# Microfluidics Expanding the Frontiers of Microbial Ecology

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## Abstract

Microfluidics has significantly contributed to the expansion of the frontiers of microbial ecology over the past decade by allowing researchers to observe the behaviors of microbes in highly controlled microenvironments, across scales from a single cell to mixed communities. Spatially and temporally varying distributions of organisms and chemical cues that mimic natural microbial habitats can now be established by exploiting physics at the micrometer scale and by incorporating structures with specific geometries and materials. In this article, we review applications of microfluidics that have resulted in insightful discoveries on fundamental aspects of microbial life, ranging from growth and sensing to cell-cell interactions and population dynamics. We anticipate that this flexible multidisciplinary technology will continue to facilitate discoveries regarding the ecology of microorganisms and help uncover strategies to control microbial processes such as biofilm formation and antibiotic resistance.

## Contents





## INTRODUCTION

Microfluidics is the technology of driving and controlling fluids at the micrometer scale. Initially stemming from microanalytical methods and microelectronic circuits in the early 1990s (119), the field has expanded dramatically in the past decade, largely due to the introduction of an easily accessible fabrication technique, soft lithography, for creating microdevices based on patterned elastomeric polymers. Polydimethylsiloxane (PDMS) is the most commonly used material to date, owing to its transparency, flexibility, gas permeability, chemical inertness, and biocompatibility (116). Because of these developments, microfluidics has revolutionized fundamental and applied research in many fields, from soft-matter physics to chemical engineering, disease diagnostics, and biomedicine. The introduction of microfluidic platforms into the study of microbial ecology is very recent and in many cases still in its infancy, yet existing studies already reveal the great potential of this technology to extend the boundaries of the field (118).

A number of features make microfluidics an appealing technology for microbial studies. As illustrated in **Figure 1**, the ability and flexibility of controlling the microbial environment at the scale of single cells or small populations set microfluidics apart from traditional tools. One can capitalize on the laminar nature of fluid flows at these scales to generate precise spatial gradients of solutes, gases, or temperature. The incorporation of porous materials (e.g., hydrogels, membranes) or nanoscale channels allows for the supply of dissolved nutrients and the removal of soluble waste by diffusion while impeding the passage of microorganisms, thus achieving selective physical confinement without chemically isolating cells. Funnels, obstacles, and barriers can be seamlessly fabricated and used to study the interaction of microbes with boundaries, leading to novel ways of concentrating motile cells. More generally, boundaries can be used to confine populations or

**PDMS:** polydimethylsiloxane

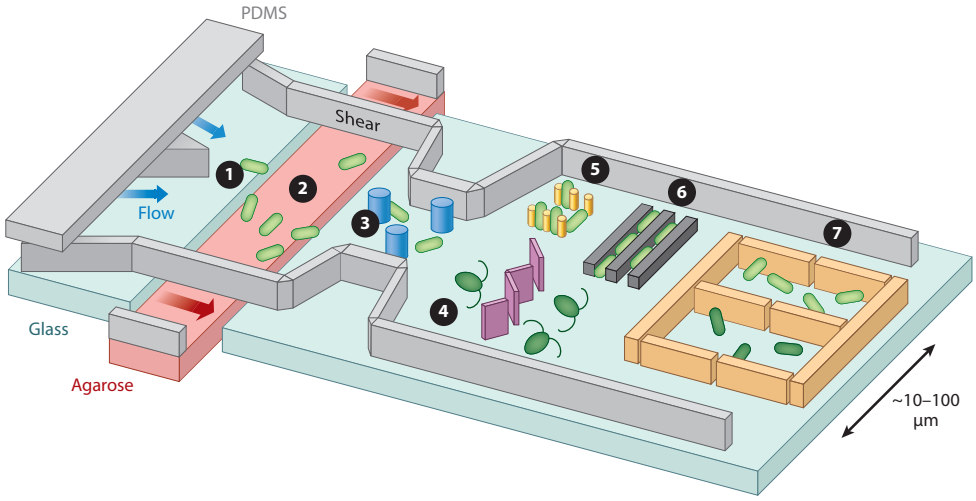


**Figure 1**

Microfluidics provides a powerful platform for microbial ecology studies. Multiple features of natural microbial habitats can be included in microfluidic studies of microbial ecology: ❶ multiple inlets and ❷ hydrogels (e.g., agarose) to impose spatial gradients, ❸ constrictions and topological features to study the effect of flow in porous environments, ❹ funnel-shaped barriers to separate and concentrate swimming microbes, ❺ microfabricated topography to study bacterial adhesion, ❻ single-cell confinements to study growth and replication, ❼ population confinements to investigate competition and population dynamics. Abbreviation: PDMS, polydimethylsiloxane.

individual cells in minuscule liquid volumes, allowing for a wide range of investigations, from short-term competition studies to multigeneration experiments on adaptation and evolution.

In this review, we describe the most recent developments in microfluidics that have opened new doors in microbial ecology, focusing on those studies that combined technical advances with new microbial insights. For reasons of focus, we do not review studies performed using traditional flow chambers, nor do we discuss microfluidic platforms for single-cell screening. For discussions of these subjects, we direct the reader to excellent recent reviews (12, 124). We begin with a discussion of bacterial chemotaxis and, more generally, microbial navigation, likely the earliest and most prolific application of microfluidics to the study of bacterial behavior. We then review microfluidic examples of how aspects of the external environment, such as fluid flow, can modify microbial motility, as well as how motility, in turn, can modify the transport properties of chemicals in the surrounding fluid. Next, we discuss transient and permanent interactions of microbes with solid boundaries, a pervasive feature in the life of many microbes and exceedingly fertile ground for microfluidic investigations. After an excursion into new microfluidic tools developed to explore and control cell growth, we focus on the microfluidic confinement of bacterial populations, which has allowed original studies on the interactions within and among microbial communities. We conclude by presenting key applications of microfluidics in the field of antibiotic resistance.

## MICROBIAL NAVIGATION

Microfluidics has changed the way we study the motility of microbes and their response to cues from the environment. Chemotaxis, the best-studied form of microbial navigation, has received considerable attention in the microfluidic literature, and its study offers a blueprint for

**Chemotaxis:** ability of an organism to sense a chemical gradient and bias its motility accordingly

the rich gamut of microbial navigation strategies, including aerotaxis, thermotaxis, galvanotaxis, and magnetotaxis, to name but a few. Microfluidics is ideally suited for such studies because it affords accuracy, flexibility, and control in creating gradients at scales relevant to microbes (hundreds of micrometers). These gradients can be steady or unsteady, linear or nonlinear, single or multiple, and, owing to the precision in the design and fabrication of microfluidic systems and the laminar nature of flow in microchannels, are straightforward to predict mathematically and easy to quantify experimentally, for example, using fluorescent dyes. The transparency of the polymers used to fabricate microchannels further allows for direct, quantitative observation of microbial responses to gradients at the level of single cells using video microscopy, automated image analysis and cell tracking. These advantages offered by microfluidics over prior methods have already been reviewed extensively (4). Here, we focus on the impact that microfluidics has had on the study of microbial navigation and highlight the most recent developments in this field.



## Chemical Attraction

Bacterial chemotaxis is one of the best-studied biological sensory systems because it is highly tractable at the molecular level and plays a fundamental role in a broad range of ecological and environmental processes, including trophic interactions, chemical cycling in natural environments, and the onset of disease. The earliest microfluidic gradient generator for bacterial chemotaxis was proposed by Mao et al. (75). Their three-inlet parallel-flow device (see also Reference 30) was based on the confluence of three streams joining into a single microchannel in a T-shaped configuration. Bacteria flowing within the middle stream could swim toward either of the two flanking streams, one containing a chemoeffector, and the other containing buffer. Chemotaxis was assessed as the preferential movement of cells toward the chemoeffector stream. Using this approach, Mao et al. (75) demonstrated that the chemotactic sensitivity of *Escherichia coli* was considerably higher (1,000-fold) than measured by traditional capillary-based chemotaxis assays, and the cells responded to amino acid concentrations as small as a few nanomolar. Kim et al. (59) recently proposed an interesting variant of this method by including arrowhead-shaped nooks along the microchannel sidewalls to concentrate bacteria, thereby magnifying the readout of the chemotactic response.

When gradients evolve by diffusion in the absence of flow, single cells are considerably easier to follow, allowing one to probe fundamental properties of chemotaxis. By tracking *E. coli* within an unsteady gradient of $\alpha$-methylaspartate, Ahmed & Stocker (5) obtained a detailed map of this organism's chemotactic velocity. The gradient, quantified using a fluorescent dye, was established by filling a side channel with a chemoattractant solution that gradually diffused out into a perpendicular main channel containing a flowing suspension of bacteria. The resulting unsteady gradient was used to assess the chemotactic velocity as a function of the local, instantaneous concentration and the gradient of chemoattractant. This work revealed that *E. coli* can achieve chemotactic velocities as high as 35% of their swimming speed, more than double the typical literature values of 5–15%, and it verified continuum models of bacterial transport based on single-cell swimming information. Unsteady gradients can also be created by the bacteria themselves through consumption of nutrients in the medium. Saragosti et al. (93, 94) explored this case by first confining bacteria in a narrow region at one extremity of a microfluidic channel using centrifugation and by then observing dense waves of bacteria migrating along the channel (**Figure 2*a***). Tracking of individual fluorescently labeled cells within the migrating front not only confirmed that their mean run length was longer in the direction of propagation, as is typical in *E. coli* chemotaxis, but also revealed that their tumbling direction was skewed in the direction of the gradient (94).

