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Microfluidics for Environmental Applications

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Contents

Abstract Microfluidic and lab-on-a-chip systems have become increasingly important tools across many research fields in recent years. As a result of their small size and precise flow control, as well as their ability to enable in situ process visualization, microfluidic systems are increasingly finding applications in environmental science and engineering. Broadly speaking, their main present applications within these fields include use as sensors for water contaminant analysis (e.g., heavy metals and organic pollutants), as tools for microorganism detection (e.g., virus and bacteria), and as platforms for the investigation of environment-related problems (e.g., bacteria electron transfer and biofilm formation). This chapter aims to review the applications of microfluidics in environmental science and engineering – with a particular focus on the foregoing topics. The advantages and limitations of microfluidics when compared to traditional methods are also surveyed, and several perspectives on the future of research and development into microfluidics for environmental applications are offered.

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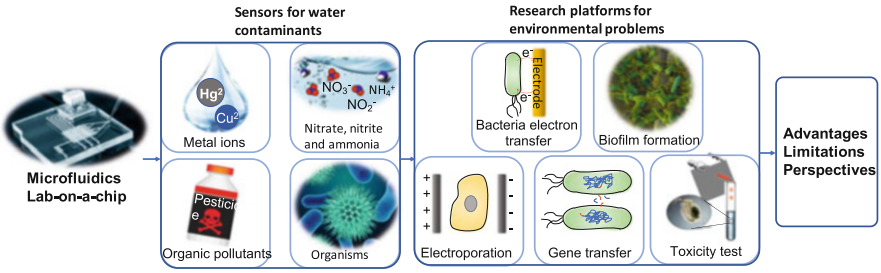
Graphical Abstract



Keywords Bacterial electron transfer, Biofilm formation, Contaminant analysis, Environmental science and engineering, Lab-on-a-chip, Microfluidics, Microorganism detection

1 Introduction

Microfluidics, or "lab-on-a-chip", is the science and technology of systems that are made using integrated circuits and/or miniaturized fluidic channels designed to realize different functions via electrical signals and/or flow manipulation [1]. When feature size and flow volume are shrunk down to microscale, surface area dramatically increases – which significantly improves the efficiency of molecular diffusion and heat transfer [1, 2]. As a result of their properties, microfluidics are increasingly finding applications in disparate areas of multidisciplinary research, including chemical [3, 4], biological [5, 6], medical (e.g., drug delivery) [2], and engineering (e.g., material synthesis) [7] fields.

Environmental science and engineering is a discipline for understanding environment-related processes and dealing with environment-related issues – such as understanding the conditions of environmental contamination and/or finding ways to affect environment remediation and protection. Some of the most widely studied environmental topics currently include pollution monitoring and analysis, research into the effects of pollutants on ecologies and human health, technologies of pollution treatment and removal, and microorganism-related challenges (such as the spread of antibiotic resistance).

Microfluidic and lab-on-a-chip devices are gaining increasing attention in this field due to their usefulness as tools for (by way of example) pollutant sensing, microorganism detection, and general environment-related process investigation (Fig. 1). Microfluidic devices offer several remarkable advantages over more conventional methods: for instance, they can more readily be used as portable detectors or analyzers due to their small size, thereby enabling on-site pollution detection and monitoring. Lab-on-a-chip devices have also provided research platforms for in situ and real-time observation of microorganisms and for visualization of other

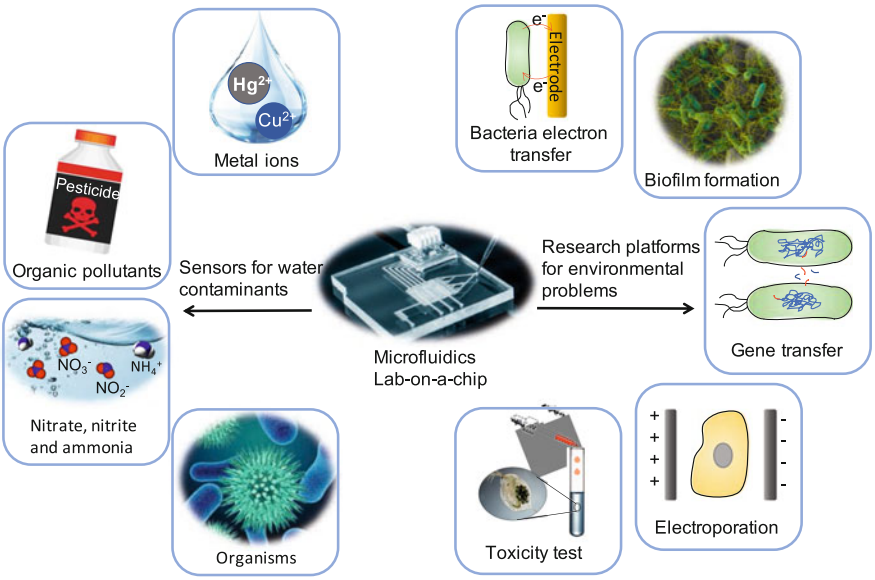


Fig. 1 Environmental applications of microfluidic and lab-on-a-chip devices (some images are from the Internet)

environmental processes. In this chapter, we will review some of the primary applications of microfluidic and lab-on-a-chip devices in environmental science and engineering. Finally, advantages, limitations, and perspectives on future development in this area will be discussed.

2 Applications of Microfluidics in Environmental Science and Engineering

2.1 Microfluidics Used for Contaminant Analysis

Conventional methods for conducting water pollutant analysis use advanced and complex instruments, such as inductively coupled plasma mass spectrometry (ICP-MS) for metal ions detection; high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) for organic compounds detection; and –ultraviolet-visible spectroscopy (UV-Vis) for nitrate or nitrite detection. Compared to these traditional analytical techniques, microfluidic systems and lab-on-a-chip sensors possess several significant advantages, namely, greater portability for on-site monitoring, smaller required sample volume, shorter reaction time, and better process control. Optical and electrochemical methods are the two main approaches that are typically used for pollutant detection in

microfluidic devices and sensors [8]. Common optical methods include fluorescent, colorimetric, surface plasmon resonance, and surface-enhanced Raman scattering (SERS) [8]. The detection techniques used in electrochemical sensors consist of amperometry, voltammetry, conductometry, and potentiometry [9]. With the sensing device miniaturization and sample volume decrease, electrochemical methods offer an inherent advantage over optical approaches. Since electrochemical methods rely on the concentration instead of absolute amount of the analyte, the sensitivity is independent of the sample volume, and more accurate determinations could be achieved due to the higher surface-area-to-volume ratios of the small probes [10].

