An assessment of the dynamic stability of microorganisms on patterned surfaces in relation to biofouling control

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Microstructure-based patterned surfaces with antifouling capabilities against a wide range of organisms are yet to be optimised. Several studies have shown that microtopographic features affect the settlement and the early stages of biofilm formation of microorganisms. It is speculated that the fluctuating stress–strain rates developed on patterned surfaces disrupt the stability of microorganisms. This study investigated the dynamic interactions of a motile bacterium (Escherichia coli) with microtopographies in relation to initial settlement. The trajectories of E. coli across a patterned surface of a microwell array within a microchannel-based flow cell system were assessed experimentally with a time-lapse imaging module. The microwell array was composed of 256 circular wells, each with diameter 10 μm, spacing 7 μm and depth 5 μm. The dynamics of E. coli over microwell-based patterned surfaces were compared with those over plain surfaces and an increased velocity of cell bodies was observed in the case of patterned surfaces. The experimental results were further verified and supported by computational fluid dynamic simulations. Finally, it was stated that the nature of solid boundaries and the associated microfluidic conditions play key roles in determining the dynamic stability of motile bacteria in the close vicinity over surfaces.

Keywords: biofouling; patterned surface; microfluidic approach; CFD simulation; dynamic stability; microwells

Introduction

Biofouling, a hierarchical process in length and time scales, starts with organic conditioning at the molecular level (mainly proteins, proteoglycans, and polysaccharides) on submerged surfaces (Railkin 2004; Rosehahn et al. 2010; Callow & Callow 2011). The attachment and growth of different microorganisms starts within hours, leading to the formation of biofilms (eg Briand et al. 2012). A biofilm consists of bacteria and other microorganisms encased in extracellular polymeric substances (EPS) (Willey et al. 2008). Bacterial biofilms have a variety of positive, negative or neutral interactions with higher fouling organisms such as algal spores and larvae of invertebrates (Dobretsov et al. 2006, 2013; Qian et al. 2007; Hadfield 2011; Mieszkoski et al. 2013). The development of biofilms and the settlement of other fouling organisms can be controlled to various degrees by altering the topography of the surface. Surface roughness and microtopographic features of artificial surfaces and their influence on larval settlement and the accumulation of biofouling has been the subject of intensive investigation in recent years (eg Petronis et al. 2000; Bers & Wahl 2004; Carman et al. 2006; Schumacher et al. 2007; Carl et al. 2012).

A number of natural biological surfaces show anti fouling (AF) characteristics with microtopographic arrangements (eg shark skin, lotus leaves, the body surfaces of different benthic invertebrates such as blue mussels, edible crabs, the egg-cases of the lessor-spotted dogfish, and brittle stars) (Bechert et al. 2000; Bers & Wahl 2004; Patankar 2004; Schumacher et al. 2008; Bhushan & Jung 2011). Biological surfaces inhibit fouling by an integrated defence mechanism that includes chemical, mechanical or physical defences, which may be found singly or in combination (Wahl 1989; Wahl & Lafargue 1990). In contrast to synthetic AF surfaces, these natural surfaces have additional defence mechanisms such as moulting, burrowing and aspects of surface chemistry (eg enzymes), which may combine with repellent surface microtopographies to form a multiple defence system (Thomason et al. 1994; Wahl 1997; Wahl et al. 1998; Bers & Wahl 2004). However, artificial surfaces with biomimetic microstructures tend to show transitory fouling resistance and their potential is still being investigated as an effective fouling prevention technology. The question arises whether the transitory AF characteristics of artificial surfaces are due to the biological responses of microorganisms towards the surface or are mediated by other physical laws.

The precise mechanism by which man-made microtopographies inhibit the settlement and colonisation of
microorganisms is still unclear. Researchers have developed different hypotheses on this question and correlated different physical parameters (e.g., wettability, surface tension, feature sizes, and shapes) with the settlement process. Callow et al. (2002) suggested that the settlement of algal spores (Ulva syn. Enteromorpha) on a surface can be controlled by surface topography and surface tension. They reported that the shape and scale of features on a surface determine where spores will colonise. Scardino et al. (2006, 2008) presented an attachment point theory based on the size of the settling larvae/spores and the wavelength of the microtexture. Moreover, microorganisms (diatom cells in their study) attached in significantly higher numbers on surfaces where the number of attachment points was the highest, thus reduced numbers of attachment points resulted in lower attachment. Chung et al. (2007) suggested that the protruding features of the surface topography provided a physical obstacle to deter the expansion of small clusters of bacteria, present in the recesses, into microcolonies. Schumacher et al. (2008) introduced the concept of nanoforce gradients based on the lateral force required to cause a 10% end deflection of microstructures in a patterned surface. They hypothesised that variations in topographic feature geometry will induce stress gradients within the lateral plane of the cell membrane of a settling cell during initial contact. Carl et al. (2012) demonstrated that wettability has a negligible effect on settlement of pediveligers of the mussel Mytilus galloprovincialis. Instead, the presence of topographic structures plays a key role in the settlement of mussels (Carl et al. 2012; Vucko et al. 2013).