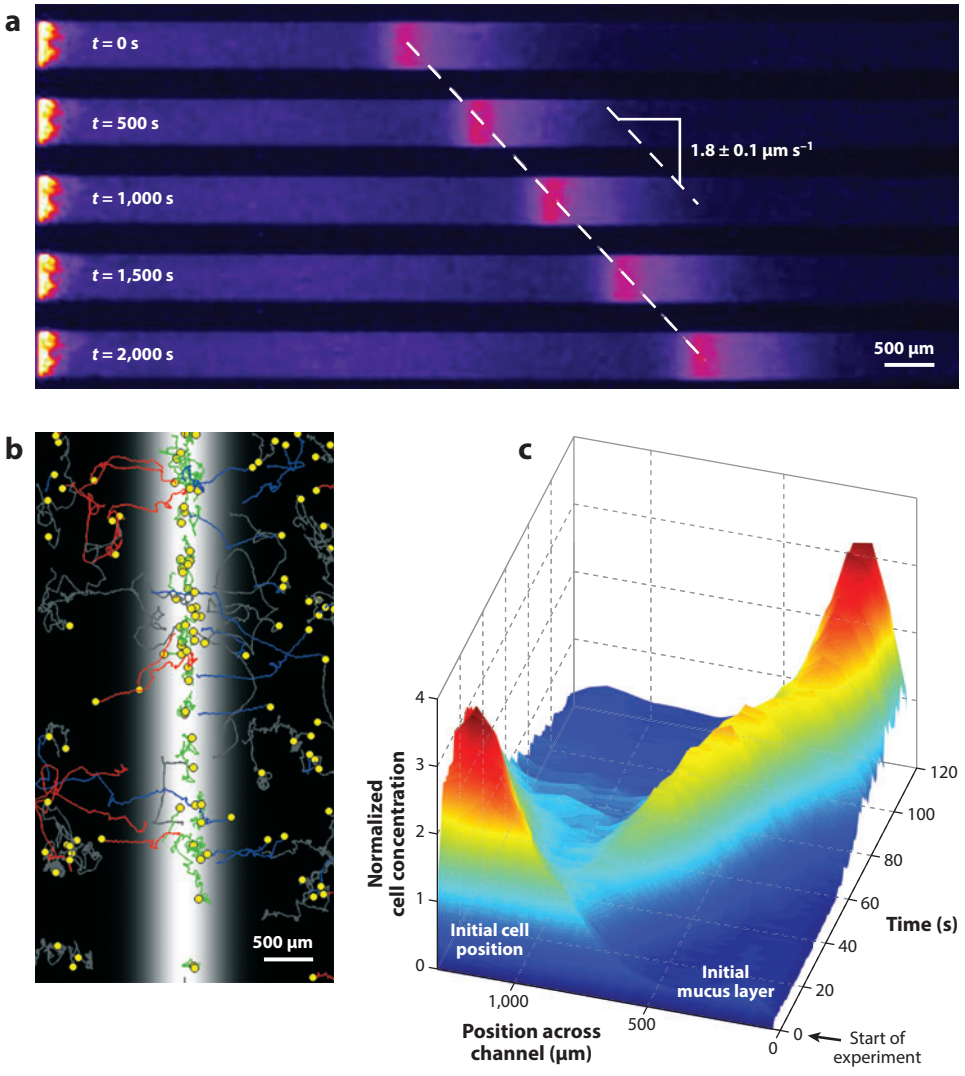


**Figure 2**

Chemical gradients reveal microbial navigation strategies. (*a*) Waves of *Escherichia coli* traveling across a microfluidic channel at a constant propagation speed. Wave crests represent high cell concentrations. Modified with permission from Reference 93. (*b*) Swimming trajectories of the dinoflagellate *Oxyrrhis marina* in response to a pulse of chemoattractant (grayscale background). Trajectories are color-coded as follows: blue and red, swimming left and right, respectively, at >85 μm $s^{-1}$; green, trajectories fully within the initial chemoattractant pulse; gray, all others. Modified with permission from Reference 99. (*c*) Time evolution of the spatial distribution of a pathogen population (*Vibrio coralliilyticus*) exposed to a diffusing gradient of coral mucus (*Pocillopora damicornis*) in a microfluidic channel. Modified with permission from Reference 35.

This previously unknown modulation of reorientations, resulting from the different number of flagellar motors undergoing a change in the direction of rotation that triggers tumbling, provides an additional, moderate increase in the chemotactic velocity.

Although self-generated or self-evolving gradients afford numerous possibilities to learn about chemotaxis, the ability to directly control gradients affords the most robust microfluidic approaches

to chemotaxis. A simple way to achieve a steady gradient is to connect two large reservoirs through a small test channel, resulting in a linear concentration profile within that channel. Masson et al. (82) recently adopted this method to track swimming *E. coli* cells and to infer their impulse-response function (the cellular response to a pulse of chemoeffector, which can be used to infer responses to more complex gradients), without needing to tether cells to surfaces as was traditionally done. One downside of this approach is the potential for fluid flow through the test section, as precise pressure equalization is challenging. The same principle can be applied with considerably more control by incorporating in the fabrication porous materials that are permeable to molecular diffusion but not to fluid flow. Such materials may be used to separate the test channel from flanking microchannels used as chemoattractant reservoirs (source and sink). Regulating the concentrations in the reservoirs affords complete control over the gradient that microbes experience in the test channel, allowing for long-term observations in steady gradients or for the temporal modulation of gradients without any confounding effects due to flow. This concept was proposed by Diao et al. (25), who used a nitrocellulose membrane, which Cheng et al. (14) later replaced with the hydrogel agarose. Ahmed et al. (3) provided a detailed characterization of the gradients by confocal microscopy. Agarose can be accurately molded, improving the fabrication of the diffusion-permeable barriers, and its transparency is ideal for microscopy. Kalinin et al. (49) subsequently adopted the device from Cheng et al. (14) and used it to quantify the steady-state distribution of *E. coli* in linear chemoattractant concentration profiles. Exploiting the device's ability to independently control the mean concentration and the gradient, they demonstrated that *E. coli* senses the logarithm of the chemoattractant concentration, a property known as log-sensing. Lazova et al. (67) used an agarose-based microfluidic device to further demonstrate that *E. coli*'s chemotactic response is invariant with respect to the intensity of the chemoattractant gradient when cells are adapted to a proportionally rescaled background concentration over a broad dynamic range, a strategy known as fold-change detection.

Suitable microchannel arrangement further allows one to establish concentration profiles of arbitrary shape (3, 120) and to overcome the otherwise long timescale for gradient establishment by exploiting fluid flow in a compartment immediately adjacent to the test channel (63). In addition, perfusion from reservoirs through permeable substrates can also be used to establish multiple simultaneous chemoeffector gradients or to modulate gradients over time. Kalinin et al. (48) exposed *E. coli* to opposing gradients of two amino acids, revealing that the chemotactic preference of *E. coli* depends on the ratio of the expression of the two receptors, which in turn varies with growth conditions. By varying the frequency of an alternated release of chemoattractant, Zhu et al. (126) demonstrated that *E. coli* could follow the resulting chemoattractant gradient and oscillate in synchrony with the environment at low driving frequencies. However, they lost the tune and began oscillating out of phase in fast-changing environments owing to their finite adaptation time.

## Aerotaxis and Thermotaxis

A special case of chemotaxis, from the point of view of gradient generation, is aerotaxis (taxis toward oxygen). In this case, one can take advantage of the gas permeability of PDMS to create steady oxygen gradients without the need for hydrogels or membranes (2). This principle was used by Adler et al. (1), who studied aerotaxis in *E. coli* under steady, linear profiles of oxygen ranging from microaerobic to aerobic conditions and showed that it does not obey log-sensing. Furthermore, the irreversible bonding that can be achieved between layers of PDMS, but not with hydrogels, makes aerotaxis an ideal model system for the study of microbial navigation in more realistic environments, such as when fluid flow is present.