2.1.1 Heavy Metal Ion Analysis

Water contamination by heavy metals is a severe environmental problem with significant implications for public health. Indeed, many lab-on-a-chip-based sensors have been developed specifically to facilitate the detection of a variety of metal ions, including Hg (II) ions [11], Pd (II) ions [12], Cd (II) ions [13, 14], Cu (II) ions [15, 16], and other metal ions [17, 18].

Wang et al. have developed a microfluidic device for quantitative analysis of trace Hg (II) ions (Hg^{2+}) based on surface-enhanced Raman scattering (SERS) [19]. A sample containing Hg^{2+} was mixed with gold nanoparticles while flowing through a wandering channel (Fig. 2a, b). The gold nanoparticles had rhodamine B dye molecules attached on the surface. Due to the strong affinity between Hg^{2+} and gold nanoparticles, the rhodamine B attached on the gold particles could be replaced by Hg^{2+} (Fig. 2c), causing a change in the SERS signal of rhodamine B in a function of the concentration of Hg^{2+}. The SERS changing was characterized by a Raman microscope system. The concentration analysis range of Hg^{2+} was estimated to be between 0.1 and 0.5 μg/L.

Another microfluidic device has been developed for continuous and on-site monitoring of Pb (II) ions (Pb^{2+}) [20]. The device is composed of cyclic olefin copolymer microfluidic channels, with silver working and counter-electrodes. The Pb^{2+} measurement was achieved by square-wave anodic stripping voltammetry (SWASV) technique. Specifically, water sample containing Pb^{2+} was first injected into the channel through an inlet. Under a certain voltage, Pb (II) ions were deposited

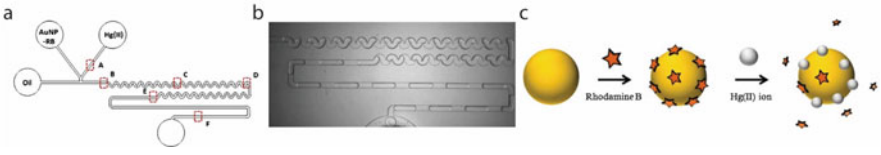


Fig. 2 Schematics of the microfluidic system for Hg ions detection. (**a**) Schematic of the microfluidic device for Hg^{2+} detection. (**b**) Photograph of the channel during operation. (c) Schematic of Hg^{2+} sensing mechanism based on the replacement of RB dye molecules through the reduction of Hg^{2+} on the surface of Au nanoparticles (reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer, [19]. Copyright (2009))

onto the Ag electrode via electrodeposition, and then plated metal was oxidized off from the Ag electrode using a square-wave anodic potential sweep. The whole electrochemical reaction is presented by $Pb^{2+} + 2e^{-} \leftrightarrow Pb$. The current generated during the stripping process was measured to identify and quantify Pb (II) ions. The detection limit was 0.55 ppb, and the correlation coefficient is 0.998 within the concentration range of 1–1,000 ppb. Furthermore, the detection performance remained stable after 43 consecutive measurements, demonstrating the sensor's reusability and great potential for real-world applications.

Microfluidic systems for water arsenic detection using both colorimetric methods and electrochemical methods have also been developed [10]. In addition, biological detection methods have been pioneered as well. A strain of genetically modified *Escherichia coli* (*E. coli*) was used as reporter bacteria for arsenic detection in a microfluidic device [21]. Polydimethylsiloxane (PDMS) was used to fabricate the microchannels. The bacteria were encapsulated in agarose beads and packed into small cages in the microchannels. When water sample containing arsenic flowed through the cages, the bacteria exposed to arsenic could produce green fluorescent proteins. The fluorescence was imaged with a microscope and processed for intensity analysis. The rate of fluorescence signal increase was linearly proportional to the arsenic concentration within the range of 0–50 μg/L. More microfluidic systems for arsenic detection were reviewed in [10].

In addition to standard silicon-based sensors, paper-based microfluidics have also been developed for metal analysis (reviewed in [22]). Paper-based microfluidics are paper substrates patterned as channels and barriers to realize different functions. Compared to traditional PMDS and glass or silicon-based microfluidics, the paper-based microfluidic devices are more cost-efficient [22]. By combining eight pyridylazo compounds, a paper-based microfluidic device could discriminate eight different heavy-metal ions (Hg^{2+}, Cd^{2+}, Pb^{2+}, Ag^{+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Co^{2+}) at concentrations as low as 50 μM [23].

2.1.2 Organic Compound Analysis

Potentially toxic organic compounds – such as phenolic compounds and pesticides – are widely used across many industries. Unfortunately, some of these organic compounds may also cause water contamination, due to wastewater discharge or leaching from soil. Microfluidic sensors for organic matter have been developed based on different detection mechanisms, including amperometry [24], enzyme-based techniques [25–27], and electrophoresis [28, 29]. A lab-on-a-chip device with layer-by-layer printing of quantum dot (QD)/enzyme microarrays was fabricated for organophosphorus pesticide (OP) detection [30]. Layer-by-layer microarrays of QDs/poly (dimethyldiallyl ammonium chloride) (PDDA) and acetylcholinesterase enzyme (AChE) were fabricated on a glass slide using inkjet (Fig. 3). Water samples and acetylthiocholine (ATCh) were added to the chip for OPs detection. AChE catalyzes the hydrolysis of ATCh, generating thiocholine (TCh), which can dissociate the electron-hole pair of QDs and quench the fluorescence.