Recently, Halder et al. (2013) proposed that microtopography-induced microfluidic disturbance (i.e., fluctuation in stress–strain rates) could inhibit bacterial (Escherichia coli) settlement processes. A few other studies also suggested that the swimming kinematics and trajectories of microorganisms could be altered by the presence of nearby boundaries (Lauga et al. 2006; Berke et al. 2008; Drescher et al. 2009; Evans & Lauga 2010; Or et al. 2011). The AF efficacy showed by microtopographies is more a hydromechanical phenomenon than a biological/physico-chemical process in this respect. Thus, the settlement (i.e., attachment) of microorganisms, their locomotion on plain and patterned surfaces and the near-surface microfluidic environment are of interest as key factors in fouling prevention technologies.

The presence of solid boundaries has profound effects on the dynamics and trajectories of microorganisms. The locomotion of microorganisms takes place at an extremely low Reynolds number (Re), a regime in which fluid motion is dominated by viscous dissipation. Near a solid surface, these effects are strongest in the viscous sublayer, lying inside the boundary layer. The swimming dynamics of microorganisms are highly dependent on hydrodynamic interactions, either between immersed cells (group locomotion), or between cells and a surface (boundary effects). For example, the complex circular swimming of E. coli (Re~10^-4) could be explained by the hydrodynamic interactions between the bacterial cell surface and solid boundaries (Lauga et al. 2006). Vigeant et al. (2002) and Lauga et al. (2006) showed that near a flat surface in a quiescent environment, conditions of force-free and torque-free swimming result in hydrodynamic trapping of motile E. coli. Cosson et al. (2003) and Berke et al. (2008) demonstrated how spermatozoa and E. coli become attached to solid surfaces that confine their domain. Hill et al. (2007) showed that hydrodynamic surface interactions enable E. coli to seek efficient routes to swim upstream. Zilman et al. (2008) studied the mechanisms of larval contact in two-dimensional Couette (linear shear flow near flat solid boundaries) and Poiseuille (laminar flow in a tube) flows. Drescher et al. (2009) investigated the waltzing dynamics of Volvox (a spherical alga) when placed next to a solid surface, where pairs of cells attract one another and can form stable hydrodynamic bound states. Evans and Lauga (2010) showed that the presence of a wall can lead to a change in the waveform expressed by a beating flagellum, which in turn results in an increase or decrease in its propulsive force depending on the type of oscillation. Or et al. (2011) investigated how a plain boundary affected the dynamic stability of low Reynolds number swimmers from the viewpoint of dynamic systems and control theory. However, most of these studies considered only plain surfaces, and did not study any patterned surfaces.

The goal of this study was to investigate the dynamic stability of E. coli on a prefabricated microwell-patterned surface compared with a conventional plain surface. The main focus was how the complex microfluidic environment developed on patterned surfaces disrupted the settlement of bacterial cells. Both laboratory experiments and computational fluid dynamics (CFD) simulations were used to characterise the swimming pattern of the cells of E. coli.

Materials and methods
Experimental design and set-up
The test set-up was analogous to that used in experiments described by Halder et al. (2013). Briefly, an in vitro flow cell model (microchannel) was designed to observe bacterial motility under controlled hydrodynamic conditions on circular well array. The flow cell was combined with light microscopic (LM) imaging and a time-lapse module to observe and quantify every stage of locomotion of E. coli under conditions of continuous flow. Leica Application Suite (LAS®) multi-time imaging
software (LAS MultiTime, Leica Microsystems Pty Ltd, Sydney, Australia) gave real-time monitoring and sequential image acquisition at specified time intervals. The dynamic stability of individual cells on plain and patterned surfaces was compared concurrently under the same conditions of flow and cell culture. The fluid flow of the microchannel was controlled precisely (in terms of shear rates) with a digital drive and microbore tubing system. The culture medium and most of the flow cell system were autoclaved. Heat-sensitive components were sterilised using a method adapted from Fisher and Petrini (1992). 