Temperature gradients have also been created within microfluidic devices and have been used to study thermotaxis and thermokinesis. One method relied on a Peltier device, together with circulation channels for hot and cold water, and on diffusion of heat through PDMS to generate a temperature gradient, which was visualized using a temperature-sensitive fluorescent dye (23, 92). Salman et al. (92) used this approach to demonstrate a subtle interplay between thermotaxis, metabolism, and chemotaxis in *E. coli*: When cells accumulate at their preferred temperature by thermotaxis, locally enhanced nutrient consumption produces a nutrient gradient that opposes the temperature gradient, causing the bacteria to migrate toward the lower temperature. Using the same approach, Demir & Salman (24) demonstrated a further response of *E. coli* to temperature gradients: thermokinesis. They showed that under low nutrient conditions, higher temperatures cause decreased intracellular pH, which in turn diminishes swimming speed, and ultimately results in a slow migration toward warmer water.



## In Sea and Soil

One group of organisms for which microfluidics has enabled a range of new insights is that of marine microbes. Their nutrient sources often come as ephemeral, submillimeter patches, which dwindle by diffusion. The utilization of these patches by microbes has been challenging to study, given the scales of traditional oceanographic approaches, but it can be conveniently investigated using microfluidics. Seymour et al. (96) devised a microinjector system to model the appearance and diffusion of a nutrient patch. In their device, a narrow band of chemoattractants was injected into a wider test microchannel. This approach has been successfully employed to quantify the microbial response by video microscopy in a range of marine processes. Stocker et al. (105) showed that the chemotactic response of the marine bacterium *Pseudoalteromonas haloplanktis* can be an order of magnitude faster than that of *E. coli*, suggesting that marine microbes are well adapted to exploiting small, ephemeral nutrient sources. Strong chemotactic responses have also been found among protists and phytoplankton, indicating that microbial trophic interactions in the ocean may occur primarily within microscale hotspots (99). The ability to produce realistic microscale nutrient pulses has further enabled the study of marine microbial responses to organic matter exuded by photosynthetic phytoplankton (95, 98); to mucus produced by corals (35); and to sulfur compounds, including the climatically active gas dimethylsulfide (97) (**Figure 2*b*,*c***).

Stocker et al. (105) used a modified version of the microinjector to simulate the nutrient plume in the wake of a settling marine particle. The particle was represented as a small PDMS cylinder, which was situated in front of the microinjector so as to turn the band into a plume. Experiments revealed that *P. haloplanktis* enhanced their exposure to nutrients by 400% over non-motile bacteria through chemotaxis in the nutrient plume of slowly sinking particles. In a different device, the addition of agarose surfaces as models for the surfaces of marine particles recently allowed Yawata et al. (123) to study the interplay between chemotaxis and surface attachment. Their results revealed a subtle competition-dispersal trade-off as the origin of the ecological differentiation among two closely related bacterial populations within the genus *Vibrio*.

The ability to fabricate complex topographies in microfluidic devices lends itself well to investigations of microbial navigation in highly structured environments such as soil and porous media. One can produce microchannels that replicate key features of porous media, for example, by using a collection of cylindrical pillars to mimic sand grains and the pores between them; these may have regular (71) or random (28) arrangements. Using the basic principle of the T-sensor—namely, injecting parallel streams of bacteria and chemoattractants—Long & Ford (71)

found that flow enhanced chemotactic migration of *E. coli* in porous media. Using a similar device, Singh & Olson (102) quantified the chemotactic migration of *E. coli* from high- to low-permeability regions, modeled experimentally as areas with and without smaller cylinders amid a regular array of larger cylinders.

**Shear rate:** spatial gradient in fluid velocity; the shear stress is obtained from the shear rate by multiplying it by the dynamic viscosity of the fluid

These microfluidic experiments on microbial chemotaxis in sea and soil provide a blueprint for the microfluidic investigation of microbial processes in other natural environments. The flexibility of investigating multiple environments within scenarios that closely mimic natural conditions suggests that a broad range of additional microbial navigation processes in diverse microbial habitats will benefit from microfluidic approaches.

## EVERYTHING FLOWS

Many microbial habitats are liquid environments characterized by frequent fluid flow. This flow can thus be a prevalent influence on microbial ecology (38). Bacteria and phytoplankton consistently experience turbulent flow in oceans and rivers, soil microorganisms are exposed to creeping groundwater flows, and human microbiota are subject to fluid flow in the digestive, respiratory, and urinary systems. Flow plays an important role in a broad variety of microbial processes, including nutrient uptake and cell encounter rates, as well as in many industrial applications ranging from wastewater treatment to seawater desalination and the production of biofuels. Despite its importance, the effect of flow on microorganisms has been investigated almost exclusively from a theoretical perspective (70, 109), largely owing to the experimental difficulties with visualizing microbial responses in accurately controlled flowing environments.

Microfluidics greatly enhances the ability to study the effect of flow on microbes by allowing one to create controlled flows over a wide range of flow speeds and shear rates and to observe microbial responses by direct imaging. Marcos et al. (76) used shallow microfluidic channels to show that shear induces a lateral drift across streamlines for non-motile, helically shaped bacteria (*Leptospira biflexa*) owing to their chiral morphology. These authors exploited the precise flow field in a straight microchannel to image bacteria at different depths: The linear variation of the shear rate over the channel depth enabled them to validate the theoretical prediction that drift velocity is linearly proportional to shear rate. Marcos et al. (77) used a similar device to observe the effect of shear on motile bacteria (*Bacillus subtilis*): The ability to precisely quantify the cells' cross-streamline migration over a serpentine channel that was more than one meter long allowed these researchers to demonstrate rheotaxis, a bias in swimming direction caused by the coupling between the fluid shear and the chirality of the bacterial flagellum. A different effect of flow on motile bacteria was recently discovered through the use of tall microchannels (i.e., channels that were deeper than they were wide), where the dominant shear was in the horizontal plane of observation. Using such a setup, Rusconi et al. (89) observed a fast, shear-trapping effect whereby bacteria strongly accumulate in the high-shear regions of the flow. By moving the microscope stage in synchrony with the average flow speed, these authors tracked individual bacteria in flow and demonstrated that trapping originated from the fluid shear rotating the cells in what are known as Jeffery orbits.

Fluid flows that can be generated in microchannels go beyond unidirectional flows and offer great flexibility in creating spatially varying flow fields such as extensional flows, hyperbolic flows, and vortex flows (44). Marcos & Stocker (78) created vortices by using a straight microchannel with a cavity on one side such that flow in the main channel drives a vortical recirculation in the cavity. This setup allowed them to study the effect on marine microbes of vortices of similar size to those found in marine turbulence and to observe the preferential alignment of elongated microbes with streamlines that had been predicted theoretically.

In natural environments, flow often occurs simultaneously with other ambient cues. This scenario is highly conducive to microfluidic analysis. For example, Garcia et al. (34) investigated the phototactic response (i.e., migration in response to light) of suspensions of the alga *Chlamydomonas reinhardtii* in a Poiseuille flow, showing that self-focusing near the channel center and migration toward the channel walls could be reversibly switched by changing the position of the light source from upstream to downstream and vice versa. Rusconi et al. (89) studied the aerotactic response of the bacteria *B. subtilis* in a Poiseuille flow, showing that the trapping caused by shear had a strong quenching effect on aerotaxis. The unexpected interactions among cues revealed by these studies, together with the plethora of cue combinations that microbes experience in their natural environments, highlight the great potential that remains to be exploited in this field.

## TINY AGITATORS

The swimming motion of microbes themselves can generate flows that enhance mixing or can be harnessed to transport objects. Kim & Breuer (60) studied the effect of bacterial motility on the diffusion of a tracer molecule using a two-inlet microfluidic channel in which transport between two adjacent streams is due solely to diffusion, when bacteria are absent. They observed that the diffusivity of dextran was enhanced by up to eightfold when *E. coli* were added and that the enhancement increased linearly with bacterial concentration. Other studies have used a modification of this approach in which bacteria were bound to the surfaces of a microchannel via their cell bodies so that the rotation of their flagella mixed the fluid (61) or pumped it along the device (62). The performance of these so-called bacterial carpets can be tuned by modulating bacterial motility through chemical stimuli or temperature.

At high cell concentrations, collective motions can not only enhance mixing, but also modify the bulk fluid properties. Gachelin et al. (32) used a two-inlet microchannel to quantify the rheological properties of concentrated suspensions of *E. coli* by measuring the position of the interface between the two streams, one with bacteria and one with the suspending fluid. The ratio of the widths of the two streams yielded the ratio of the viscosities of the two fluids. This method revealed the presence of a shear-thickening behavior (an increase in viscosity with increasing shear) at low shear rates and a shear-thinning behavior (a decrease in viscosity with increasing shear) at high shear rates.

Microbes in microchannels have also been put to work as micro-oxen (80). Weibel et al. (117) demonstrated that the alga *C. reinhardtii*, guided by phototaxis, can push microbeads through microchannels. Similar effects were reported for the bacterium *Serratia marcescens* when exposed to a chemoattractant gradient (56) and for the gliding bacterium *Mycoplasma mobile* while moving along asymmetrically patterned substrates (41). Microfabricated gears decorated with asymmetric teeth can be made to rotate using the same process (103). Although these observations remain to be integrated into applications, they show that microfluidics can be used not only to understand but also to control and exploit microbes.