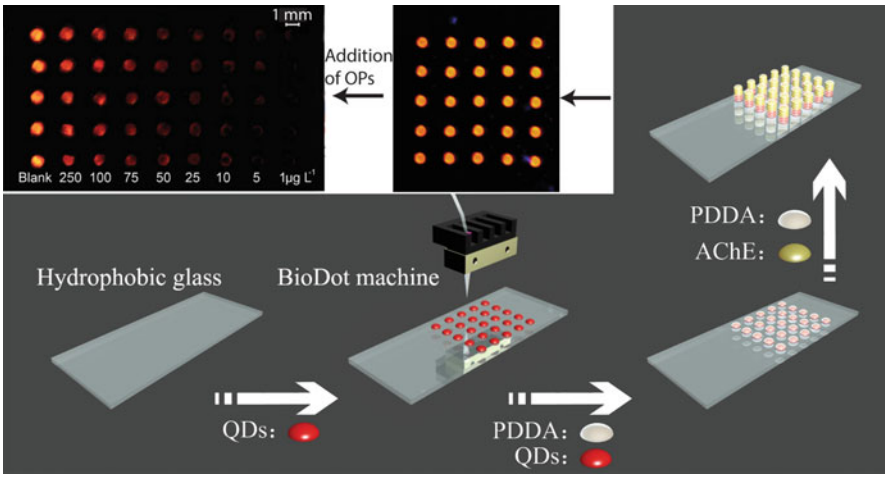


Fig. 3 The schematic of the fabrication process of the OPs detection chip and the image of QDs after OPs are added. (Reprinted from [30]. Copyright (2016), with permission from Elsevier)

When OPs are present, the activity of AChE was inhibited; thus the fluorescence of QDs will not be quenched. A detection limit of 1 μg/L of Ops was achieved with this device, which was much lower than levels specified by standard tests and other colorimetric detection methods.

2.1.3 Nitrate and Ammonia Analysis

Nitrate and nitrite are ubiquitous water contaminants in both surface and groundwater, and they each can impose harmful effects on human health. A miniaturized microfluidic sensor has been developed to facilitate nitrate determination using a double-potential-step chronocoulometry (DPSC) method [31]. Two potential steps, E_1 and E_2, were applied sequentially to obtain oxygen reduction charge Q_1 and both nitrate and oxygen reduction charge Q_2. The nitrate reduction charge was calculated by subtracting Q1 from Q2, which is directly related to nitrate concentration in the sample. A silver sensing electrode, silver oxide reference electrode, and platinum counter electrode were then deposited on a silicon substrate. A polyimide passivation layer was also deposited to prevent short circuit and improve reliability. The microchannels were fabricated via deep reactive ion etching, which enabled the flow-through analysis. The lower and upper detection limit for nitrate were 4–75 μM and 500–2,000 μM, and the linearity (R^2) was >0.99. Other microfluidic-based sensors for nitrate [32, 33] and ammonia [34, 35] analysis were also reported.

Lab-on-a-chip systems have also found applications in a wide variety of disparate environments, including marine pollution analysis [36–39], air pollutant detection [40, 41], and bioaerosol monitoring [42–44].

2.2 *Microfluidics Used for Microorganism Detection*

Pathogen contamination of drinking water remains a serious public health concern worldwide, especially in less developed areas. Waterborne pathogens can include bacteria, viruses, and some protozoa. Some of these biological agents are highly infectious and resistant to water treatment processes and accordingly pose a severe risk to human health. Different detection approaches have been developed to facilitate pathogen detection on-chip, such as nanomechanical cantilever sensing [45, 46]; surface-enhanced Raman spectroscopy [47]; impedance-based sensing [48]; amplification-based sensing, including PCR [49–56] and loop-mediated isothermal amplification [52]; and quartz crystal microbalance-based sensing [57]. Both optical signals [58–61] and electrical signals [62, 63] are used in microorganism sensing techniques. The applications of microfluidics in waterborne pathogen detection are reviewed in [64]. Microfluidics for pathogen detection are also being developed as point-of-care devices, for diagnostic purpose [65]. Although the samples analyzed in diagnostic devices (e.g., saliva and blood) are different from environmental samples, the detection techniques and approaches are still valuable as references.

2.2.1 Virus Detection

An ultrasensitive virus detection sensor based on the Young interferometer has been reported [66]. The sensor is a silicon chip consisting of four light channels. Si_3N_4 and SiO_2 layers were deposited on a silicon substrate via chemical vapor deposition. The SiO_2 layers were etched to form windows for antibody functionalization and virus detection. The Si_3N_4 layer beneath served as a pathway for light (Fig. 4a). Monochromatic light from a laser source was coupled to an optical channel and guided into the four parallel channels (Fig. 4b). Antibodies for different viruses' detection were coated onto the channels. The light interfered on a screen after exiting from the four waveguide channels, generating an interference pattern. Virus binding to the antibody would be probed by the evanescent field of the guided modes, thus causing a phase change which could be measured as a change in the interference pattern. The pattern reflects the amount of the viruses bonded on the antibodies. Figure 4c shows the specific detection of herpes simplex virus type 1 (HSV-1) realized by the specific reaction between HSV-1 and the antibodies. The sensor specifically and sensitively detected HSV-1 with the concentration as low as 850 particles/mL and the detection sensitivity of the sensor was estimated to approach one single HSV-1 particle.

