different hydrodynamic environments when they swim near agar surfaces and ox-PDMS surfaces, because hydrodynamic interactions fall off slowly with distance. When the channel height was 10 μm, about equal numbers of cells swam to the right (closer to the microchannel floor) and to the left (closer to the microchannel ceiling); that is, there was no preference for side. These result are consistent with other studies indicating that cells are affected by a surface (here, the gel) only when they swim within 10 μm of the surface[6,19,20].

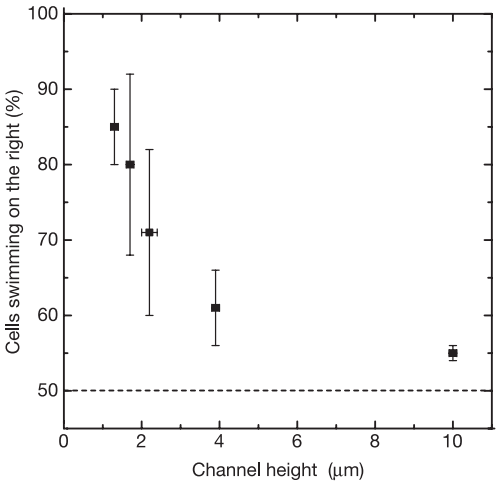

Figure 4 | Preference of cells to move on the right in microchannels as a function of the height of the channel. Channels had a floor composed of nutrient agar, and a ceiling and sidewalls composed of ox-PDMS. For each datum, at least 10^3 cells were counted in seven or more separate channels. Error bars show s.d. for the different channels measured.

Agar and agarose are porous gels with a wide distribution of pore sizes[21]. We propose that cells experience less resistance to their movement when they swim close to the porous agar surface than when they swim close to the non-porous ox-PDMS surface. It has been shown that a translating and/or rotating sphere experiences less hydrodynamic drag near a porous boundary than near a solid boundary[22]. The speed at which a cell swims provides a direct indicator of the hydrodynamic drag it experiences. We measured the swimming speed of 50 individual cells (strain HCB437) in channels with porous agar floors and in channels with solid ox-PDMS floors (Table 2). Cells moving on the right in channels with agar floors swam at an average speed of 31 ± 3 μm s^{-1} and cells in channels with ox-PDMS floors swam at 27 ± 4 μm s^{-1}; these mean velocities were statistically different within a 95% confidence limit. These results further suggest that the hydrodynamics of swimming is different in composite agar/ox-PDMS microchannels and in microchannels in which all of the walls are ox-PDMS.

To determine whether there were phenotypic differences between cells that swam along the left or the right wall in shallow agar/ox-PDMS channels, we compared the lengths and swimming speeds of cells at each wall. In an agar/ox-PDMS channel that was 1.4 μm tall and 10 μm wide there was no significant difference in the average length of cells travelling along the right or left sidewall (Table 2). However, cells moving along the right wall swam faster (by 15%) than cells moving along the left, which is consistent with the hypothesis that cells experience less resistance when they swim closer to the agar surface than to the PDMS surface. In an ox-PDMS/ox-PDMS channel that was 1.4 μm tall and 10 μm tall, there was no significant difference in the average length or speed of the cells travelling on the left or right wall (Table 2).

The finding that cells move preferentially along the surface of polysaccharide hydrogel for a longer period than along a solid surface might help to rationalize behaviours that are important in environmental and medical microbiology. Various bacteria often produce polysaccharides to enhance flagella-dependent swarming motility, pili-dependent twitching motility, and gliding motility on surfaces[2]. Uropathogenic *Proteus mirabilis* cells, which are often of the swarming phenotype, migrate up the urinary tract, which is lined with polysaccharide-coated uroepithelial cells[23]. *Vibrio fischeri* symbiotically colonize the light organ of the squid *Eupryma scolopes* by migrating through narrow ducts (15 μm wide) that are coated with mucus produced by the squid[24].

Table 2 | Length and swimming speeds of cells travelling on right versus left

Floor material	Ceiling material	Side of channel	Cell length (μm)	Swimming speed (μm s^{-1})
Nutrient agar	Ox-PDMS	Right	3.3 ± 0.8	31 ± 3
		Left	3.4 ± 0.9	27 ± 4
Ox-PDMS	Ox-PDMS	Right	2.7 ± 0.7	26 ± 5
		Left	2.6 ± 0.4	26 ± 5

Lengths and swimming speeds were measured for smooth-swimming HCB437 cells[27] in microchannels that were 1.3–1.5 μm tall and 10 μm wide; $n = 50$ cells for each determination. Errors are s.d. for 50 measurements. Histograms showing the distribution of the lengths of cells and swimming speeds are given in Supplementary Information. Nutrient agar contained 3 g l^{-1} beef extract, 10 g l^{-1} peptone, 5 g l^{-1} sodium chloride, 4.5 g l^{-1} Eiken agar and 0.5% glucose.

The composite agar/ox-PDMS channels developed here can be extended to study the growth or movement of a range of motile, free-swimming microorganisms (or perhaps higher organisms) in a confined but nutritive environment. Observing a preference for swimming along the right or left wall provides an experimentally convenient method for examining the hydrodynamics of movement close to proximal surfaces, and to determine the chemical and physical dependence of these interactions. The ability to design channels by using materials that affect the hydrodynamics of swimming differently offers a strategy with which to direct the motion of motile cells in microchannels. This strategy would not require external pumping, valving or chemical gradients to direct the movement of cells. We believe that directed motion is a first step towards the development of self-contained microdevices that use moving bacterial cells[25] for cell-based bioassays and biosensors.

Received 4 February; accepted 21 April 2005.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank W. S. Ryu, D. Ryan, M. P. Brenner and H. A. Stone for discussions, and S. Rojevskaya for technical assistance. This research was supported by the NIH and DOE. W.R.D. acknowledges an NSF-IGERT Biomechanics Training Grant. M.M. acknowledges a postdoctoral fellowship from the Swiss National Science Foundation. P.G. thanks the Foundation for Polish Science for a postdoctoral fellowship. D.B.W. thanks the NIH for a postdoctoral fellowship.

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