## A WORLD WITH BOUNDARIES

Given the pervasive presence of surfaces in microbial habitats, how microbes interact with boundaries is a fundamental issue in microbial ecology. Surfaces may act as physical barriers that limit motility and dispersal, as nutrient hotspots in resource-limited environments, or as substrates that cells colonize and infect. Although surface interactions play crucial roles in a wide range of ecological, medical, and industrial processes, including biofouling, fertilization, infection, cell sorting,

and bioremediation, the biophysical mechanisms governing microbe-surface interactions remain largely unexplored. Once more, microfluidics provides a broad set of experimental platforms to obtain deeper insights on the effects of surfaces on microbes.

**Ratchet:** device or mechanism that favors motion in one direction of an otherwise isotropically moving organism

## Hitting a Wall

Exquisite control over geometry provides a means to direct and collect microbes in microfluidic devices. For example, bacteria near surfaces swim in circular trajectories, and they experience a surface-attracting force resulting from the counter-rotating motion and differential drag of the cell body and flagella (66). *E. coli* cells that were confined between an agar surface and a PDMS surface in microchannels only slightly taller than the width of one bacterium preferentially swam along one sidewall, "driving on the right" (26). Their behavior was a result of circular swimming and the lower drag near the porous agar surface. Related hydrodynamic considerations explain the pattern of swimming upstream that *E. coli* cells demonstrate near surfaces in the presence of fluid flow (53).

Appropriately arranged microstructures result in the directional motion of swimming microbes (termed rectification)—in a microfluidic analog of Maxwell's demon—and thus in the possibility of concentrating them (**Figure 3*a***). The best-studied arrangement is the so-called funnel wall, a line of wedge-shaped barriers with spaces between them through which microbes can swim. Rectification occurs because the probability of crossing the funnel wall is larger in one direction than the other. Galajda et al. (33) used a series of funnel walls to concentrate motile bacteria and to separate them from non-motile ones (**Figure 3*b***). Concentration also occurs in the presence of flow (7) and can be destroyed by chemotaxis, for example, when cells create their own gradient through nutrient consumption and, by following it, escape from the trap (65). Funnel walls allowed Kantsler et al. (50) to show that the surface scattering of *Chlamydomonas* algae is governed by steric interactions between the algaes' flagella and the surface, not by far-field hydrodynamic interactions, as was previously believed. Taking advantage of the ease of microfabricating multiple geometric configurations, these authors further optimized the funnel geometry for maximal rectification, thereby providing a blueprint for the concentration, separation, and eventual collection of different microorganisms (**Figure 3*c***).

Microstructures have further been used to sort motile bacteria by length. Hulme et al. (45) took advantage of the "swimming on the right" behavior observed in *E. coli* to create a continuous, unidirectional movement of cells in a system of arrowhead-shaped ratchets (**Figure 3*a***). By alternating ratcheting sections with sorting junctions, these authors designed a system that exploited the correlation between the cell length and the radius of curvature of the cells' circular trajectories (26). In doing so, they successfully enriched a population of short, and thus young, cells, a promising step toward obtaining synchronized cells to study genetic and behavioral changes across generations.

## Flatlanders

Microbes can attach to nearby surfaces through a complex process that involves electrostatic, van der Waals, and hydrophobic interactions and that is mediated by appendages such as pili, fimbriae, and flagella. Despite much attention and broad applicability, the mechanisms governing microbial adhesion to surfaces remain poorly understood.

The small dimensions of microfluidic channels allow one to study surface adhesion under a wide range of shear forces and to monitor the attachment of individual cells. Accurate control of flow conditions enabled Lecuyer et al. (68) to show that increased shear stress diminished the number of

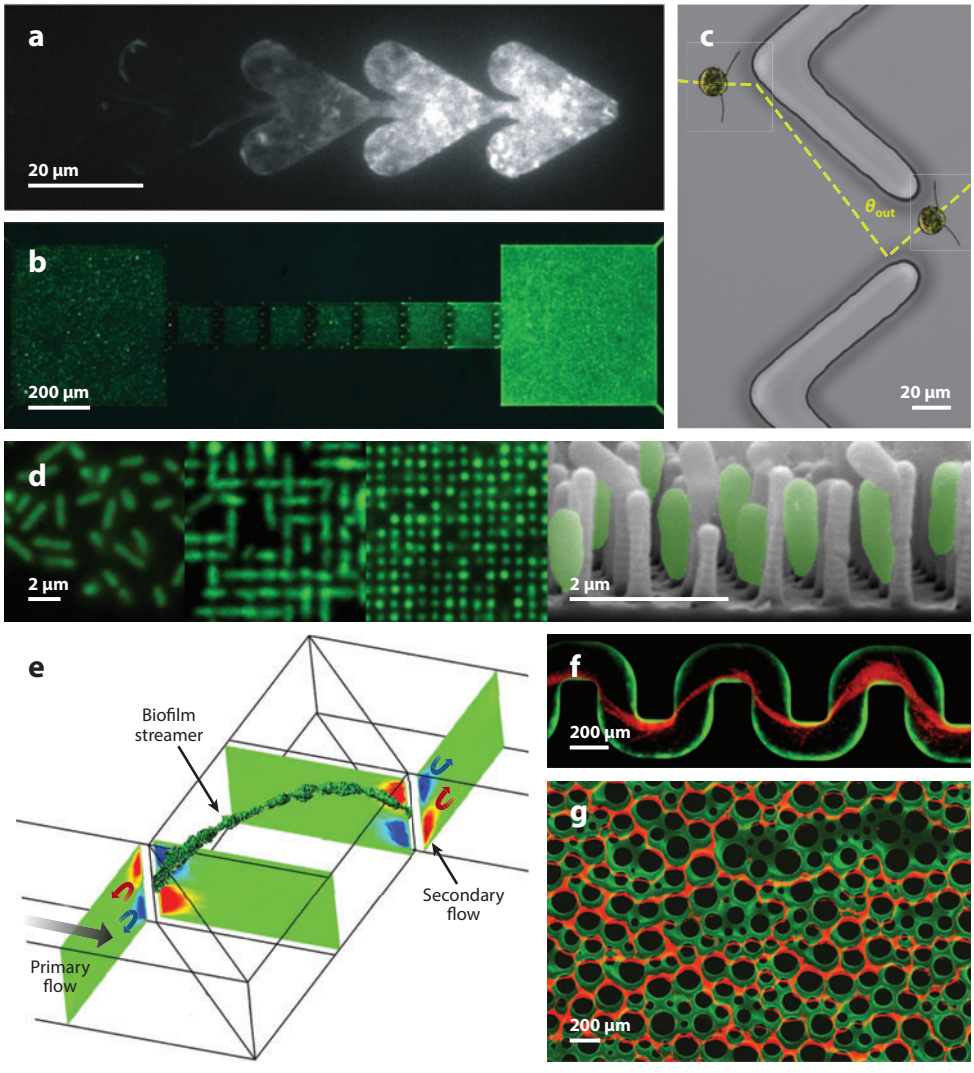


**Figure 3**

Fine control over microscale topography sheds light on microbe-surface interactions. (*a*) Fluorescent *Escherichia coli* bacteria trapped and concentrated in arrow-shaped microfluidic ratchets. Modified with permission from Reference 45. (*b*) Series of funnel arrays used to separate motile from non-motile *E. coli* cells and to concentrate the motile fraction. Modified with permission from Reference 33. (*c*) Rectification of the locomotion of *Chlamydomonas reinhardtii* algae in microfluidic ratchets via secondary scattering. Modified with permission from Reference 50. (*d*) Fluorescent images of *Pseudomonas aeruginosa* bacteria adhering to structured surfaces; spacing between posts decreases from left to right. (*d*) Cross-sectional scanning electron microscopy (SEM) image of the same process. Cells are false-colored to highlight their orientation. Modified with permission from Reference 42. (*e*) Schematic visualization of the mechanism underlying the formation of *P. aeruginosa* biofilm streamers (rendering from confocal images) in curved microchannels. Modified with permission from Reference 90. (*f*) *P. aeruginosa* biofilm streamers highlighted by the subsequent injection of red-fluorescent cells. Modified with permission from Reference 27. (*g*) Patterns of biofilm growth (*E. coli* autofluorescence; *green*) and fluid flow (*red*) in a microfluidic device modeling a porous soil environment. The fluid flow signal (*red*) is slightly shifted to the bottom to better reveal the pattern of growth (*green*). Modified with permission from Reference 28.