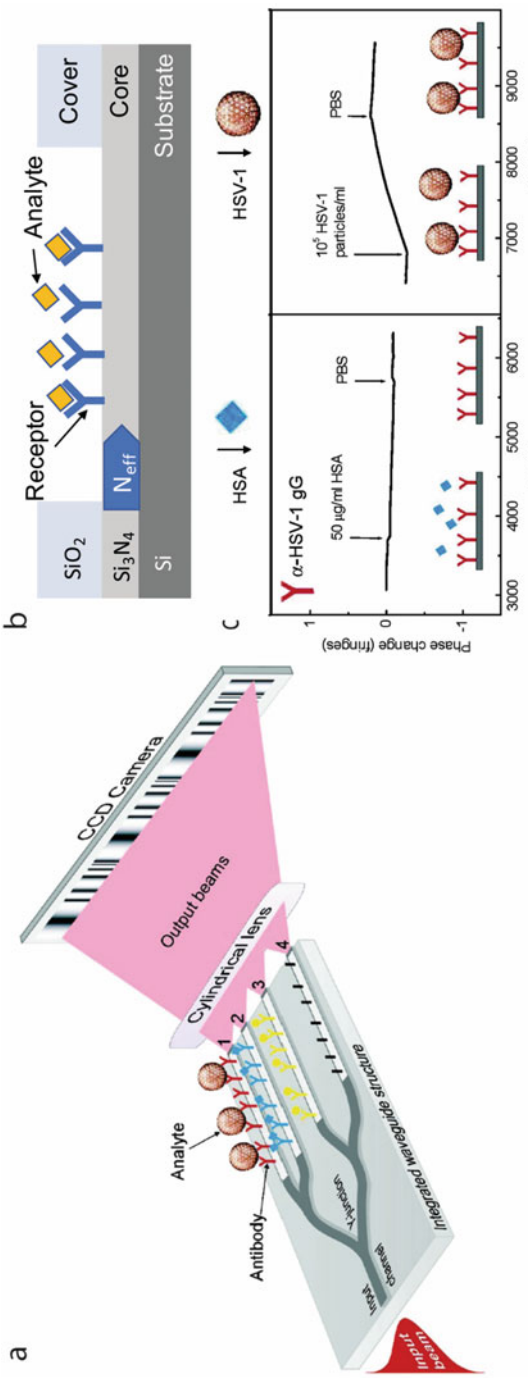


Fig. 4 (**a**) Schematic of the sensor for virus detection. 1, 2, and 3 are the measuring channels, and 4 is the reference channel (reprinted with permission from [66]). Copyright (2007) American Chemical Society]. (**b**) Cross section of the chip along the direction of the channels (Adapted form [67]). (**c**) Specific and selection detection of HSV-1. The figures indicate phase changes as a function of time. The phase change does not increase when human serum albumin (HSA) is added but increases only after HSV-1 is added, which is due to the specific interactions between HSV-1 and α-HSV-1gG (reprinted with permission from [66]. Copyright (2007) American Chemical Society)

2.2.2 Bacteria Detection

Bacteria are another kind of major waterborne pathogen that poses risk to human health. Mannoor et al. have reported a microfluidic system for real-time on-chip bacteria detection using impedance spectroscopy [68]. A gold electrode array was deposited onto a silicon substrate via standard microfabrication methods. The flow channel for real-time monitoring was fabricated using PDMS and bonded to the substrate. The electrode surface was functionalized with magainin I, which is a kind of antimicrobial peptide (AMPs) used for bacteria binding. When the bacteria contained in water samples were recognized by the AMPs and bonded to the electrode surface, the impedance of the electrode array changed, which was analyzed by a spectrum analyzer. Since the binding activity was directly proportional to the variation of impedance, the bacteria concentration could be analyzed. The detection limit of *E. coli* was about 1 bacterium/μL. The system showed sufficient selectivity toward pathogenic and Gram-negative bacteria, and also maintained broad detection capability for other bacteria. Furthermore, the flow system enabled real-time bacteria monitoring for a continuous water sample.

2.2.3 Protozoa Detection

In addition to viruses and bacteria, protozoa – especially some parasites – can pose a significant risk to human health. *Cryptosporidium* is one of the parasites of greatest concern on this front, due to its low infection dose and resistance to common water treatment approaches [69]. Several techniques have been integrated to miniaturized fluidic chips for *Cryptosporidium* detection, including optical methods such as target trapping combined with immunofluorescence or microscopy detection; mass-based methods such as quartz crystal microbalance sensing and cantilever sensing; and electrical techniques such as bioimpedance and dielectrophoresis methods. The detection of cryptosporidium in microfluidic devices is reviewed in [69].

2.3 Microfluidics Used as Research Platforms

Understanding environmental-related natural processes is another important component of environmental science and engineering. These widely studied processes are encompassed within, but certainly not restricted to, the fields of environmental microbiology, ecotoxicology, and contaminant transportation. Microfluidic and lab-on-a-chip devices are increasingly being used in environmental research laboratories, since they provide ideal research platforms for in situ and real-time observation. The combination of lab-on-a-chip devices and observation techniques (such as microscopy) enables in situ visualization, characterization, and simulation of a wide

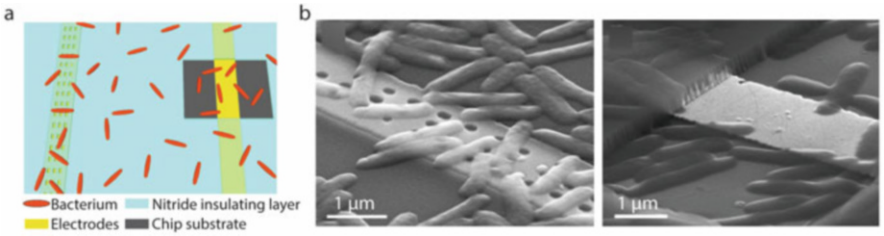


Fig. 5 Schematic of the design of the electrodes for *Shewanella* electron transfer study. (**a**) The silicon nitride insulating layer (blue) with nanoholes or large window openings is deposited over electrodes (yellow) to prevent or enable direct contact with bacteria (orange). (**b**) SEM images of the bacteria cells on the electrodes with nanoholes (left) and large window openings (right). (Reprinted with permission from [73], Proceedings of the National Academy of Sciences)

range of environment-related processes, thus becoming a valuable investigation approach in environmental studies.

2.3.1 Mechanisms of Bacteria Electron Transfer

Microbial fuel cells, which use microorganisms colonizing electrodes to catalyze electrochemical reactions and convert chemical energy into electrical power, are being intensively studied in the environmental technology field since they possess the potential capability of converting organic or inorganic waste into power via an environmentally friendly microbiological process [70, 71]. Understanding the mechanisms of electron transfer from bacteria to electrode is accordingly imperative for the further development of potential microbial fuel cells.

