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Discussion: Embracing microfluidics to advance environmental science and technology

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ABSTRACT:

Microfluidics, also called lab-on-a-chip, represents an emerging research platform that permits more precise and manipulation of samples at the microscale or even down to the nanoscale (nanofluidic) including picoliter droplets, microparticles, and microbes within miniaturized and highly integrated devices. This groundbreaking technology has made significant strides across multiple disciplines by providing an unprecedented view of physical, chemical, and biological events, fostering a holistic and an in-depth understanding of complex systems. The application of microfluidics to address the challenges in environmental science is likely to contribute to our better understanding, however, it’s not yet fully developed. To raise researchers' interest, this discussion first delineates the valuable and underutilized environmental applications of microfluidic technology, ranging from environmental surveillance to acting as microreactors for investigating interfacial dynamic processes, and facilitating high-throughput bioassays. We highlight, with examples, how rationally designed microfluidic devices lead to new insights into the advancement of environmental science and technology. We then critically review the key challenges that hinder the practical adoption of microfluidic technologies. Specifically, we discuss the extent to which microfluidics accurately reflect realistic environmental scenarios, outline the areas to be improved, and propose strategies to overcome bottlenecks that impede the broad application of microfluidics. We also envision new opportunities and future research directions, aiming to provide guidelines for the broader utilization of microfluidics in environmental studies.

Keywords: *Microfluidics, lab-on-a-chip, real-time in situ detection, microscale dynamics, environmental interfacial processes, microbial assay*

Microfluidics is an emerging miniaturized device for manipulating small volumes (10^{-3} to 10^{-12} μL) of fluids within micrometer-scale channels (Gao et al., 2023). Unlike conventional laboratory methodologies (e.g., column tests and pot experiments), microfluidic devices provide unique platforms to access microscopic, *in situ* visualized, dynamic outcomes by building reaction micromodels on-chip and integrating complementary analytical techniques like absorption spectroscopy (Moragues et al., 2024), electron microscopy (Torino et al., 2023), etc. In the past three decades, this lab-on-a-chip technology has contributed significantly to multiple disciplines ranging from chemical synthesis (Gimondi et al., 2023) and micro- and nanofabrication (Qi et al., 2023) to point-of-care diagnostics (Zhang et al., 2023), with an increasing number of publications every year (Figure 1a). Nevertheless, for environmental science and engineering, the incorporation of microfluidics is still at an early stage but will possibly revolutionize this field.

The below research area exemplifies to benefit the most from microfluidic technology both currently and in the near future, including i) miniaturized samplers or analytical devices for on-site environmental surveillance or rapid toxicity assessment of particular chemicals (Lu et al., 2024; Spatola Rossi et al., 2023; Tan et al., 2023; Yuan et al., 2024). It presents the most straightforward avenues for utilizing microfluidics in research endeavors addressing environmental challenges. For example, Aryal et al. first designed an inexpensive capillary flow-driven microfluidics combined with a paper device for fast user-friendly heavy metal detection in water (Aryal et al., 2023). This device can generate a consistent color signal within 8 seconds of sample insertion, featuring outstanding detection limits (e.g., 0.3 ppm for Cu(II). ii) controllable microreactors for exploring complex environmental interfacial processes (Jia et al., 2023; Wang et al., 2022; Xiao et al., 2023), which represents one of the most advanced yet underexplored frontiers of microfluidic technology for environmental science. Zhu et al. managed to employ a coupled microfluidic reactor and an online high-resolution mass spectrometry system to elucidate the intricate dynamics of dissolved organic matter with iron oxyhydroxide at a molecular level (Zhu et al., 2023). and iii) portable cultivation and analysis tools for high-throughput microbial screening and bioassays (Burmeister and Grünberger, 2020). As an illustration, Liu et al. established a protocol enabling cell cultivation within specialized microfluidic devices while allowing the time-resolved measurement of intracellular reactive oxygen species (ROS) levels following exposure to ambient particulate matter (Liu et al., 2020). This subcellular data would enable a direct reflection of cellular health and functionality, encompassing metrics such as the fraction of responsive cells, as well as the visualization of dynamic response behaviors and distributions at single-cell resolution — an ability currently unavailable in alternative assays like plate reader or flow cytometry. Despite these advances, there still exists a risk of errors and inaccuracies arising. Therefore, it is crucial to exercise caution and critically examine its role in environmental research.