**Microchannel flow cell system**

Bacterial motility was observed in a rectangular polydimethylsiloxane (PDMS) microchannel (1 mm wide, 0.11 mm deep and 50 mm long). The channel bed was a thin glass slide, coated with SU-8 epoxy patterned with a rectangular array of circular wells (microwells, depth 5 μm). The prefabricated microwell array was (272 × 272) μm², composed of microwells with 10 μm diameter and 7 μm edge to edge spacing (indicated as D10S07). Halder et al. (2013) stated that the geometric feature sizes of a prefabricated micropattern need to consider the size of the microorganisms for higher AF efficiency. In their experiment with a series of microwells with sequential size and spacing (1–10 μm), it was found that feature size smaller than the cell body size showed negligible effect on settlement of E. coli. Conversely, substantially larger features than the cell body size can provide physical protection to the settling cells against hydrodynamic forces (Scardino et al. 2006; Sweat & Johnson 2013). Hence, in this study the microwell configuration D10S07 was chosen to be slightly larger than the body size of E. coli (~2–5 μm). The microchannel was connected with a ‘once-through’ flow tube, fed from a mixing chamber. Details of the microchannel flow cell set-up can be found in Halder et al. (2013) and in Supplementary materials (Figures S1 and S2). [Supplementary material is available via a multimedia link on the online article webpage.]

**Flow cell fabrication**

The flow cell device consisted of two components: a microwell array fabricated on a glass slide using an epoxy-based negative photoresist SU-8 2005 (MicroChem Corp., Newton, MA, USA) and a microchannel made of PDMS (Figure S2). The microwell array (16 × 16) was fabricated on a surface area of (272 × 272) μm². The PDMS microchannel was then placed over the microwell array to form a closed rectangular channel with one inlet and one outlet at the ends (Figure S2). The steps involved in the flow cell fabrication can be found in detail in Halder et al. (2013).

**Flow cell hydrodynamics**

The fluid velocity was carefully controlled to obtain sequential images of bacterial motility. As this study aimed to investigate the motility of individual bacterial cells in relation to fluid–structure interactions, a very low flow rate was applied (0.02 μl s⁻¹) for this experiment. The critical strain rate for suppressing the self-propulsion of E. coli is > 10 s⁻¹, as suggested by Hill et al. (2007). The selected flow rate ensured that the induced hydrodynamic forces did not manipulate the self-propulsion of cells significantly. Instead, a tractable path line of a bacterium could be identified within the field of view (FOV) of the objective lens (40 ×) and compared for both plain and patterned surfaces using a time-lapse imaging system. The given flow condition was necessary to propel and track individual cells as they crossed the smooth surface and then encountered the patterned surface without disrupting their self-propulsion capability significantly.

The developed wall shear stress at the channel bed was ~ 0.01 Pa. The calculated Reynolds number, hydrodynamic entry length and the shear strain rates were ~ 0.03, 0.3 μm and 10 s⁻¹, respectively. The steps involved in these calculations can be found in Halder et al. (2013).

**Image analysis**

For the cell motility experiment, real-time locomotion of bacteria was monitored continuously using LM (phase contrast) and a digital microscope camera (Leica DFC310 FX). The video microscopy system was supported by a Leica application suite (LAS MultiTime® module) with time stamping option. A total of seven video clips (labelled as Observations 1–7) were obtained using the video microscopy system. The duration of each video clip was 20 s with a 1 s interval of time stamping. Still images were captured and saved separately at every second from the acquired video clips. The frame rate was ~20 frames per second (fps) for most of the video clips. Thus, it was easy to retrieve suitable still images from the series of frames embedded in the video clips. In total, 147 still images (ie 21 images × 7 video clips) were retrieved from the acquired clips. All still images were analysed further to identify bacterial swimming trajectories using IMAGEJ software (http://rsbweb.nih.gov/ij/). Images were also taken using an environmental scanning electron microscopic (ESEM; FEI Quanta 200, FEI
Company, Hillsboro, OR, USA) to characterise the microwell array in relation to the elevation difference from the adjacent plain surface (Figure 1). All ESEM images were taken at 30° tilt to get a clear view of the elevation difference and microwell depth (5 μm).

To characterise the effect of solid boundaries on cell dynamics, it was essential to find out at what depth the bacterial cells were swimming. It was difficult to assess the swimming depth using LM as cells changed focal planes very frequently. In this study, swimming depth was assessed based on the focal plane set-up of the microscope at a known depth. For example, to observe cells on the plain surface at a higher swimming depth, the focal plane of the microscope was adjusted to the top of the microwell array (Figure S3, see also Figure 1). This ensured that the observed bacterium (within LM field of view) was swimming at a distance ~ 5 μm above the bottom surface.