Three possible electron transfer pathways have been proposed: via direct contact, via conduct pili, and via diffusion of soluble redox-active molecules serving as "electron shuttles" [72]. Jiang et al. have reported a lab-on-a-chip device with microelectrodes as a platform to investigate the electron transfer between *Shewanella oneidensis* and electrodes [73]. Finger-shape electrodes were defined by photolithography and deposited onto a cover glass using metal evaporation and lift-off methods. A passive Si_3N_4 layer was deposited by chemical vapor deposition and patterned to have nanoscale openings on one electrode and a big opening on the other electrode (Fig. 5a, b). The nanoholes were small enough to prevent direct contact between bacteria and the electrode but allowed the indirect contact through pili or diffusion of extracellular redox-active molecules. A SU-8 (a commonly used epoxy-based negative photoresist) chamber was fabricated to improve reliability and environmental control. In situ cell image/tracking with a microscope and current recording revealed that the currents could be detected even without direct contact between bacteria and electrodes, suggesting that electron transfer was realized by pili or a mediator's diffusion. In addition, the removal of the diffusible mediators caused a rapid drop of the current, which further supported that electron transfer occurs predominantly by diffusion of mediators.

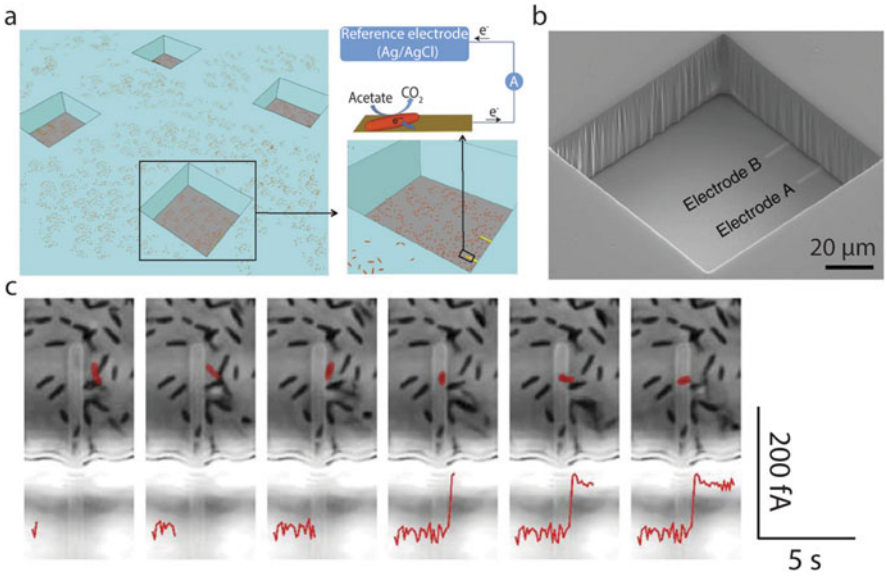


Fig. 6 (**a**) Schematic of experimental design for *Geobacter sulfurreducens* electron transfer. (**b**) SEM image of a well containing two finger electrodes. (**c**) In situ microscopy images of *Geobacter* cells around and on the measured electrode and the current changes at the same time. The cell that contacts the electrode at the same time with the current increases is marked in red. (Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer, [74]. Copyright (2013))

For other kinds of bacteria, however, the electron transfer mechanism may need to be altered. A similar lab-on-a-chip device was fabricated to probe the charge transport from *Geobacter sulfurreducens* to electrode [74]. Gold electrodes were deposited via metal evaporation and lift-off. Thick SU-8 was then fabricated to form wells around the electrodes to allow direct contact between bacteria and electrodes (Fig. 6a, b). Simultaneous recording of cell position and currents indicated that the contact of a cell to the electrode directly caused a stepwise increasing of current (Fig. 6c). The current of a single *Geobacter* was 92 fA, and the current density was estimated to be ~10^6 A m^{-3}. In addition, when the diffusible redox mediators were removed, the current was not affected. These measurements together indicated that, different from *Shewanella*, the electron transfer between *Geobacter* and electrode was mainly due to direct contact. Ding et al. reported a nanoelectronics lab-on-a-chip system to investigate the electrical conductivity of both *Shewanella* and *Geobacter* and indicated that electrochemical electron transfer at the cell/electrode interface was the origin of the conductive current for both microbes [75].

As researchers have started to gain a deeper understanding of bacterial electron transfer, the role of bacterial self-assembled nanostructures for extracellular electron transfer has also garnered increased attention. For example, to elucidate the effects of microenvironment on the intercellular microbial nanostructures (nanowires)

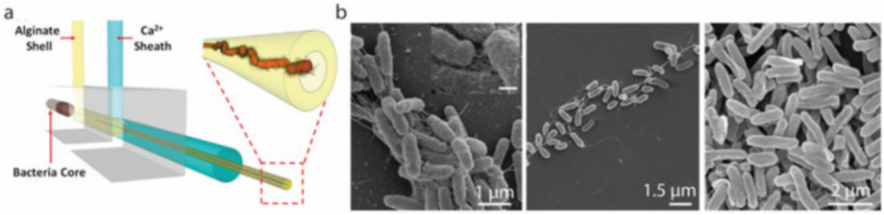


Fig. 7 (**a**) Schematics of the flow-focusing device for core/shell bacterial fiber generation. The bacteria-containing core stream (brown) is focused before entering the alginate shell stream (yellow), and then a $CaCl_2$ sheath flow is introduced to cross-link the alginate to form the cord. (**b**) SEM images of high (left) and low (middle) bacteria density networks as well as high-density networks cultured in electron acceptor rich conditions (right). (Reprinted with permission from [76]. Copyright (2018) American Chemical Society)

formation, a one-dimensional core/shell bacterial cable has been developed – which allows rational control of the microenvironments [76]. The fabrication method of this cable was different from common microfluidics fabrication processes. The cable was generated through a flow-focusing device with coaxially aligned glass capillaries and multiple inlets for different solutions. Bacteria solution flow was focused into a narrow stream, and alginate was injected to the device to form the scaffolding for bacteria encapsulation. A Ca^{2+} containing sheath flow was exploited to cross-link alginate to become a solid hydrogel (Fig. 7a). The results revealed that the formation of intercellular structures is closely related to the fiber diameters. More densely and closely packed bacteria produced more self-assembling microbial nanowires, which directly increased the extracellular electron transfer efficiency (Fig. 7b). Furthermore, lack of electron acceptors can enhance the production of the nanowires (Fig. 7b).