*P. aeruginosa* cells attached to a surface but increased their residence time on the surface regardless of the presence of surface appendages. De La Fuente et al. (22) used microfluidics to study the role of pili in anchoring bacteria to the substrate for the bacterial plant pathogen *Xylella fastidiosa*. This anchoring can result in cells being flipped by flow and consequently walking upstream (84, 100). Adhesion can be further studied by taking advantage of microcontact printing, a microfluidic technique to spatially control surface chemistry. For example, Holz et al. (43) used PDMS stamps to print bovine serum albumin (BSA) patches onto glass slides and filled the surrounding surface with fluid-like lipid membranes to study the transition between spreading and clustering in the human pathogens *Neisseria gonorrhoeae* and *N. meningitidis*.

**Extracellular polymeric substances (EPS):** "slime" produced by surface-attached cells for adhesion and protection

**Biofilm streamers:** tethered filamentous aggregations of bacteria held together by the extracellular polymeric substances they secrete

Beyond surface chemistry, microfabrication allows accurate control of surface topography at the scale of individual microbes. Given that the effects of nanometer-sized structures on bacterial surface adhesion have been reviewed elsewhere (8), we only briefly highlight the capabilities offered by surface micropatterning. Hochbaum & Aizenberg (42) fabricated arrays of high-aspect-ratio polymer posts to study the growth of *P. aeruginosa* on structured surfaces. Cells spontaneously assembled into patterns dictated by the spacing between neighboring posts. As the spacing approached the size of the cells, the orientational and positional order were enhanced such that bacteria eventually became aligned along the length of the posts (**Figure 3*d***). This assembly phenomenon was likely driven by the cells' tendency to maximize their contact area with the surface, a hypothesis supported by experiments showing reduced adhesion upon the addition of a microtexture that minimized cell-surface contact area (51). Further work in this area could result in the development of materials with controlled surface topography and could have broad implications for antibiofouling and other biomedical and industrial applications.

## Hanging On by a Thread

A characteristic consequence of microbial attachment to surfaces is the formation of biofilms, surface-attached communities encased in a self-secreted matrix of extracellular polymeric substances (EPS). Bacteria within biofilms have higher chances of resisting chemical insults and phagocytosis by immune system cells than do their planktonic counterparts. The resulting difficulty in combating biofilms makes them a major problem in medicine and industry, where they reduce the lifetime of prosthetic devices, cause chronic infections, and enhance drag and corrosion in pipelines.

Microfluidics has enabled biofilm studies to go beyond flow chambers and to address, in particular, the effect of topographical features (e.g., porous media). Much attention has been devoted to biofilm streamers. These bacterial structures, frequently found in natural and artificial systems, result from the coupling of complex topography and fluid flow and are responsible for increased uptake of organic molecules (11) and membrane biofouling (113). Fabrication of curved channels, coupled with time-lapse confocal microscopy, allowed Rusconi et al. (91) to demonstrate that *P. aeruginosa* streamers developed where the channel curvature induces secondary, vortical flows that drive the accumulation and extrusion of the EPS that form their backbone (**Figure 3*e*,*f***). The sharper the curvature of the channel, the more quickly the streamers develop (39, 90). By capturing floating biomass, the sieve-like streamers caused rapid microchannel clogging (27). Similar streamers were observed when vortical flows were produced by the oscillation of a bubble trapped in a horseshoe microstructure and driven by a radio frequency signal (122). These dynamics have also been investigated in more complex geometries, such as those modeling porous media environments (64, 81, 110), underscoring the potential of microfluidics to mimic multiple components of natural environments in which topographic complexity and fluid flow are important.

## GROWING UP

Microfluidics provides unique opportunities to study and control the growth of microbes with unprecedented resolution by allowing researchers to fine-tune environmental conditions and spatial confinement. This capability has already enabled new insights into important biological and ecological processes ranging from cell morphology during replication to the control of long-term population dynamics to culturing previously unculturable or rare populations. Takeuchi et al. (108) trapped nonseptating *E. coli* in individual microchambers, which were made of agarose to keep cells hydrated and supplied with nutrients, and demonstrated that cells could bend during growth to adopt the shape of the chamber. When released from confinement, the cells retained both the shape of the chamber and their motility (although shape markedly affected swimming).

Under more extreme confinement conditions, bacteria can lose motility yet still traverse constrictions by growth and division. This phenomenon was demonstrated by Männik et al. (73), who fabricated microfluidic chambers connected by channels for which one dimension was as narrow as a few hundred nanometers. Even when flattened considerably, *E. coli* cells were able to grow and divide within these channels such that the lineage of a single ancestor penetrated through constrictions as narrow as half the cell diameter (**Figure 4*c***). The shapes and sizes of bacteria exiting

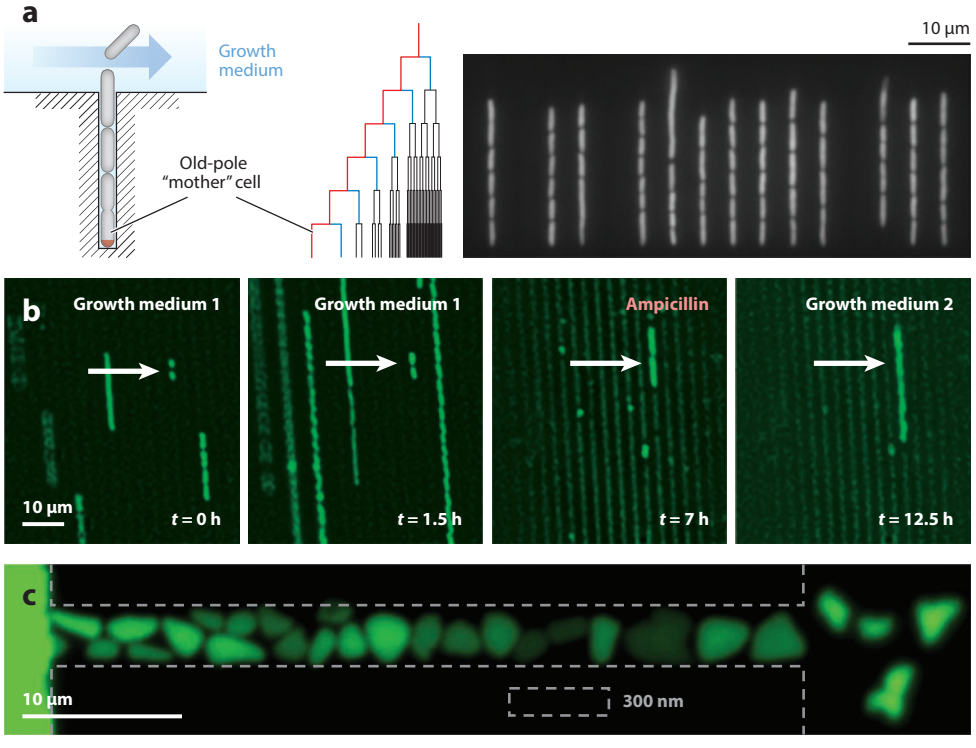


**Figure 4**

Single-cell microconfinement opens new doors for understanding growth and persistence. (*a*) Schematic representation, lineage tree, and snapshot of the microfluidic "mother machine." Modified with permission from Reference 115. (*b*) Time-lapse images of a high-persistence mutant of *Escherichia coli* growing in a microfluidic chamber and exposed to the antibiotic ampicillin, showing the location of slow-growing persisters (*arrows*). Modified with permission from Reference 9. (*c*) Morphogenesis of *E. coli* in ultrathin microfluidic channels; dashed lines show approximate channel boundaries. Modified with permission from Reference 73.

the constriction were substantially different from their regular shape, which, along with motility, was recovered after a few days. This remarkable plasticity of *E. coli* suggested that cell morphology is mostly inconsequential for the accurate partitioning of cell volume during division (74). These studies highlight the power of microfabrication for understanding the role of mechanical stress on bacterial growth and morphogenesis and suggest that submicrometer pores may be more prolific bacterial habitats than previously thought.

Similar channel-design principles have helped the study of long-term growth and division patterns in bacteria. Wang et al. (115) used a high-throughput microfluidic device—called the mother machine—to follow the growth of single *E. coli* cells for hundreds of generations. The device consists of a series of growth channels, each of which is sufficiently narrow to prevent movement of the mother cell (**Figure 4*a***). These channels are connected at the distal end to a trench channel used to replenish nutrients and remove emerging daughter cells. The authors observed that the growth rate of mother cells remained remarkably constant with increasing replicative age, in contrast to previous evidence (104), although the apparent conflict may vanish upon accounting for the partitioning of nongenetic damage across the two cell poles (88). Long et al. (72) devised a modified mother machine with narrower, open-ended growth channels that enables faster loading of cells, faster replenishment of media, and greater immobilization for subdiffractive intracellular measurements. This device allowed these authors to probe the viscoelastic properties of the nucleoid by tracking the movement of fluorescently tagged chromosomal loci. Siegal-Gaskins & Crosson (101) used a different approach to multigenerational studies. They exploited the robust adhesion of *Caulobacter crescentus* bacteria to surfaces for long-term imaging and showed that growth rates were significantly correlated between mother and daughter cells, providing evidence of epigenetic inheritance of cell-division behavior.

