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Microfluidic chips for microbially induced calcium carbonate precipitation: Advantages, challenges, and insights

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Abstract: Microbially induced calcium carbonate precipitation (MICP) has garnered significant attention as a biomineralization process with diverse applications spanning from construction to environmental remediation. To propel MICP research and deepen our comprehension of MICP mechanisms, microfluidic chips have emerged as potent tools offering precise control over environmental parameters and real-time observations. Herein, we explore the benefits and challenges associated with employing microfluidic chips as a platform for investigating MICP. The advantages of microfluidic chips lie in their capacity to create controlled microenvironments conducive to emulating specific conditions crucial for MICP. The high-throughput nature of these devices accelerates experimentation by facilitating simultaneous testing of various microbial strains and nutrient compositions. Throughout the MICP process, observations were made on the behaviors of both bacterial cells and $CaCO_3$ cementation. The inherent reduction in reagent consumption offered by microfluidics is both cost-effective and environmentally friendly. However, scaling up from microscale findings to practical applications necessitates careful consideration. Fully replicating the three-dimensional complexity and heterogeneous structures of the soil matrix, which influence microbial behavior, mineral distribution, and overall precipitation dynamics, using microfluidic chips remains challenging. Additionally, certain environmental complexities, including macroscopic soil components such as organic matter and various particle types, which significantly affect microbial activities and mineral precipitation patterns, may be difficult to replicate in microfluidic setups. However, microfluidic chips stand as invaluable tools for advancing MICP research. By addressing the advantages and disadvantages outlined here, researchers can harness the capabilities of microfluidic systems to unravel the intricacies of MICP, ultimately bridging the gap between fundamental understanding and real-world applications.

1.Introduction

Microbially Induced Calcium Carbonate Precipitation (MICP) stands out as a remarkable biotechnological process, emerging at the intersection of microbiology and geotechnical engineering. Understanding the primary mechanisms underlying MICP is pivotal for its effective application. This process relies on the metabolic activities of microorganisms, often specifically selected for their ability to produce enzymes such as urease [1]. These enzymes catalyze the hydrolysis of urea, releasing carbonate ions (CO_3^{2-}) that subsequently react with calcium ions (Ca^{2+}) in the surrounding environment. This chemical interplay ultimately leads to the precipitation of calcium carbonate, binding soil particles, and reinforcing geological structures [2].

$$\mathrm{CO(NH_2)_2 + 2H_2O \xrightarrow{urease} 2NH_3 + H_2CO_3} \tag{1}$$

$$\mathrm{2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^-} \tag{2}$$

$$\mathrm{HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O} \tag{3}$$

$$\mathrm{Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3} \tag{4}$$

The significance of MICP spans a diverse spectrum of applications, making it a subject of paramount importance in various fields. In the realm of geotechnical engineering, MICP has found utility in slope stabilization, ground reinforcement, and mitigation of liquefaction in seismic-prone regions [3–8]. Its ability to enhance soil strength without compromising permeability renders MICP an appealing choice for soil improvement projects. Furthermore, it has proven instrumental in repairing soil cracks and restoring historical structures and monuments [9,10].

MICP is a multifaceted biogeochemical process driven by microbial metabolism, mineral precipitation, environmental factors, microbial interactions, transport phenomena, temporal dynamics, spatial heterogeneity, and practical applications, [11–15] offering valuable advantages for effectively studying this complexity. Traditional MICP experimental methods typically involve post-process testing of the final mechanical and permeability properties of samples to detect the MICP transport reaction, lacking real-time dynamic detection capability. Traditional methods also fall short in elucidating the microscale mechanisms of MICP, making it challenging to visualize processes such as bacterial migration, distribution, and proliferation, as well as the nucleation, growth, and crystalline changes of calcium carbonate. Microfluidic systems confine liquids within channels ranging from microns to millimeters, thereby facilitating operation, flow, visualization, and analysis at the micron scale. Consequently, leveraging microfluidic technology to investigate MICP processes holds tremendous potential. Microfluidics enables real-time visualization of MICP processes, providing insights into temporal and spatial dynamics [16,17]. It also allows precise control over environmental conditions, facilitating the investigation of factors such as pH, temperature, and nutrient gradients on MICP. Furthermore, microfluidics operates at a microscale, making it an ideal tool for replicating and studying MICP processes at an appropriate scale. It generates highly detailed and precise data, [18]helping researchers unravel the intricacies of MICP mechanisms and interactions, and encouraging interdisciplinary collaboration among microbiologists, engineers, and geoscientists, thereby facilitating a comprehensive understanding of MICP complexity and its practical applications.

2.Background of Microfluidics

Microfluidics is a highly versatile and interdisciplinary field that involves the science and technology of manipulating and controlling fluids in channels or devices with dimensions ranging from micrometers to millimeters [19]. It has rapidly evolved from its early beginnings and has found applications in diverse research domains. Initially, microfluidics was not conceived for the study of MICP but rather emerged to address fundamental challenges in various fields [20,21]. The history of microfluidics can be traced back to the early 1960s when researchers began exploring the potential of small-scale fluid manipulation. The advent of microfabrication techniques, inspired by the semiconductor industry, played a pivotal role in the development of microfluidic devices [22,23]. The seminal work of George A. Whtpical et al. in the late 1980s and the early 1990s laid the foundation for microfluidics by demonstrating the capabilities of microscale channels and their potential for a wide range of applications [24,25].