This discussion aims to draw from our microfluidic research experience and demonstrate how this cutting-edge tool can be leveraged for tackling environmental question concern areas while also highlighting current challenges and opportunities. We hope to stimulate researchers' interest in microfluidics to advance environmental science.

■ WHAT WE FOUND VALUABLE AND WHERE WE SEE POTENTIAL

***In Situ* Real-Time Monitoring and Analysis.** Using microfluidics as *in situ* sensors or analyzers to provide point-of-use environmental monitoring (e.g., contaminants, nutrients, microorganisms, and microplastics), has attracted increasing interest over the past few years (Cioffi et al., 2021; Faramarzi et al., 2024; Yang et al., 2020; Yuan et al., 2023). Traditional measurements of natural environments entail manual sample collection followed by centrale laboratory analysis, which is burdened by time-consuming and labor-intensive processes, as well as bringing about inherent risks of sample contamination and transformation during the transportation process, or need cold-chain storage which increase the cost (Hara and Singh, 2021). Microfluidic devices present a multitude of significant advantages over traditional methods and are reshaping environmental analysis with their enhanced sensitivity, and rapid and multiple analysis capabilities. Microfluidic very significantly helps in reducing sample and reagent consumption, thereby contributing largely towards the associated costs and waste disposal management. Microfluidic technology circumvents intermediate steps (such as enrichment, separation, and purification) between sampling and determination, fundamentally eliminating uncertainties inherent in these processes. This substantially enhances the accuracy and reliability of environmental monitoring data. Furthermore, it empowers precise manipulation of sample volumes and reaction parameters, offering a distinct advantage in detecting trace contaminants from complex environmental samples. For example, microfluidics was utilized to separate and detect a list of organophosphate compounds in river water and achieved a limit of detection in the micromolar range (Kempa et al., 2020). As a high-throughput sensor, the microfluidic chip is particularly valuable for analysts who need to quickly extract extensive information from complex environments. Kamnoet et al. fabricated microfluidic paper analytical devices (μPADs) featuring dual pretreatment zones, allowing for the simultaneous determination of Cu(II), Co(II), Ni(II), Hg(II), and Mn(II) (Kamnoet et al., 2021). Of note, certain colorimetric detection-based chips have remained confined to detecting only one type of analyte, such as macromolecules or micromolecules. By using a UV exposure method, Xiong et al. constructed a μPAD to enable simultaneous multiplexed detection of heavy metal ions Fe(III), Ni(II), and proteins (Xiong et al., 2020). Another valuable feature lies in the integration of multiple analytical functions, such as sample pretreatment and detection hardware, onto a single chip. This capability greatly streamlines analysis protocols and enhances workflow efficiency compared to conventional field-to-lab procedures. For example, several pilot studies have showcased the remarkable efficiency of spectrophotometric (Wu et al., 2020), chromatographic (Li and Chang, 2020), and electrochemical (Song et al., 2022) microfluidic setups for continuous water quality monitoring. Moreover, they can also be coupled with wireless technologies e.g., smartphone apps, unmanned aerial vehicles, and digital control circuits (Chen et al., 2023a; Pal et al., 2022; Sun et al., 2018; Xiong et al., 2022; Zhang et al., 2024), facilitating long-term data acquisition and real-time monitoring for on-site environmental surveillance in remote or hazardous areas. For instance, in a recent study, Ozer et al. engineered a portable sensor by amalgamating ion-selective electrodes, Internet of Things (IoTs) technology, and solar panels onto an automated sampling microfluidic chip for *in situ* and long-range monitoring of ferrous ions in acid mine drainage (Ozer et al., 2024). However, it is worth noting that the implementation of this smart sensing platform in soil analysis have significantly lagged compared to water and air, possibly attributed to the technical challenges in handling inhomogeneous solid matrices on chips.