2.3.2 Biofilm Formation

Biofilm formation is a natural process that occurs during bacteria growth. On one hand, biofilms play important roles in some environmental engineering processes, including in wastewater biological treatment and microbial fuel cells. However, biofilm can also cause environmental and public health problems – including by contaminating or clogging drinking water pipelines or fouling water treatment systems. As a result, the process of biofilm formation is gaining more attention in environmental science and engineering. Drescher et al. have developed a microfluidic device to investigate biofilm formation in fluidic channels [77]. A meandering microfluidic channel was fabricated with PDMS and sealed with a cover glass. *Pseudomonas aeruginosa* bacterial solution flowed through the microfluidic channel and the biofilm formation process in the channel was observed with a microscope. This work demonstrated that the 3D biofilm streamers that bridged the space between obstacles and corners caused major clogging of the channel, instead of the biofilm attached on the inner surface. The 3D biofilm streamer was first

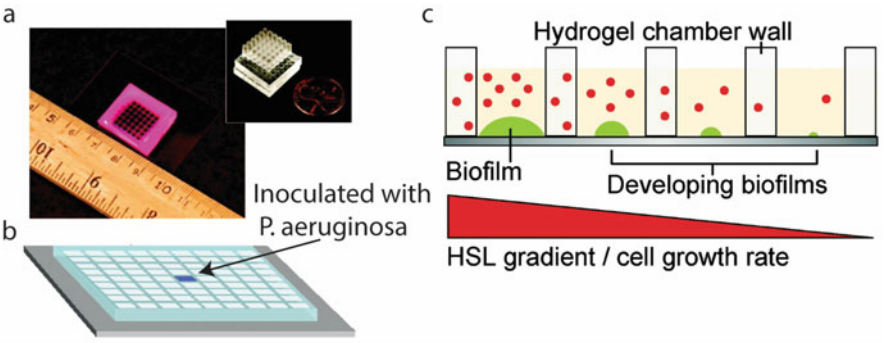


Fig. 8 (a) An image of the chamber for quorum sensing study with hydrogel chamber wall (stained with red dye) on a glass coverslip (upper). An image of the PDMS stamp used to make the chamber (lower). (**b**) The center chamber was inoculated with *P. aeruginosa*. (**c**) Schematic of the experiment. HSL diffuse through the hydrogel chamber wall, which is detected by biofilm in each chamber. (Reprinted with permission from [78]. Copyright (2011) American Chemical Society)

formed by the extracellular matrix shed from the attached bacteria and then worked as a network to catch the flowing bacteria and biomass, leading to a rapid clogging. With this microfluidic chip that enabled in situ observation of the biofilm formation, this work demonstrated a biofilm formation process which is independent of and much faster than bacteria growth. The results also suggested that the biofilm streamers may contribute more to the clogging of flow through systems such as water pipelines.

During biofilm formation, the bacteria within microbial communities can sense chemical signals from other cells and regulate their own gene expression as a response. This process is referred to as quorum sensing, and it is an important factor in regulating biofilm formation that is a current subject of intense study in the environmental microbial field. Flickinger et al. have reported a lab-on-a-chip platform to study quorum sensing between microbial communities [78]. The lab-on-a-chip device contained an array of spatially confined chambers fabricated with poly (ethylene glycol) diacrylate (PEGDA) on a silanized cover glass using a PDMS mold (Fig. 8a). *Pseudomonas aeruginosa* (*P. aeruginosa*) was used as a model bacterial strain and inoculated in the center chambers for biofilm growth (Fig. 8b). The molecule regulators secreted from the biofilm for quorum sensing, homoserine lactones (HSLs), can diffuse inside (filled with 15% PEGDA) and between the PEGDA chamber to form spatial and temporal gradients, thus enabling analysis of the relationship between the diffusion of HSLs and formation of nascent new biofilm (Fig. 8c). The results showed that HSL was detected by the bacteria cells within a distance of 8 mm. The new biofilm growth within 3 mm away from the existing biofilm, where the HSL concentration was higher than 1 μM, was enhanced due to the detection of HSL, while further biofilms were not affected. In addition to regular on-chip chambers, 3D cavities with various geometries were fabricated using 3D printing strategy to study the mechanisms of community regulation [79].

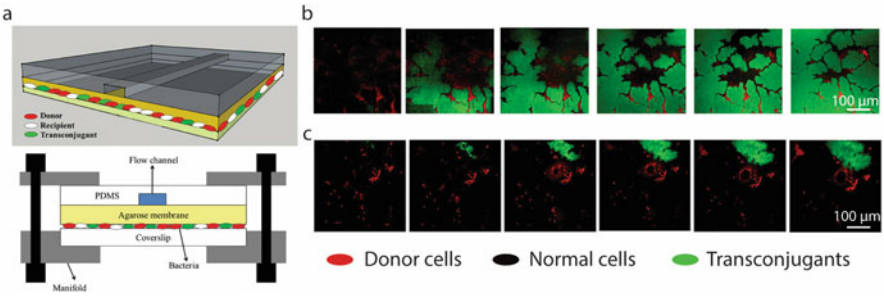


Fig. 9 (**a**) Schematic of the device to study antibiotic resistant genes transfer (upper) and the device setup (lower). (**b**) Gene spread in pure *E. coli* culture. (**c**) Gene spread in activated sludge community. For both (**b**) and (**c**), the donor cells *P. putida* KT2440 are red; normal *E. coli* or active sludge cells are colorless, while transconjugants emit green fluorescence. (Reprinted with permission from [80], https://pubs.acs.org/doi/abs/10.1021/acs.est.8b03281. Copyright (2018) American Chemical Society. Further permissions related to the material excerpted should be directed to the ACS)