Statistical analysis
Statistical differences between the swimming velocities of cells over plain/patterned surfaces were analysed using a two-way ANOVA followed by the Tukey’s multiple comparisons test. Here, two different factors were considered, which included the surface patterning (plain/pattern) and the swimming depths (1 μm/5 μm). Parametric t-tests (paired) were also conducted for all identified bacterial cells without discriminating them in relation to swimming depths to see the effect of surface patterning only on cell dynamics. In all tests, statistical differences were considered significant at the 95% confidence level.

Bacterial cells were also considered independent for all statistical analyses. All these analyses were performed using GraphPad Prism software (version 6.00 for Windows; GraphPad Software, La Jolla, CA, USA; www.graphpad.com).

CFD analysis with patterned surfaces
To investigate the effects of solid boundaries (plain/patterned) on the swimming trajectory and the dynamic stability of E. coli, a commercially available CFD software package (ANSYS CFX, Version 14.5, ANSYS Inc., Canonsburg, PA, USA) was applied to calculate flow fields around the moving bacterial cells. The bacteria were modelled as immersed solids near to the surface and the complex microfluidic environment was solved using a finite volume method. The immersed solid technique involves the use of an immersed solid domain that is placed inside a fluid domain to represent the motile bacterium, including its shape, position, orientation and velocity (Figure S4). As the simulation proceeds, CFX-solver calculates which parts of the fluid domain are overlapped by the immersed solid (ie within the immersed solid domain), and applies a source momentum to the fluid inside the immersed solid domain in order to force the immediate outside fluid to move with the solid object. Thus the flow condition around a bacterium was simulated, as if it were flowing around an immersed solid boundary.

The entire channel was simulated initially to find the developed wall shear stress (~0.01 Pa) on the plain
surface, which was validated by the analytical solution described in Halder et al. (2013). In this regard, the inlet flow rate was set at 0.02 μl s⁻¹ and the channel walls were considered plain surfaces with no-slip boundary conditions. Other studies (Lee et al. 2008; Tovar-Lopez et al. 2010; Chen et al. 2012) also applied no-slip boundary conditions for the channel walls to simulate the flow field in a microchannel using CFD. Wall shear stress was ~ 0.01 Pa at the maximum area of the channel bed (Figure S5). To save computational time, the domains were subsequently considered differently in the two sets of simulations for plain and patterned surfaces. In both cases, a segment (100 μm wide, 100 μm deep and 150 μm long) of the entire microchannel was taken as a computational domain. For patterned surfaces, 5 μm circular wells were additionally included in the channel bed. The flow condition around a bacterium was simulated by an immersed solid placed in this computational domain (Figure S5).

To model a self-propelled microorganism (an individual cell), both the translational and rotational velocities of the organism were considered. *E. coli* was modelled as a rod-shaped bacterium 2 μm long × 1 μm diameter, with a 10 μm long × 0.5 μm diameter flagellum (tail). The characteristic length was 12 μm, as described in Koch and Subramanian (2011). It was considered to swim parallel to an infinite plain wall at three different distances of 2, 5 and 15 μm from the bottom in static and flowing fluid conditions. The different swimming depths were taken to see the effects of wall distance on bacterial motility. *E. coli* moved with a translational and rotational (flagellar bundle) velocity of 30 μm s⁻¹ and 100 Hz (62.83 radian s⁻¹) respectively, as found by Berg (2004) and Damton et al. (2010). The collective (population) hydrodynamics (in the case of group locomotion) could also influence the flow field substantially (Koch & Subramanian 2011; Guasto et al. 2012), but that aspect was not considered in this simplified modelling. A transient scheme (second-order backward Euler) with an immersed solid solution technique was taken for a total duration of 1 s with 0.01 s time steps. A high resolution scheme (HRS) was selected to solve convection terms of the Navier–Stokes equations. Diffusion terms were solved using shape functions (parametric) defined by ANSYS-CFX.

A grid independence analysis (mesh convergence) was conducted by increasing the number of nodes and calculating the maximum wall shear for all the cases. Figure S6 shows the percentage difference between the maximum wall shear calculated for subsequent refinements (relative error) as a function of the number of nodes. Plots are shown for fluid domains with plain boundaries and immersed solids (model bacterium with self-velocity of 30 μm s⁻¹) at distances of 2, 5 and 15 μm from the bottom. Mesh convergence was assumed satisfied when variation in the maximum wall shear values was < 5%, although a finer mesh was used to have a better description of the complex flow-field around a moving microorganism. Mesh statistics showed that in all cases > 3 million nodes were generated. Mesh elements were mostly hexagonal (94% hexahedrons and 6% prisms). The mesh orthogonal quality was in the range of 0.67–0.99 (acceptable value ≥ 0.1) and skewness was in the range of 3.99E-04–0.6 (acceptable value ≤ 0.8).