Microreactors for Probing Dynamic Processes. The core idea behind microfluidics fabrication is to elucidate transient microscale phenomena by rationally simulating real reaction environments on chips, enabling more profound insights into the macroscopic processes, which are not comparable to other traditional bulk-scale tests (e.g., batch sorption experiments). This exciting feature makes it particularly valuable as a research platform for exploring complex systems characterized by high levels of structural heterogeneity, such as the heterogeneous catalytic oxidation driven by nanomaterials (Dong et al., 2022) and interfacial processes occurring in soils e.g., microbes, roots, minerals.(Lee et al., 2022; Wielinski et al., 2022; Zhu et al., 2024; Zhu et al., 2023). For example, the microenvironments (e.g., pH, pores, charged soil aggregates) in artificial soil chips (also called "soil-on-a-chip") can be well-fabricated and precisely controlled by multiple techniques, decoupling complicated subsurface heterogeneities into a simple and well-demarcated layout (Walton et al., 2022). It is helpful for biogeochemical researchers who need to minimize the inherent complexity and uncertainty when dealing with real soil samples while digging into molecular-level mechanisms and evidence under defined boundary conditions. More than that, the flexible integration of microfluidics with a range of advanced imaging and analytical instruments creates new avenues for obtaining *in situ*, visualized, and dynamic information, surpassing the limitations of relying solely on effluent analysis for endpoint data. Researchers focused on plant and microbial ecology can benefit greatly from this capability. For example, our group previously developed a microfluidic rhizosphere chip and combined it with confocal microscopy, which allows us to directly observe how extracellular ROS fluctuate periodically, bringing us one step closer to what happens in the "black box" of real soil (Figure 1b) (Dai et al., 2022). In the study by Walton et al., they introduced a novel methodology for the *in situ*, spatiotemporal measurement of amino acids from bacterial biofilms and plant root exudates via liquid microjunction-surface sampling probe-mass spectrometry (LMJ-SSP-MS) in conjunction with a porous membrane microfluidic framework (Walton et al., 2022). The entire platform being optically transparent facilitates the correlation of chemical signal maps with brightfield and fluorescence images of the microfluidic habitat and its inhabitants. Uniquely, to better reflect the rhizosphere-like environment without harming its function, *Populus* was cultivated in soil-analogous microfluidic habitats and chemically imaged. Of particular interest to environmental chemists is the innovative concept of "catalysis-on-a-chip", which has been recently devised for addressing refractory organic pollutants (Chen et al., 2023b; Wang et al., 2023). In the realm of treating urban wastewater effluents using electrochemical advanced oxidation processes, conventional undivided macroreactors frequently encounter limitations. These include higher overall power consumption attributed to significant ohmic drops between electrodes and the necessity of adding extra electrolytes to facilitate the mass transfer of pollutants. Fortunately, the utilization of microfluidic configurations enables the enhancement of inherent mass transfer without the necessity for supporting electrolytes, greatly augmenting removal efficacy while simultaneously reducing operational costs (Adnan et al., 2023; Hakizimana et al., 2023; Lan et al., 2021).

Bioanalytical Platforms. As a versatile and automated platform, microfluidics has started to drive a new direction that may miniaturize and innovate environmental biochemical research. The past decade has witnessed encouraging achievements in using microfluidics for mining the microbial "dark matter" with specific functions, for example, droplet microfluidic enables the cultivation of uncultured microorganisms from environmental samples by confining microbes individually in droplets (Liu et al., 2023). Alternatively, adopting a microfabricated approach to traditional bioanalytical methods presents unprecedented prospects for mitigating the high-throughput screening costs by, for example, conducting millions of parallel analytical tasks on an inexpensive chip-based device. Most importantly, however, these innovative platforms allow for precise control over the physicochemical environment during the spatiotemporal analysis of molecules, cells, tissues, and small model organisms that cannot be achieved with conventional equipment. Furthermore, another minor but growing direction is that the microfluidics is enabling lower costs while providing the ability of single-cell resolution imaging (Zheng et al., 2022) and straightforward integration with laboratory infrastructure (e.g., mass spectroscopy) has sparked increasing interest among ecotoxicological researchers. Some notable recent examples include *in vitro* cell-based toxicity bioassays using bacteria and algae, as well as in vivo toxicity analysis using crustaceans and lower vertebrate embryo models (Xu et al., 2020).