Integration of additional microfluidic elements, including peristaltic pumps and micromechanical valves to supply media, remove waste, and recover cells, has enabled the long-term culturing of extremely small populations of bacteria in microfluidic chemostats. Balagaddé et al. (10) used microchemostats to study the planktonic growth of *E. coli*, and they prevented biofilm formation by alternating circulation and cleaning cycles to eliminate adherent cells. By monitoring individual cells carrying a synthetic genetic circuit, these authors observed greater stability in the programmed behavior compared with that of macroscale batch cultures, as well as a correlation between oscillations in cell density and specific cell morphologies. Groisman et al. (37) devised a microfluidic chemostat (and thermostat) made of shallow chambers to monitor bacterial growth. Their device used auxiliary channels, connected to the chambers via submicrometer conduits, to continuously supply fresh media and remove metabolic waste by diffusion while the conduits' small dimensions prevented cell escape. The compliance of PDMS was ingeniously exploited to load cells into the chambers by temporarily inflating the conduits through an increase in pressure inside the device.

The ability to use extremely reduced volumes or to recreate specific microenvironments, in conjunction with direct visualization and parallelization, has been exploited to isolate unculturable microbes and to grow cells that could not be cultured with traditional methods. Marcy et al. (79) identified a rare subset of microbes from the human mouth by isolating cells with a rod-like morphology, amplifying their genome, and performing high-throughput sequencing on a microfluidic chip. By combining microfluidics with cell confinement in aqueous plugs surrounded by an immiscible fluid, Liu et al. (69) succeeded in isolating and cultivating slow-growing cells from mixed bacterial populations. Further, Kim et al. (58) showed how a biomimetic microdevice (**Figure 6*a***) composed of two channels separated by a porous, flexible membrane and lined with human intestinal epithelial cells could mimic the complex structure and physiology of live intestines, allowing the study of the dynamic symbiotic interactions between human and microbial cells.

## LIVING TOGETHER

In the natural world, a microbial cell commonly finds itself in the vicinity of other cells. Elucidating the mechanisms underlying the interactions among microbes is fundamental to our understanding of how small-scale encounters influence larger-scale processes such as ecosystem function, organismal health, or industrial remediation. Microfluidic devices provide an excellent platform for probing these interactions because they can confine cells in controlled and appropriately designed geometries, allowing one to dissect the specific physical, chemical, and biological ingredients of cell-cell interactions.

**Quorum sensing (QS):** a form of chemical communication among microbes that occurs when secreted signaling molecules exceed a threshold concentration

**Green fluorescent protein (GFP):** a protein frequently used to monitor microbiological processes through epifluorescence microscopy

### Bumping Into Each Other

In several environments, including the light organs of some squid species and bacterial swarms on surfaces, bacteria can grow to extremely high densities. In such crowded conditions, mechanical interactions can generate population-scale patterns. Self-organization can be advantageous for bacterial populations, yet the role that direct cellular contact plays in this process has received little attention owing to the challenges in visualizing it. Microfluidics has enabled real-time studies of physical interactions in dense bacterial suspensions by providing control over flow and nutrient delivery and by allowing single-cell-resolution imaging. Using a microfluidic device containing shallow chambers of varying shapes and sizes, Cho et al. (15) observed that *E. coli* cells self-organized over multiple generations to enhance nutrient transport, favoring growth in dense colonies. In another study, similarly shallow microchannels enabled non-motile cells to be grown and imaged as a monolayer, allowing researchers to probe the biomechanical interactions arising as a result of cell growth and division (112). This study showed that the rapid increase in cell density observed after a few generations created an expansion flow that triggered a transition from a randomly oriented initial cell distribution to an orientationally ordered phase (**Figure 5*a***). The flow of growing cells inside microfluidic traps can be inhomogeneous and characterized by narrow, fast streams amid regions of stagnant cells (83). This phenomenon was attributed to the strong dependence of bacterial mobility on cell size and nutrient availability: Larger cells grown in favorable conditions experienced greater friction from the channel walls and were mostly immobile, whereas smaller cells grown farther from the nutrient sources could move around the clusters of stagnant cells, producing the streams.

### Social Sense

Cells interact through a number of chemical signaling pathways, the most heavily studied of which is quorum sensing (QS). Microfluidics has enabled a number of advances in our understanding of cellular signaling, primarily owing to the ability to work in confined environments and with small numbers of cells. For instance, using microfabricated maze topologies, Park et al. (87) found that in *E. coli*, chemotactic self-attraction due to secreted amino acids could drive rapid accumulation of cells in small confined spaces, triggering quorum-dependent behaviors (**Figure 5*b***). Following nutrient deprivation (86), such self-induced chemotactic behavior triggered the formation of solitary waves of bacteria that collapsed into the smallest confining structures in their path, emphasizing that a population's ability to induce cell-cell communication under stressful nutrient conditions is a critical component of its survival (**Figure 5*c***).

Until recently, QS was believed to occur only at high cell numbers. In contrast, Boedicker et al. (13) used microfluidics to produce arrays of droplets, each containing only a few *P. aeruginosa* cells, and visualized QS by green fluorescent protein (GFP) expression. Confinement resulted in the onset of QS despite the low cell numbers, owing to the rapid accumulation of cell-secreted

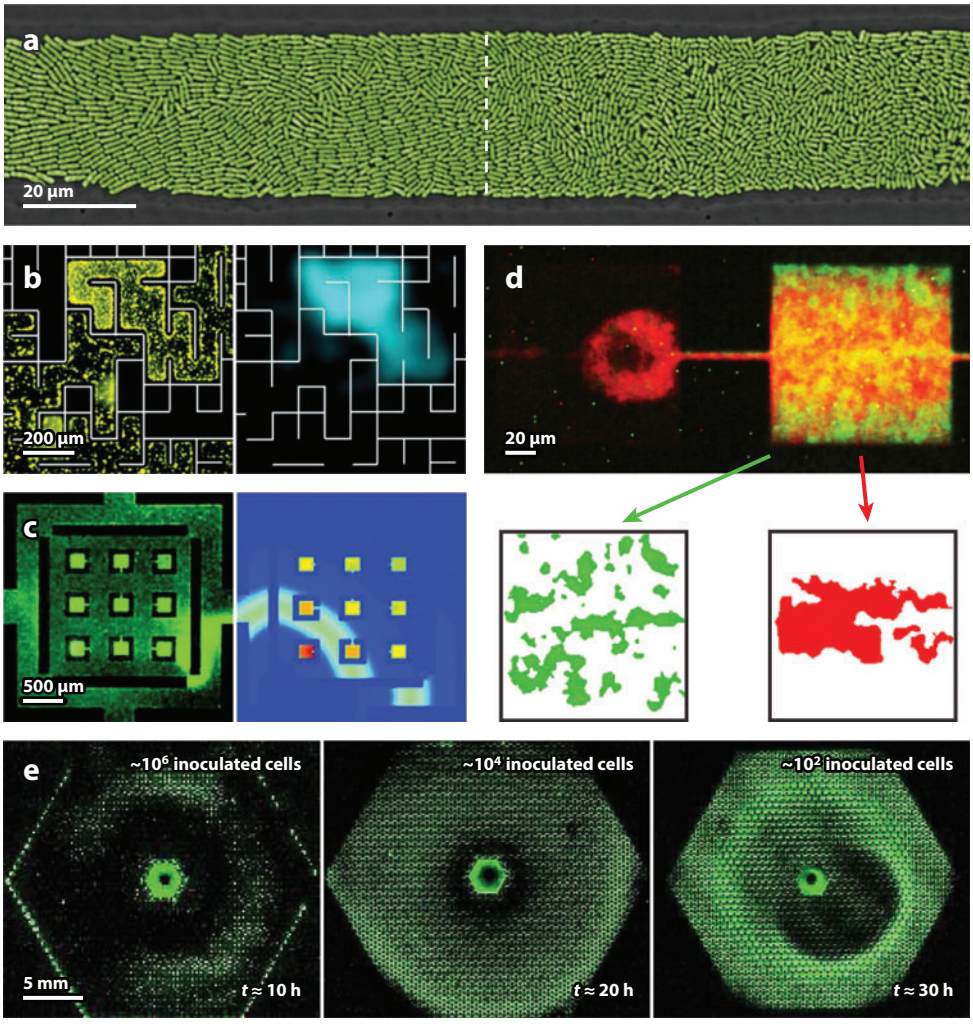


**Figure 5**

Multiple-cell microconfinements reveal new population-scale microbial processes. (*a*) Bacterial growth and ordering in a quasi-two-dimensional microfluidic open channel. Modified with permission from Reference 112. (*b*) *Vibrio harveyi* accumulation in a microfabricated maze inducing quorum sensing (*left*, dark-field image; *right*, photon-counting image of the intrinsic quorum sensing luminescence). Modified with permission from Reference 87. (*c*) Wild-type *Escherichia coli* collapsing into confining microfluidic chambers (*left*, experiments; *right*, simulations). Modified with permission from Reference 86. (*d*) Fluorescence images of competing *E. coli* populations [*red*, growth advantage in stationary phase (GASP) mutant cells; *green*, wild-type cells] in microhabitat patches. Modified with permission from Reference 54. (*e*) Large array of connected microchambers showing the rapid emergence of antibiotic resistance within an antibiotic gradient for different times and inoculation densities. Modified with permission from Reference 125.