### Results

The motility pattern of cells changed with the nature of the nearby solid boundaries (ie plain or patterned). In most cases, video microscopy indicated that *E. coli* cells had more dynamic control over plain surfaces than over patterned surfaces. The trajectory of an individual bacterium, which could be identified over both plain and patterned surface regions, showed that a cell remained for a longer time period over a plain surface than over a patterned surface. Generally it was observed that circular swimming, the pattern described by Lauga et al. (2006), was more common over the plain surface. In contrast, less curved swimming trajectories of cells were observed over the patterned surface.

Still images captured from video clips were analysed for positions and velocities of cells using IMAGEJ software. Firstly, an arbitrarily selected Cartesian coordinate was taken on the starting image (ie image at 0 s) as a reference point and then the following images were analysed in relation to that reference point. For example, in Observation 1 (Cell 1), the initial position (at 0 s) of the observed microorganism was at 36 μm and 60 μm in the *x* and *y* axes, respectively (Figure 2A). All distances were measured from the reference coordinate axes (0, 0) and then plotted into a graph to obtain a motility path line for individual cells (Figure 2B). Following these steps, three different sets of data were generated from three individual cells in each observation. In total, 21 different motility path lines (ie 3 cells × 7 observations) of 21 cells were identified (Table 1).

Residence time on plain and pattern surfaces was averaged from the datasets obtained and expressed as a percentage of the total time (Table 1). Firstly, individual cell residence times were calculated from individual observations. For example, in Observation 1 (Cell 1), the observed individual cell (as shown in Figure 2B) remained for 14 s on the plain surface compared with only 6 s on the patterned surface. So, the residence time was calculated as 75% on the plain surface and 25% on the patterned surface. Similarly, residence times for Cells 2 and 3 of Observation 1 were calculated (Cell 2: 77.44% and 22.56%, Cell 3: 57.27% and 42.73%, time on the plain and patterned surfaces, respectively). Following these steps, the residence time (%) was calculated for all identified cells and the average values were taken.
based on swimming depths and surface patterning. The individual cell velocity was determined from the distance travelled by *E. coli* cells divided by the associated residence time. An average cell velocity of three different cells was obtained from each observation at particular depths for a comparison of cell dynamics over surfaces (see details in Table 1).

Table 1 shows that the mean swimming velocities of cells on the plain surface were lower in all the cases than on the patterned surface. When the swimming depth was higher (~5 μm), the mean difference (8.25 μm s⁻¹) was comparatively lower than that (~5.97 μm s⁻¹) of the surface-close swimming (~1 μm) in relation to the surface patterning. The lowest mean difference (5.71 μm s⁻¹) was observed in the case of cells over patterned surfaces at 1 μm depth compared with plain surfaces at 5 μm depth. The motility patterns were also less curved at a greater distance (~5 μm) above the surface (general observation). For example, a comparison of the motility pattern in Observations 2 and 3 (Cell 1) clearly indicated that at surface-close swimming, *E. coli* showed a circular trajectory (Figure 3A), while at a greater distance (~5 μm) less curved trajectories were observed regardless of the surface pattern (Figure 3B).

Table 1 also shows that the residence time was generally higher on the plain surface than on the patterned surface. Cells remained on plain surfaces for a longer time period than on patterned surfaces (62.52% and 37.48% of the total observed time on plain and patterned surfaces respectively, at ~1 μm swimming depth). Figure 3 shows an example of motility and cell residence time for an individual cell over surfaces. In Observation 2 (Cell 1) the individual cell remained on a plain surface for 16 s compared with 2 s on a patterned surface (Figure 3A). The lower residence time on patterned surfaces indicated that bacteria were out of focus more frequently at some points due to moving to the higher fluid strata.

**Table 1. Comparison of bacterial locomotion and residence time over different surfaces at different swimming depths.**

| Approximate swimming depth | Observation labels | Individual cell velocity (μm s⁻¹) | Mean velocity ± SD (μm s⁻¹) | {Average residence time (%)}* |
|---------------------------|-------------------|-----------------------------------|-----------------------------|--------------------------------|
|                           | Cell 1 | Cell 2 | Cell 3 | Cell 1 | Cell 2 | Cell 3 | Plain | Pattern | Plain | Pattern |
| 1 μm                      | Observation 1   | 9.95  | 18.22 | 11.36 | 26.32  | 31.18 | 17.30 | 17.26 ± 6.85 | 62.52 | 25.51 ± 7.75 | 37.48 |
|                           | Observation 2   | 11.95 | 30.61 | 30.75 | 14.23  | 39.17 | 36.41 | 31.22 ± 6.07 | 60.40 | 37.19 ± 9.82 | 39.60 |
| 5 μm                      | Observation 3   | 32.67 | 29.94 | 42.28 | 41.66  | 32.50 | 50.71 | 31.22 ± 6.07 | 60.40 | 37.19 ± 9.82 | 39.60 |