■ WHAT IS CHALLENGING AND WHERE WE COULD IMPROVE

Microfluidics, while yielding striking microscopic breakthroughs by reducing environmental complexities, also raises concerns about the reliability of this simplified artificial device. Extensive efforts have been made to extract representative natural parameters, for example, modifying microfluidic channels with specific beads to mimic soil geometries or injecting nutrient solutions to emulate rhizosphere habitat (Guo et al., 2020). Nonetheless, it is not technically feasible to precisely replicate the entire real environment on a chip, consequently increasing the risk of erroneous or false outcomes. Given these facts, when designing microfluidic devices, researchers should firstly consider which environmental characteristics need to be highlighted according to their specific scientific requirements (Figure 1c). There is no universal chip for all scenarios, we thus call for extensive reference experiments to verify the stability and precision of the device across varying environmental contexts. Also, cross-validating microfluidic results against both field experiments and theoretical calculations is recommended to facilitate the holistic grasp of intricate reaction systems at diverse scales.

Inherent microscale structural properties determine that microfluidics may introduce inherent biases, including the occurrence of microchannel clogging caused by particles or biological samples, along with the formation of small bubbles, which can disturb fluid flow and affect the accuracy and reproducibility of results. Particularly, enhanced demands on meticulous operation (e.g., filtration and sterility) arise when conducting biogeochemical studies or field monitoring on a chip. Similarly, due to their small internal volumes, it's typically challenging for microfluidic devices to meet the minimum sample volume requirements of mainstream analysis instruments, rendering both sampling and multiplexing difficult to control. Indeed, researchers have proposed seemingly viable strategies, such as prolonging the individual sampling time or increasing the flow rate, unfortunately, this also brings traps of the limited precision of time-resolved data or being not environmentally relevant. In our view, a principal solution is to further innovate suitable on-chip *in situ* analysis methods, such as exploring how to unite chips with microelectrodes or spectrometers via assembly. This could be more technically challenging, yet offer more informative data with precise spatial and temporal resolution. The robustness, operational performance, reliability, and applicability of the microfluidic system also require further improvement to effectively detect in complex environments e.g., aging, fouling, strong winds, and large waves (Figure 2).

Besides, compatibility concerns with specific chemicals or biological samples may pose limitations on the use of certain chips, such as glass and silicon fall short of fulfilling all the essential characteristics (particularly gas permeability) needed for handling live cells, necessitating the exploitation of alternative materials like paper-based microfluidic analytical devices (μPADs) (Cao et al., 2024; Zhou et al., 2021). The μPADs, typically crafted from cellulose paper substrates, are adept at handling liquid volumes ranging from 0.1 to 100 μL within millimeter-scale fluidic channels. Cellulose paper is highly acclaimed for its abundance, affordability, light-weight, and biodegradability, making it the most prominent natural platform in microfluidic applications (Silva-Neto et al., 2023). Despite potentially lacking the mechanical resilience of traditional materials like glass, silicon, or polymers, μPADs which do not need power and tubing to drive liquid, boast unique advantages compared to traditional microfluidics. Leveraging cellulose's inherent hydrophilicity and porosity, they exploit capillary action to drive fluid flow autonomously, eliminating the need for external pumps or power sources (Noviana et al., 2021). Moreover, various patterning techniques enable the creation of well-defined channels through several fabrication methods. The μPADs find particular favor in point-of-need scenarios, characterized by the necessity for rapid analysis, cost-effectiveness, and user-friendly operation. More importantly, the μPADs suit for the detection in the low-resource setting, e.g., low- and middle-income countries.