2.3.3 Antibiotic Resistance Gene Transfer

It is now known that horizontal gene transfer is an important pathway by which antibiotic resistance spreads from one organism to another. Microfluidic devices are promising platforms for facilitating gene transfer study, since they enable the in situ and real-time monitoring of the process dynamics. A microfluidic device was reported to investigate the plasmid-mediated horizontal gene transfer within the same species and between different species [80]. The microfluidic chip consisted of a cover glass with a layer of agarose and a PDMS cover on top (Fig. 9a). A drop of mixed bacteria solution containing the gene donor strain (*Pseudomonas putida* harboring an antibiotic resistance plasmid) and recipient strain (*E. coli* or bacteria extracted form activated sludge) was sandwiched between the agarose and cover glass. The PDMS cover had a channel in it for broth delivery and waste removal for bacteria growth (Fig. 9a). The gene donor bacteria carried plasmid RP4, which was labeled with GFP, but also tagged with red fluorescent genes that repress the expression of GFP. So, the donor bacteria emitted red fluorescence. When the plasmids were transferred to acceptors, the acceptors would emit green fluorescent from GFP carried with the plasmids. The gene horizontal transfer process on the chip was monitored with a fluorescence microscope. The results showed that the horizontal gene transfer was highly dependent on the structure and composition of the biofilm. The plasmids were first successfully transferred from donor species *Pseudomonas putida* to acceptor *E. coli*. Within the pure *E. coli* colony, the transfer from the first transconjugants to other cells was very efficient, leading to a cascading gene spread within the single-strain biofilms (Fig. 9b). In comparison, for the activated sludge biofilm consisting of different species, vertical gene transfer appeared to be the dominant route instead of horizontal transfer (Fig. 9c). It is also found that many species that showed horizontal gene transfer were associated with human pathogens.

Other microfluidic systems for gene transfer and antimicrobial resistance related studies were also reported, including using microfluidic devices to study gene transfer on the single-cell level [81], dissect horizontal and vertical gene transfer [82], test antimicrobial susceptibility [83], and investigate the modulation of antibiotics on horizontal gene transfer [84]. More studies are reviewed in [85].

2.3.4 Electroporation

Electroporation is the phenomenon whereby pores form on a cell membrane when the cell is exposed to an external electric field. It is commonly used to control cell membrane permeability when molecular intracellular transfer is desired. In addition, electroporation is also a widely used method for cell inactivation and lysis. Researchers in environmental fields are also increasingly exploring the possibility of using electroporation as a bacteria inactivation approach for drinking water disinfection [86–89] and hazardous wastewater decontamination [90]. Understanding the electroporation process has therefore become another important environmental study topic. Since the actual formation of these pores is difficult to observe, Sengel et at. have developed a lab-on-a-chip device to image the dynamics of individual electropores [91]. The experimental setups are shown in Fig. 10a. A cover glass was coated with agarose and placed in a recess. Lipid solution was added and associated with the agarose to form a lipid monolayer. An aqueous droplet with lipid monolayer was brought onto the cover glass. Two monolayers at the contact area formed a lipid bilayer, which is similar to cell membrane. Two electrodes were placed at the two sides of the lipid bilayer to monitor the current, and the pore formation was labeled by a fluorescent dye and recorded with a fluorescence microscope (Fig. 10b). With this platform, researchers found several interesting phenomena of electroporation. When the potential difference across the bilayer reached 100 mV, the membrane permeability started to change. With higher

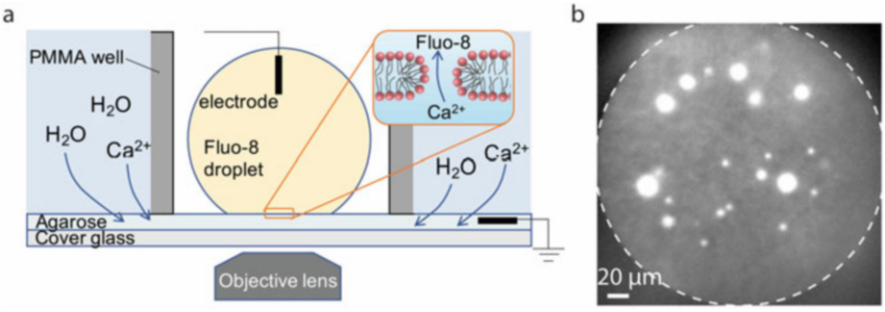


Fig. 10 Schematic of the experimental setup. (**a**) A lipid bilayer is formed on the interface of the droplet and the substrate. When a pore is formed due to electroporation, Ca^{2+} ions flow into the drop, which could be detected by the Ca^{2+} sensitive dye fluo-8 and visualized by a microscopy. (**b**) A microscopy image of pores formed on lipid bilayer. (Adapted and reprinted with permission form [91], Proceedings of the National Academy of Sciences)

transmembrane potential, larger pores formed, but a large number of small pores still existed. In addition, the pores fluctuated (opened and closed) in a variety of modes, and higher potential did not lead to more stable pores. Two adjacent pores did not tend to combine, while anti-combination was found since the potential across the lipid bilayer would be released when a nearby pore gets larger. A lab-on-a-chip device to rapidly determine the electroporation threshold for bacteria inactivation was also reported [92].

2.3.5 On-Chip Toxicity Test

Ecotoxicology focuses on identifying the toxicity level and impact of environmental pollutants on creatures and human health. Compared to traditional toxicity testing approaches, the emerging on-chip toxicity tests enabled by microfluidics are significantly more compact, convenient, and labor-efficient – and as a result they are quickly gaining substantial attention in this field.