*The average residence times (%) are shown in the brackets {}.
The mean swimming velocities (as shown in Table 1) of cells were further analysed statistically to find out if any significant difference existed among them. The 2-way ANOVA analysis \((p = 0.05)\) followed by Tukey’s multiple comparisons test showed that the cell velocities were significantly different over a plain surface compared with a patterned surface \((p < 0.05)\) at a distance of 1 μm from the bottom surface (Figure 4A). On the other hand, the cell velocities showed no significant difference at a higher swimming depth (5 μm) in relation to the bottom surface patterns (ie plain vs patterned). This result indicated that the surface pattern can influence the cell dynamics only at a closer distance. Multiple comparisons also showed that cell velocities over the plain surface at a distance of 1 μm were significantly different from cell velocities over the plain \((p < 0.01)\) and patterned \((p < 0.0001)\) surfaces at a distance of 5 μm. This result was consistent with the conventional law of fluid dynamics that fluid velocity increases at a greater distance from the bottom solid boundaries. However, cell velocities over a patterned surface at a distance of 1 μm were not significantly different from cell velocities over a plain surface at a distance of 5 μm. Physically, this means that cells immediately over a patterned surface attained swimming velocities similar to their higher depth swimming.

Another comparison of cell velocity was conducted using parametric t-tests (paired), where all observations were taken into account without considering the
swimming depth separately \((n=21\), values were normally distributed). The test result showed that the cell velocities were significantly different over plain compared with patterned surfaces \((p<0.0001)\). The mean cell velocities were 21.25 (SD: 9.15, SEM: 1.99) \(\mu\text{m s}^{-1}\) and 28.84 (SD: 9.77, SEM: 2.13) \(\mu\text{m s}^{-1}\) on plain and patterned surfaces, respectively (Figure 4B).

From all these results it was clear that the swimming speed of \(E.\ coli\) decreased closer to the surface and increased with an increase in swimming depth. The results also showed that cell velocity over a plain surface could increase in close proximity to the surface while crossing over the pattern due to the presence of a particular type of surface patterning (eg microwells, as used in this study).

**Discussion**

From the experimental results it is evident that the dynamic stability of \(E.\ coli\) shows dependency on surface patterning. Furthermore, the swimming depth (distance from the boundary wall) determines the degree of interaction between microorganisms and solid boundaries. Generally, the surface selection preference of \(E.\ coli\) is based on its ambient microfluidic environment. Studies by Rosehahn and Sendra (2012) and Heydt et al. (2012) showed that microorganisms (algal spores) need to reduce their swimming speed for surface selection and temporary settlement. However, in the case of a patterned surface with a microwell feature, \(E.\ coli\) cells attained higher velocities and periodic microfluidic disturbances while crossing over the microwell array.

In a viscous dominated zone, convection is almost negligible and hence a motile rod-shaped bacterium rotates its body (including the flagellar bundle) continuously for locomotion. Bacterial locomotion in viscous liquid has been described extensively (Ramia et al. 1993; Lauga et al. 2006; Berke et al. 2008; Lauga & Powers 2009; Guasto et al. 2012). From simulations of bacterial motility near a plain surface, it is clear that fluid particles around submerged bodies (\(E.\ coli\)) are driven by viscous forces only. In the case of a patterned surface, kinematic components are more active in the surrounding fluid domain. These components include vorticity (from angular velocity), linear strain rate (due to acceleration/deceleration of the fluid particle) and shear strain rate (due to angular deformation of the fluid particle). The near-surface boundary effect on the velocity field was compared through CFD simulations for a rod-shaped bacterium swimming at a distance 2 \(\mu\text{m}\) (from body centre) on plain and patterned surfaces in both static and dynamic flow conditions (Figure S7). These four cases are further delineated by vector field (Figure 5A–D) and graph (Figure 5E). The velocity vectors varied rapidly around the body of the bacterium modelled, and developed recirculation zones inside the microwells for both static and dynamic conditions (Figure 5B and D). In the case of a plain surface, the velocity vectors were parallel to the locomotion of the bacterium (Figure 5A and C). The local velocity magnitudes at 1 \(\mu\text{m}\) below the cell