Despite the advantages of microfluidics in microscale environmental research for scientists from diverse backgrounds, unfortunately, its utilization has been limited to a few skilled laboratories due to the intricacy and specialized expertise involved in device fabrication and operation (e.g., photolithography and wet etching). Access to this lab-on-a-chip technology for newcomers is poised to grow as commercially available microfluidics continue to emerge, and universities establish an increasing number of micro/nanofabrication facilities (e.g., 3D printing) (Noviana et al., 2021). Exploring strategies that can aid in the simplification of operation processes, development of standardized protocols, and reduction of the cost barrier is also expected to inspire more researchers to integrate microfluidic approaches into their work. Moreover, assimilating and nurturing emerging techniques early on in the design process can deliver valuable knowledge concerning the desired size, portability, and pricing of the final product. Activities related to machine learning (ML) in this space are advancing rapidly (Lashkaripour et al., 2021; McIntyre et al., 2022; Moragues et al., 2023). One such example is electronic design automation software (from providers like Ansys and Cadence Design Co.), an open-source simulation tool that leverages ML to assist engineers in conceptualizing, testing, refining, and predicting circuits before chip fabrication. Beyond technical skills, the exchange of case studies and ideas on microfluidic devices through workshops, training sessions, webinars, or open-source websites is highly encouraged. Meanwhile, fostering interdisciplinary collaboration among diverse scientific domains—spanning fundamental researchers, technology development engineers, product designers, data scientists, business professionals, and policymakers—is pivotal for propelling microfluidics technologies and "lab-on-a-chip" innovation forward.

■ OUTLOOK

Microfluidic technology will offer great — perhaps even revolutionary — novel methodologies for future environmental research and analysis by automating and streamlining processes. Despite its utility, it is a simple fact that microfluidics remains in its infancy. Most of the works reported are proof-of-principle concepts in nature rather than demonstrations of real large-scale applications. Concerns like scalability or mass production arise because there is a need to bridge the gap between these concepts and real-world suitability. This gap is clearly evident across various sensing platforms where researchers often ignore or neglect testing the sensors in real environmental matrices. It is important to acknowledge that the performance and applicability of microfluidic devices tested in controlled lab conditions (i.e., Millipore water, controlled chambers, controlled soil conditions, etc.) may not always translate directly to real-world scenarios (Aryal et al., 2024). Firstly, as discussed, there is still a need for technical innovation to enhance analytical throughput, sensitivity, stability, and operational sophistication. For instance, detecting all major categories of emerging pollutants, particularly compounds exhibiting stark similarity in structural and chemical interaction properties (e.g., congeners and isomers) (Yuan et al., 2023), with a single chip is currently quite challenging. In this regard, the optimal integration of high-resolution, information-rich detectors and ML algorithms is poised to play a pivotal role in facilitating the swift execution of complex chemical and biological workflows with unrivaled efficiency and precision. Secondly, microfluidics is not a flawless vessel for replicating environmental interfacial processes in many scenarios, and thus it is critical to cautiously select the appropriate component materials capable of maximally emulating the features and biological function of natural conditions. In this regard, the emergence of bespoke surfactants signifies a notable recent breakthrough, effectively tackling both mass transport and adsorption of macromolecules at the chip interface (Chowdhury et al., 2019). Another recommended practice involves cross-referencing the results obtained from microfluidics with those derived from field trials, mesocosm tests, and computational modeling. Regrettably, few studies have bridged the gap between the processes observed in microfluidics and those occurring at the macroscale. Finally, the shortage of awareness and easy-to-follow procedural guidelines and protocols for integrating microfluidics technology into current works may be the major barrier impeding its broader adoption.

Going forward, we believe that microfluidics technology has already affirmed its paradigm-shifting potential, empowering environmental physicists, chemists, and biologists to reconsider and reconceptualize the structure and complexity of experimental workflows. We hope that this discussion has, to some extent, underscored the previously overlooked yet significant implications of microfluidics in environmental studies. As a largely unexplored tool, we should embrace and leverage its innovative capabilities to explore and advance research and development findings, while also exercising prudence to mitigate pitfalls and obstacles that may arise along the way. Effective analyses and data mining via the advanced and sophisticated microfluidic devices have tremendous potential to fill the information gap that existing technologies have so far failed to unravel. The extent to which microfluidics accelerates scientific discovery hinges on our actions and approaches. Hopefully, the discussion in this article will significantly contribute to advancements in microfluidics and global environmental resources, eventually inciting the trajectory towards "*The Rise of Environment-on-a-chip*".