Fine particles are major pollutants in the air, which makes them crucial indicators of general air quality. A microfluidic device aiming at recognizing the toxicity of fine particle matter ($PM_{2.5}$) on human lung epithelial cells was reported [93]. A porous membrane was bonded to the PDMS chamber, and the human lung epithelial cell line (BEAS-2B) was cultured on the membrane. Medium flowed under the membrane to replenish nutrient for cell growth (Fig. 11). The cell viability remained above 98% after 21 days of culturing, which demonstrated that this lab-on-a-chip is capable of retaining the viability of cells for the toxicity test. The air liquid interface mimicked the pulmonary natural microenvironment, which enabled the in vitro cytotoxicity test. Particles were added to the cells using an aerosol nebulizer, and the cytotoxicity was analyzed by several different approaches after exposure. The results showed that some metabolic pathways of the cells contributing to inflammation reactions were activated after the exposure. The cell apoptosis rate was also increased from 3.8% to 66.7% after 24 h of exposure. This configuration is also applicable for cytotoxicity test of other pollutants.

Engineered metal nanoparticles are being used for a variety of applications in many different fields. However, the potential hazards of these nanoparticles to human health and environment remain topics of hot debate and active research. To investigate the effects of silver nanoparticles on microorganisms' behavior, a

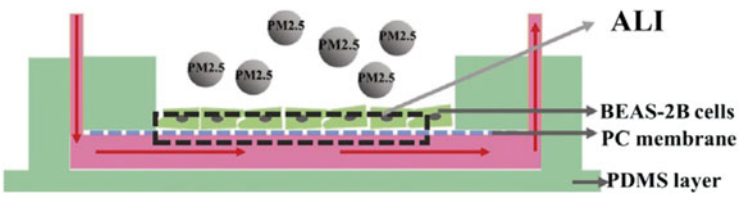


Fig. 11 Schematic of the designed microfluidic chip for ambient particle toxicity test. (Reprinted from [93]. Copyright (2019) with permission from Elsevier)

microfluidic device was reported to study the swimming response of algae to silver nanoparticles [94]. PDMS containing microchannels was fabricated via soft lithography and bonded to a glass slide, forming the microfluidic device. The microfluidic device used in this study was previously designed for a bacterial chemotaxis test, which contained a concentration-gradient generator and a chemotaxis observation channel [95]. Solution with and without silver nanoparticles were added to the two inlets, respectively. After flowing through the mixing part, nanoparticle concentration gradient was created and maintained in the observation channel. Algae was then added to the observation channel and exposed to the nanoparticle gradient. The algae swimming response was observed and recorded with a microscope. The results showed that algae moved away from the area containing 10^8 silver particles/mL, but no significant aversive swimming was found to gold nanoparticles at the same concentration. The toxicity of the released Ag ions may be the main reason leading to the avoidance behavior.

Microfluidics also have applications in aquatic toxicity tests on bacteria [96, 97], nematodes [98], crustacea [99, 100], and fish embryo [101]. More applications and future perspective were discussed in the review paper [102].

3 Perspectives on Microfluidics' Applications in Environmental Science and Engineering

Microfluidic and lab-on-a-chip devices are increasingly being used as tools in the fields of environmental science and engineering. Miniaturized microfluidic or lab-on-a-chip devices evidence remarkable sensing abilities, because sensing electrodes can be miniaturized without losing sensitivity and the configurations are compatible with thin layer operations [9]. Flow manipulation and compound separation can also be enabled by incorporating additional electrodes in existing channels, without adding additional parts. When used as detection equipment, lab-on-a-chip systems offer several comparative advantages over traditional mechanisms – including shorter analysis time, smaller sample volume, and online and real-time monitoring. All of these benefits are attributable to their small size, precise flow control, and low cost compared to traditional instruments. Some sensors have already been commercialized, such as test strips based on electrochemistry for arsenic detection [103] and sensors based on stripping square-wave voltammetry for metal analysis [104]. A DNA electrochemical biosensor has been combined with sample processing platforms for online pathogens monitoring in natural water [105]. IBM is also working on the development of sensors for environmental pollution detection, such as methane leakage [106]. Since real-world samples are often complex and signal characterization systems are still required for the systems, real-world implementation of these detection devices remains limited in some instances. In addition to further improving the performance of sensors, future studies will undoubtedly focus on developing more integrated systems that combine

sampling, pre-treatment, and signal interpretation on a single chip – which will increase the viability of on-site, real-time applications across a wide variety of real-world settings. Furthermore, exploring cost-efficient materials (e.g., paper-based devices), simpler fabrication processes and easier operation approaches will also undoubtedly continue to bring the cost associated with these devices down, which will also further facilitate the feasibility of on-site and point-of-use applications.

But compared to their use as sensors and analyzers, microfluidic devices offer even more remarkable advantages as research platforms for environment-related process investigation. Their miniaturized size and the flexible configurations for realizing various functions provide them with unique capabilities for visualizing and unveiling the secrets of numerous environmental-related processes, which are not comparable by other approaches. Therefore, we believe the future growth of lab-on-a-chip devices as research platforms will be focused on exploring novel and clever designs to realize more functions based on different investigation purposes. Nano structures, such as nanoholes, nanoparticles, nanowires, and coating layers with nanoscale thickness, are providing lab-on-a-chip devices with more features and functions. Nanofabrication techniques, including electron beam lithography and atomic layer deposition, are becoming widely used for chip fabrication. In addition, more and more lab-on-chip devices for research purpose are not restricted to standard chamber or channel on-chip configurations. Various 3D geometries are enabled by thriving 3D fabrication techniques, such as 3D printing, two photon polymerization, and micron/submicron stereolithography. To improve the capabilities of lab-on-a-chip devices, the performance of their basic functions, such as flow control, cell manipulation, cell culturing, and target tracking, is also worth improving. Finding the environmental problems and processes that could be investigated using lab-on-a-chip platforms is also important. In addition to visualizing small-scale process, such as bacterial-related phenomena mentioned in Sect. 2.3, lab-on-a-chip systems are also ideal for mimicking and simulating large-scale ecological processes, such as fate and transport of nanoparticles in soil and groundwater [107, 108]. The findings of the on-chip simulations could provide valuable experimental data for modeling and further on-site studies.

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