**Autoinducer molecule (AI):** a molecule that triggers quorum sensing among bacteria when in sufficiently high concentrations

autoinducer molecules (AIs), the concentration of which could not become diluted by diffusion into surrounding liquid. The insight that a few cells can initiate QS—because the concentration of inducer molecules and not cell abundance per se is the primary driver of this behavior—further highlights the importance of confinement and chemical transport at the microscale.

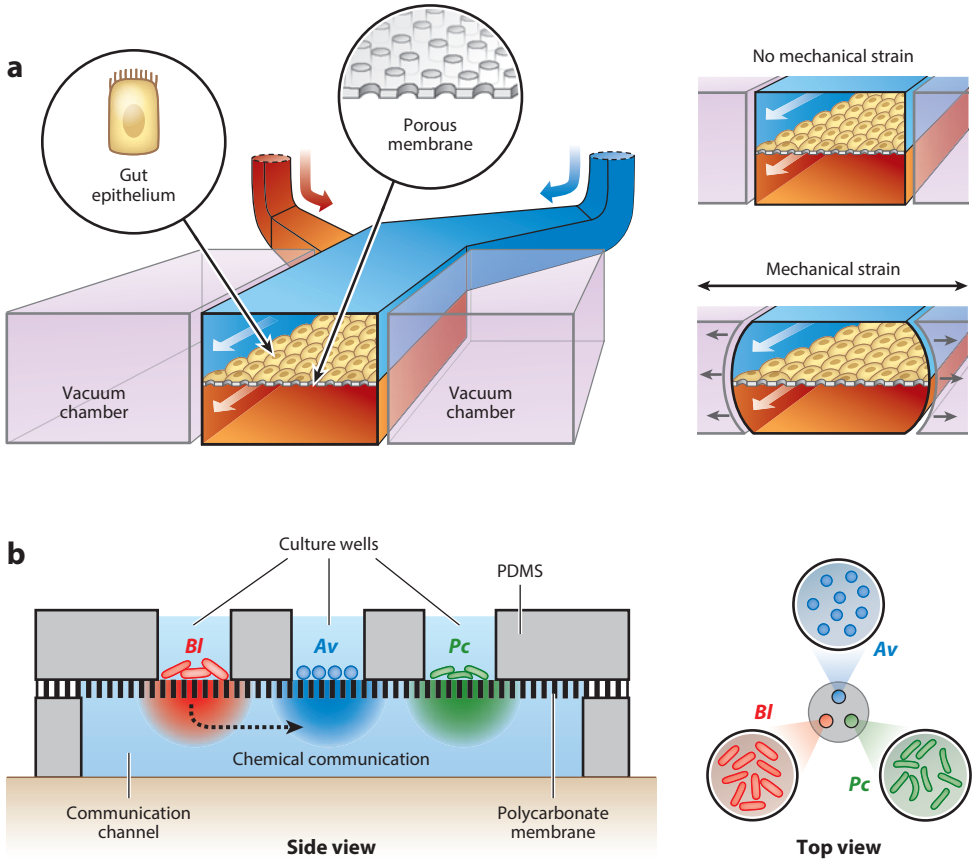


**Figure 6**

Multilayer microfluidic devices allow coculturing. (*a*) Schematic of a gut-on-a-chip device showing the porous membrane lined by gut epithelial cells with or without mechanical strain exerted by suction. Modified with permission from Reference 58. (*b*) Schematic of the microfluidic device used to coculture three species of soil bacteria by imposing spatial structure on three culture wells and providing a chemical communication channel. Modified with permission from Reference 57. Abbreviations: *Av*, *Azotobacter vinelandii*; *Bl*, *Bacillus licheniformis*; *Pc*, *Paenibacillus curdlanolyticus*; PDMS, polydimethylsiloxane.

By producing reliable chemical signals, microfluidic confinements have been instrumental in demonstrating new synchronization properties of bacterial communities. Danino et al. (21) achieved a prototypical form of coordination in *E. coli* communities by engineering a synchronizable network of oscillating genes, in which neighboring cells exchanged information while keeping the same phase in the oscillation. Trapping the cells in a network of microchannels that supplied them with nutrients while avoiding interference from external flow led to the observation of intense synchronization waves above a threshold colony size. This work expands on a seminal study of engineered synthetic time keepers (oscillators) (29) that did not display synchronization.

Studies of microspatial patterning of bacteria led to the discovery that spatial clustering among bacteria favors the secretion of signaling molecules that induce coagulation in human and mouse blood (52). In this study, prescribed numbers of *B. cereus* and *B. anthracis* cells were patterned in different spatial configurations, including a spatially homogeneous arrangement and

small and large clusters. Only large clusters initiated the coagulation cascade, whereas for other configurations, the threshold of signaling molecules required to induce coagulation was not reached. This elegant result illustrates the power of microfluidics in modeling the microscale spatial structure of bacterial communities and in revealing its macroscopic consequences, a theme we believe to be ubiquitous and poorly understood across microbial ecology.

## A DYNAMIC NEIGHBORHOOD

Many questions have remained unanswered with regard to microbial population dynamics, in large part owing to the difficulty of observing these dynamics under realistic microenvironmental conditions. Microfluidics is opening new experimental windows to overcome this historically recalcitrant barrier and to begin building a mechanistic understanding of critical questions such as how a population adapts to a changing environment and what processes determine the stability of multispecies consortia.

### Metapopulations

The dynamics of metapopulations—namely, collections of populations living in distinct habitat patches connected by dispersal—represents one of the most intriguing topics in ecology. This topic is ideal for microfluidic exploration because microfluidics affords the ability to create landscapes of patches with great control and flexibility. An early example is the nanofabricated landscape of Keymer et al. (55), who constructed an array of microscale habitats connected by small corridors and monitored the cell concentration in every habitat. These authors used a series of nanoslits to ensure nutrient supply to and waste removal from each habitat and introduced controllable heterogeneity in habitat quality by varying the number of nanoslits. They found that an *E. coli* population could adapt rapidly to regions of intense nutrient-limitation stress in a landscape with high niche diversity. Using the same device, Keymer et al. (54) discovered that two strains of bacteria that competed for the same resources to the point of extinction in a homogeneous culture actually cooperated in a nanofabricated heterogeneous landscape (**Figure 5*d***). These results suggest that in natural environments, such as soil, dominant strains may benefit from the continued existence of less competitive strains.

The same fundamental principle—allowing the diffusion of physiologically important molecules while preventing cell migration out of habitats—was used by Kim et al. (57) to study the interactions of three strains of soil bacteria in a synthetic community. The three strains were separated physically but could exchange solutes through a porous membrane that allowed diffusive transport among the habitats of each strain (**Figure 6*b***). In bulk liquid, the community was highly unstable. In the microfluidic device, however, "fences made good neighbors." When the wells were placed at intermediate distances from each other, the three populations stably coexisted. More recently, Connell et al. (20) used a three-dimensional printing technique to create a core–shell arrangement of two physically segregated but chemically interacting populations of bacteria. They showed that the resistance of one species to an antibiotic can enhance that of a second species. These studies illustrate how microfabrication opened an important window for the study of microbial chemical communication in the absence of physical interaction.

### Predation

An application of microfluidics to microbial ecology that is still in its infancy, yet has great future potential, is the ability to readily visualize individual cell-cell interactions among species, in particular predator-prey and virus-host interactions. For example, Park et al. (85) exploited the facile

generation of gradients in microfluidic devices to study predator-prey interactions among bacteria. They used a microfabricated array of picoliter chambers to study the interactions between the bacterial predator *Bdellovibrio bacteriovorus* and its prey, *E. coli*. A novel way of integrating two existing microfluidic approaches—namely, a Christmas tree gradient generator (46) to create a gradient of prey concentration and an array of arrow-shaped ratchets (45) to sequentially concentrate the predator and the prey cells—resulted in a systematic study of the predation rate as a function of the initial concentrations of predator and prey cells.

Microfluidics has also begun to benefit the study of virus-host interactions by providing highly controlled environments that help overcome the challenge of studying the extraordinarily small-scale processes surrounding viral interactions with their hosts (at ~100 nm in size, viruses are smaller than the wavelength of visible light) (114). Advances have been made through the use of devices that allow for trapping and imaging of viruses in addition to real-time monitoring of host infection. For example, incorporating dielectrophoretic filters within microfluidic chips has enabled the capture and real-time imaging of viral particles from a flowing system (6), a useful technique for separating and concentrating viruses from mixed communities. Multilayer devices have been employed to regulate the delivery of viruses to a host population and to image the infection dynamics in real time (18, 121).