![Figure 5](image_url)

**Figure 5.** CFD simulation of velocity vectors around a moving rod-shaped bacterium: (A) over a plain surface in a static flow condition; (B) over D10S07 microwells in a static flow condition; (C) over a plain surface in a dynamic flow condition (D) over D10S07 microwells in a dynamic flow condition. Flow direction from left to right (C–D). (E) Local velocities for the above four cases (values taken at a distance of 1 \(\mu\text{m}\) below from the cell body centre).
body centre were also plotted (Figure 5E) to compare the differences between plain and patterned surfaces for both static and dynamic conditions. The magnitude of local velocity was always higher (maximum 55 μm s\(^{-1}\)) for a patterned surface in dynamic flow conditions. The lowest velocity field (~10 μm s\(^{-1}\)) was experienced by the modelled bacterium in the case of static flow conditions on a plain surface, which seemed a favourable flow field for the cell to control its motility.

Strain rates were also shown for the above four cases in Figure 6A–D and in the graph (Figure 6E). The bacterium modelled experienced the highest strain rates (maximum 20 s\(^{-1}\)) (Figure 6D) from the fluid particles in the case of patterned surfaces under dynamic flow conditions. On the other hand, for a plain surface under static flow conditions, the shear strain rate was below the critical limit (< 10 s\(^{-1}\)) of self-propulsion (Hill et al. 2007) (Figure 6A). This result further emphasised the notion that the dynamics of fluid particles on a plain surface were not adequate to alter the self-propelled trajectory of a motile rod-shaped bacterium such as \textit{E. coli}. In other cases of static and dynamic flow conditions on patterned surfaces, the shear strain rate exceeded the critical limit of 10 s\(^{-1}\) (Figure 6B and D).

The velocity field around rod-shaped bacterial cells showed lower convection very close to the plain surface (Figure 7A). The damping effect of fluid viscosity, which is more pronounced near a solid boundary, restrains fluid particles and microorganisms from higher convections. On the other hand, when the bacterial cells were swimming at higher elevations (5 and 15 μm as shown in Figure 7B and C), the fluid particles around the cells attained higher velocities. The developed velocity fields around the bacteria were less affected by the surface boundaries. At higher elevations, the local velocity around cells increased and the bacteria moved rapidly. In the case of a patterned surface, bacteria experienced higher convection even in close proximity to the boundary as shown in Figure 7D. They were subjected to a rapid change of their swimming depths and moved to upper strata. This phenomenon was also evident from the experiment where \textit{E. coli} was moving to higher positions over the patterned surface and moving out of focus quickly (see also Figure 3). Generally, the fluctuating swimming depths due to the presence of a patterned surface make motile rod-shaped bacteria dynamically unstable. In this situation, even in a nearly static flow condition (eg wall shear stress < 0.01 Pa, as used in this study), the bacteria lost their control over self-propulsion and were thus washed away. Velocity streamlines over a patterned surface showed more perturbations of fluid particles in comparison to a plain surface (Figure S8). This microfluidic disturbance might affect the trajectories of a bacterium and could cause deflection (ie changing swimming depths rapidly) over the patterned surface.

**Effect of microwell array platform edge**

The microwell array used in this study developed an elevated platform (5 μm) on the base planar surface (glass slide). Hence, a question is whether the resulting platform edge would influence the cell dynamics differently.

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Figure 6. CFD simulation of shear strain rates around a moving rod-shaped bacterium: (A) over a plain surface in a static flow condition; (B) over D10S07 microwells in a static flow condition; (C) over a plain surface in a dynamic flow condition; (D) over D10S07 microwells in a dynamic flow condition. Flow direction from left to right (C–D). (E) Shear strain for the above four cases (values taken at a distance of 1 μm below the cell body centre).
from a flush edge microwell array. CFD simulations of the microchannel for these two cases (ie platform edge and flush edge, as shown in Figure 8A and B) showed that fluid particles attained an instantaneous velocity fluctuation at the beginning of the platform edge which dampened immediately after crossing the entry length (~0.3 μm) (Figure 8C). As the flow condition was laminar and the entry length was too small, it was evident that the *E. coli* trajectories were mostly unaffected by the platform edge. However, experiments using holographic particle image velocimetry (HPIV), which allows recording of three-dimensional information of a flow (Heydt et al. 2007, 2009; Weiße et al. 2012), might assess the edge effect on motile microorganisms more precisely. In this study, as the bacterial cell velocities were determined separately for the plain and pattern surface regions, it was possible to exclude the platform edge effect from these cell velocity estimates. Regarding the effect from the top wall, it can be stated that the bottom wall of the microchannel remained unaffected, being a considerable distant apart (~100 μm).