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Competing interests

The authors declare no competing interests.

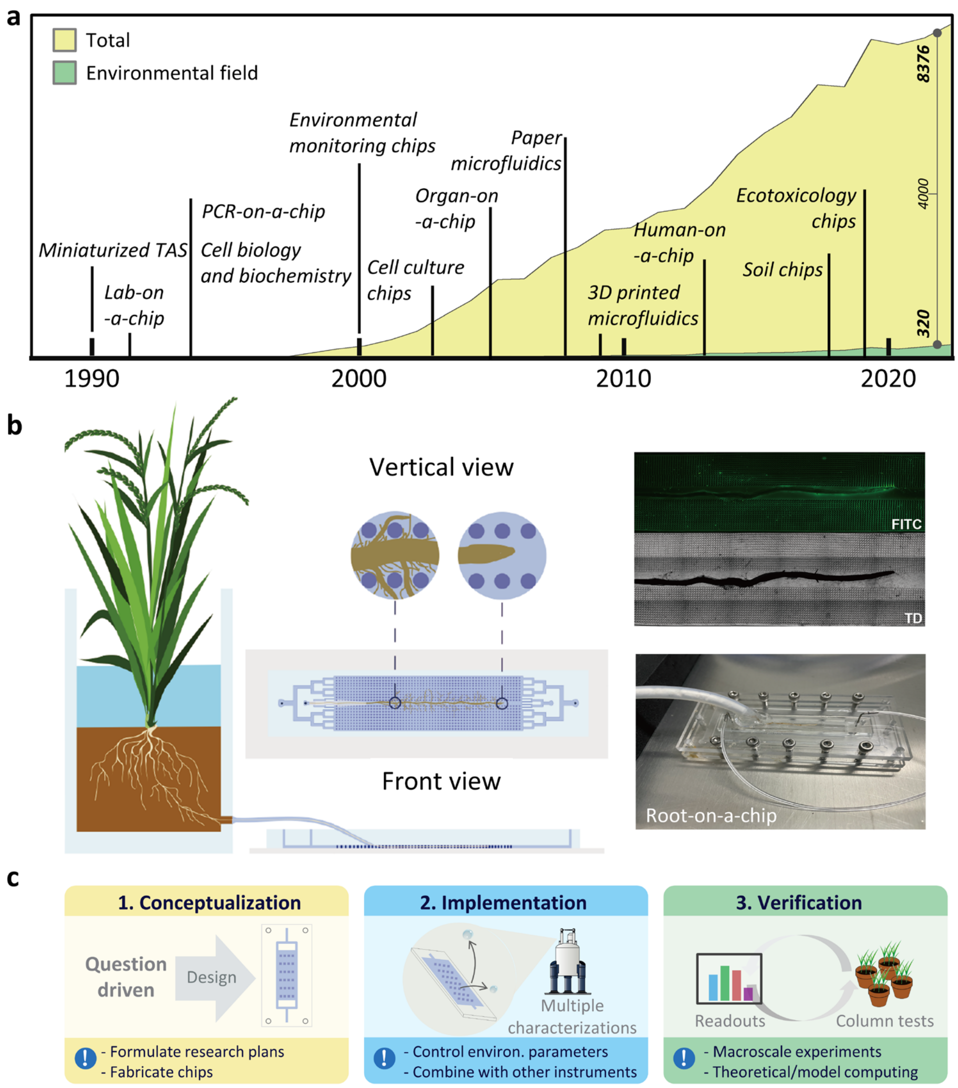

Figure 1. (a) Timelines of historic events in microfluidics. Filled areas depict number of yearly publications in Web of Science using "microfluidics" or "lab-on-a-chip" as search terms (last access: 28 October 2023; yellow: total publications; green: environmental field; TAS: total analysis systems; PCR: polymerase chain reaction). (b) A typical microfluidic chip, fabricated using polydimethylsiloxane (PDMS), is designed for the observation of reactive oxygen species (ROS) in the rhizosphere. (c) A conceptual framework for applying microfluidics in environmental research.

Figure 2. Proposed strategic goals and best practices for overcoming challenges in microfluidics.

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