A major open challenge in microbial ecology is gaining a better understanding of bacteria that are not culturable (these comprise more than 99% of naturally occurring bacteria) and the viruses that regulate these populations. An important advance came when Tadmor et al. (107) adapted a droplet-based microfluidic PCR method to probe viral infections of uncultured bacteria. By amplifying viral and bacterial genetic markers from single cells (using degenerate primers and the SSU rRNA gene, respectively), the authors were able to sequence amplicons and identify host-virus pairs directly from environmental samples. They discovered remarkably structured and specific host-virus relationships, even though these organisms came from a mixed assemblage in the termite hindgut, in which ample opportunity for lateral gene transfer seemingly exists. This application of high-throughput microfluidic PCR opens a new research avenue into the virus-host relationships of unculturable microbes.

**PCR:** polymerase chain reaction

**MIC:** minimum inhibitory concentration

**Persisters:** cells within a population that survive antibiotic treatment without genetically acquiring resistance

## ANTIBIOTIC RESISTANCE

Antibiotic effectiveness is a major societal challenge in the 21st century. This issue will affect human health and industries such as agriculture and aquaculture as greater numbers of microbes develop resistance to antimicrobial compounds. Furthermore, resistance can be transmitted among microbial populations and through different ecosystems, potentially heightening the exposure of human and wildlife populations to such organisms. A range of microfluidic devices has opened a new door for high-throughput evaluation of the effects of antibiotics on bacteria. In this section, we focus on selected advances and refer the reader to a recent review for more information (40).

Microfluidics-based methods for determining minimum inhibitory concentrations (MICs) have included a wide range of designs, from microwells containing preloaded antibiotic compounds into which cells are added (19) to droplet-based systems (17) and agarose microparticles (31). The effect of antibiotics on cellular repair mechanisms was studied by immobilizing cells on surfaces (47) or in agarose (16) within microfluidic chambers. The consequences of low doses of antibiotics on cell morphology during growth have been studied in high-throughput devices made of multiple channels, each equipped with dozens of growth microchambers (106).

Any isogenic population of bacteria may contain dormant cells that tolerate antibiotics, called persisters, which are likely an important reservoir for chronic and recurring infections. A handful

**Microhabitat:** confined, microscale volume of fluid within which microbes typically experience uniform environmental conditions

of microfluidic studies have recently tackled the question of how these persisters arise within a population. A microfluidic chemostat allowed Vega et al. (111) to identify the critical role that bacterial chemical communication plays in the formation of persisters. These authors observed that *E. coli* cells exposed to physiological levels of indole, a chemical signal that affects cellular stress responses, exhibited levels of persistence that were one order of magnitude higher than unexposed cells. By engineering a mutant strain with a fluorescent reporter for this pathway of chemical sensing, the authors could track the indole levels sensed by individual cells in the microfluidic chemostat and correlate those levels with each cell's subsequent ability to survive antibiotic treatment. Their study revealed that indole sensing is heterogeneous within a population, and it plays a significant role in the formation of persister cells.

The growth dynamics of *E. coli* persisters were analyzed at the single-cell level by Gefen et al. (36) using a microfluidic device that allowed them to modulate the supply of growth media and antibiotics over time. Cells were held in a microfluidic chamber that allowed for continuous supply of growth media and real-time imaging. Using fluorescent reporters, the authors observed that (*a*) persisters continued to synthesize proteins for 90 minutes at the beginning of what was previously believed to be a dormant stage and (*b*) this phase represented a time window when persisters were vulnerable to antibiotics. This window of vulnerability may prove important for clinical treatment of persistent infections. The observation of single cells under controlled environmental conditions has also led to a degree of predictability regarding which cells become persisters. Balaban et al. (9) used a multilayer microfluidic device to supply nutrients and antibiotics from one layer through a transparent membrane into a second layer containing an *E. coli* population arranged in narrow grooves, a precursor to the mother machine (**Figure 4*b***). The authors were able to predict which cells would become persisters before antibiotics were introduced on the basis of a phenotypic switch in growth rate: Cells that eventually became persisters grew more slowly and passed on the slow-growth phenotype to daughter cells for several generations. This work has shown that persistence can be a phenotypic switch—gained and then lost again within a single cell lineage—rather than a result of genetic heterogeneity, as in the case of antibiotic resistance.

The creative use of microfluidics is broadening our understanding of how environmental conditions and habitat niches influence the development of resistance, a major open question pertaining to the effectiveness of antimicrobials. This is exemplified by the work of Zhang et al. (125), who used a hexagonal device composed of 1,200 hexagonal wells (**Figure 5*e***), which were connected to each other through small corridors. Nanoslits along the periphery allowed for nutrient and waste exchange, as well as antibiotic dosing. This landscape of microhabitats, which contained both nutrient and antibiotic gradients, resulted in "Goldilocks points," i.e., wells in which the conditions were just right for selecting for resistant bacteria. In the absence of the antibiotic gradient, no resistance emerged. At Goldilocks points, resistance developed in as little as ten hours, and four single-nucleotide polymorphisms responsible for the resistance attained fixation.

## SUMMARY POINTS

1. The study of microbial navigation, and chemotaxis in particular, is an area of microbial ecology that has substantially capitalized on microfluidic technologies, in large part through the tracking of single cells within spatiotemporally controlled gradients, created, for example, by incorporating porous materials in the microfabrication. This approach has enabled studies to closely mimic natural environmental conditions and has thereby shed light on a plethora of microbial behavioral responses ranging from logarithmic sensing to fold-change detection to adaptation.

2. The seamless integration into microbial studies of carefully controlled fluid flow, afforded by microfluidics, has begun to reveal a broad range of new biophysical interactions, given that the transport and hydrodynamic forces exerted by fluid flow on individual microbes can considerably influence fundamental microbial processes such as cell motility and surface attachment.
3. A distinguishing feature of microfluidics is the flexibility to fabricate unconventional geometries and topographical features such as corners, funnels, corridors, nanoslits, and posts. This capability has allowed researchers to examine and control the emergence of patterns and self-organization, particularly in the context of interactions with boundaries, adhesion to surfaces, and the rectification of bacterial motion.
4. The ability to confine single cells or small populations in highly controlled microenvironments has opened new avenues of research into biological and ecological questions about growth, morphogenesis, inheritance, cell signaling, and antibiotic resistance. This ability has also provided novel tools to culture rare or mixed populations of cells.
5. The ability to directly visualize the dynamics of single or multiple populations of microbes and to segregate populations physically while allowing them to communicate chemically (achieved through membranes, hydrogels, or nanoslits) has provided new insights into the role of spatial heterogeneity in coexistence and cooperation.
6. The ability to impose and manipulate multiple gradients simultaneously using microfluidic technologies has resulted in studies of microbial ecology within environments that reproduce with increasing accuracy the essential physical, chemical, and biological features of a range of natural microbial habitats, from the ocean to soils to the human body.
7. The studies discussed in this review exemplify how the application of microfluidics to microbial ecology provides a level of environmental control and a richness of data that are nearly impossible or exceedingly time-consuming to obtain in ecological studies at larger scales. They also indicate that microbial ecology, when paired with microfluidics, can serve a considerably more powerful function in developing and testing general ecological theories than has occurred to date.



## FUTURE ISSUES

1. Navigational abilities, responses to chemical cues, and adaptation dynamics remain unknown for most bacteria, with the exception of selected model systems such as *E. coli*. Thus, researchers may use microfluidic platforms to facilitate the acquisition of quantitative information about a broad range of microbes and environments to understand the dynamics, diversity, and impacts of microbial navigation and sensing behaviors.
2. Microfluidic studies of microbial interactions with fluid flow and with confining surfaces, two features that are ubiquitous in natural microbial habitats and that are easily mimicked through microfabrication, are still in their infancy. Such studies promise many new insights into microbial ecology and potentially practical solutions to challenging problems, such as biofouling.

3. Microfluidic devices have been underutilized for the study of biofilm formation and development, a complex topic that can greatly benefit from the ability to control the local environment and obtain real-time data at the single-cell level.
4. Exploiting microbes to perform microscale tasks has been elegantly demonstrated in proof-of-concept experiments. Future work is needed to determine whether these dynamics can be harnessed and implemented in engineering systems.
5. Microbial ecology will greatly benefit from the continued integration of microfluidics with advanced optical methods and spectroscopy techniques to further probe behavioral and metabolic responses of cells under known environmental conditions.
6. The spreading of microfluidic technologies to biological and ecological studies is currently limited by the engineering involved in their fabrication and the physics involved in the understanding and control of flow and transport. In addition to interdisciplinary collaborations, simplified and easily integrated devices with standardized analysis tools will enable a broader cross section of researchers to take advantage of this powerful approach to microbial ecology.



## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Annual Review of Biophysics
Volume 43, 2014

# Contents

**Index**



**Errata**

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