**Microwells vs micropillars**

From the experimental results it was evident that bacterial cells attained higher convections over the microwell...
Various studies (Goldstein & Tuan 1998; Bechert et al. 2000; Friedmann 2010; Weiße et al. 2011) have shown that an alteration in surface topography could increase/decrease the local convection based on the resulting roughness geometry and thus could be successfully applied in drag reduction. Friedmann et al. (2010) showed that riblet-like structures could impede the cross flow and redirect it into the longitudinal direction. Through this redirecting, very slow rotating spirals are formed in the riblet valleys and the particles above the riblets flow away with the stream with higher velocity. The particles in the riblet valleys rotate slowly inside while new particles come and slip over them. Similarly, in this study the depression zones of microwells developed recirculation regions (see Figure 5B and D), which conceivably created effective velocity slip of the fluid particles over them. On the other hand, surface roughness (eg micropillars) which creates a direct obstacle to the longitudinal flow can decrease the streamwise local convection compared to the flat wall case. Weiße et al. (2011) studied the effect of micropillars on the assembly of artificial actin networks using digital in-line holographic microscopy (DIHM). They found that the pillar field (62,500 pillars: 5 μm in diameter, 15 μm in height and 20 μm spacing) caused the deceleration of fluid particles which eventually reduced the velocity of tracer particles in the pillar region compared to the flat wall. A comparison of flow fields over micropillars, microwells and plain surface was conducted by CFD simulation (Figure S9), which showed that the fluid particles at a 1 μm distance from microwells attained higher convection than on a plain surface. Micropillars, on the other hand, reduced particle velocities compared to microwells and plain surface. The increased local velocity over microwells was believed to increase the bacterial cell velocities as found in the experiment.

The development of effective velocity slip with microstructures (recirculating fluid particles inside the depressed regions) can be an important design consideration for fabricating synthetic AF surfaces. The entrapping of air bubbles (plastrons) inside the grooves of the microstructure, for example, also causes effective velocity slip (Derek et al. 2004; Ou et al. 2004; Ou & Rothstein 2005; Hwang & Song 2008) of fluid particles over them. Air entrapment can also reduce attachment points and surface accessibility for the settling microorganisms (Wu et al. 2013). However, the limited underwater stability of plastrons reduces its scope for commercial applications (Poetes et al. 2010). Other design considerations for AF surfaces could be its ability to change the swimming depths of microorganisms and to develop high shear bounded isolated areas. An enclosed pattern (eg circular, rectangular or hexagonal wells) fragments group settlement (gregariousness) by developing high shear bounded zones along the peripheries of the pattern geometries (Halder et al. 2013). Microstructures capable of keeping microorganisms away from the surface, moving them to higher convection zones and giving no shelter against fluctuating microfluidic forces could be considered effective for AF surfaces.

**Conclusion**

This study demonstrated that the dynamic stability of *E. coli* cells depends on the nature of the nearby surface boundaries, swimming depths and ambient fluid flow conditions. Microorganisms over patterned surfaces experience a complex microhydrodynamic environment, which includes differential strain rates, fluctuating velocity distribution and recirculation, and associated force distribution on submerged bodies according to the structural pattern of the boundary. Numerical simulations of a motile rod-shaped bacterium showed that the swimming behaviour could be altered by changing the surface microtopography. As the dynamic stability condition of swimming microorganisms is disrupted, the individual settlement process could be restrained. The characterisation of the swimming pattern of microorganisms over microtopographies is believed to be more complex than over plain surfaces in real-world scenarios, involving a number of interrelated parameters, including species morphology, the presence of other organisms, nutrient supply, surface chemistry, swimming depths and ambient fluid properties. This study explained only the hydromechanical development around an individual motile rod-shaped bacterium in a near-surface microfluidic environment due to the presence of microstructures. In relation to biofouling, it can be stated that microtopographies can alter the dynamic stability of self-propelled microorganisms, which could in turn affect the subsequent stages of biofilm development. However, blocking or delaying the initial stage of biofilm development may not necessarily reduce or eliminate macrofouling, which typically develops later (Mieszkin et al. 2013). The present study was intended to highlight only the transitory AF mechanism of microtopographies in relation to the dynamics of *E. coli*, leaving apart numerous biotic and abiotic factors. In studying the dynamics of microorganisms on different surface patterns, the present approach of numerical simulation is convenient and simple. A bi-directional fluid–structure interaction (FSI) method, which is still in development, could be an enhanced alternative of this current immersed-solid solution technique.
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