Over the years, microfluidics has found relevance in numerous research fields. In analytical chemistry, it revolutionized sample handling, mixing, and analysis, enabling high-throughput screening and miniaturized laboratory-on-a-chip systems [26,27]. In biology, microfluidic devices have been instrumental in studying cell behavior, single-cell analysis, and DNA sequencing [28–30]. Additionally, microfluidics has made significant contributions to the fields of materials science, physics, and even environmental monitoring [31,32].

While microfluidics was originally developed for different applications, its precision and control over fluid dynamics have made it a valuable tool for investigating complex processes, such as MICP. Researchers have adopted microfluidic systems to simulate and analyze the intricate mechanisms of MICP in controlled laboratory settings [33,34]. These microscale platforms offer a unique advantage for studying how microorganisms interact with calcium ions, urea hydrolysis, and carbonate precipitation [35–37]. By providing a controlled environment, microfluidic experiments can yield valuable insights into the kinetics and factors influencing MICP, thereby enhancing our understanding of this biogeochemical process. Its precise control over fluids has opened up new avenues for studying complex phenomena, including MICP, contributing to advancements in various scientific disciplines.

3.Advantage of using microfluidics to study MICP

Microfluidics represents a powerful and versatile tool for investigating MICP mechanisms. Its precise control, real-time visualization capabilities, scalability, reproducibility, and high-throughput screening potential have revolutionized MICP research [38–40]. By leveraging these advantages, researchers can unlock the full potential of MICP for diverse applications ranging from soil stabilization to environmental remediation and contribute to the advancement of biogeochemical engineering. Over the last five years, the technology for utilizing microfluidic chips to study MICP has seen significant development. Wang et al. [37][18] pioneered the use of microfluidic chips in MICP studies to observe bacterial behavior and patterns of crystal change. Subsequently, various types of microfluidic chips have been gradually introduced into MICP studies, including droplet microfluidics [41], soil particle-filled microfluidic chips [42] and long-distance (1-meter) microfluidic chips (Figure 1).

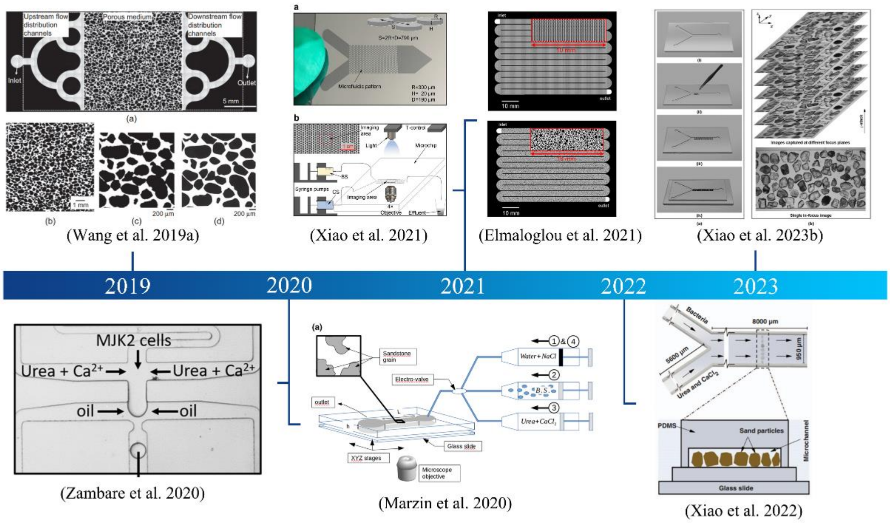

Figure 1. History of microfluidic chips applied to MICP research.

Microfluidic devices provide an unparalleled level of control compared to traditional MICP research methods. Researchers can precisely manipulate factors such as fluid flow rates, reactant concentrations, and environmental parameters like pH and oxygen levels [39,43]. This control enables the creation of well-defined microenvironments that closely mimic natural conditions, facilitating accurate and controlled MICP experiments. An advantage of microfluidics is its compatibility with real-time visualization techniques, including microscopy. Within microfluidic channels, researchers can observe and record MICP processes as they unfold at the microscale level. This dynamic visualization not only offers valuable insights into the temporal and spatial aspects of MICP but also enables the monitoring of microbial activity, mineral nucleation, and growth [34,43,44]. Microfluidic systems inherently operate at the microscale, aligning with the dimensions of MICP processes. This feature allows for the detailed investigation of microbial behavior, urea hydrolysis, and mineral precipitation on the same scale as they occur in natural settings [35,44]. Microfluidic platforms can be adapted for high-throughput screening of various microbial strains, environmental conditions, and reactant combinations [17]. This accelerates the discovery process by enabling researchers to rapidly identify the most efficient MICP-inducing conditions and microbial strains [35]. Optimizing MICP parameters is crucial for practical applications, and microfluidic devices facilitate this optimization by simulating diverse environmental conditions relevant to MICP applications. Researchers can recreate the complexities of soil, groundwater, and subsurface reservoirs to closely mimic real-world scenarios [45,46]. This capacity for environmental simulations provides critical insights into the applicability and adaptability of MICP in geotechnical and environmental settings [47]. With the help of microfluidics, researchers have gained high-resolution insights into the intricacies of MICP mechanisms, thereby enhancing our understanding of this process. The mechanisms of MICP that can be investigated include bacterial growth after injection (Figure 2a), bacterial aggregation and detachment after the injection of the cementation solution (Figures 2b and c), the correlation between bacterial distribution and $CaCO_3$ distribution (Figure 2d), the growth of $CaCO_3$ crystals with the injection of the cementation solution (Figure 2e), and the crystal morphology and bacterial traces in the microfluidic chip under a scanning electron microscope (Figure 2f).

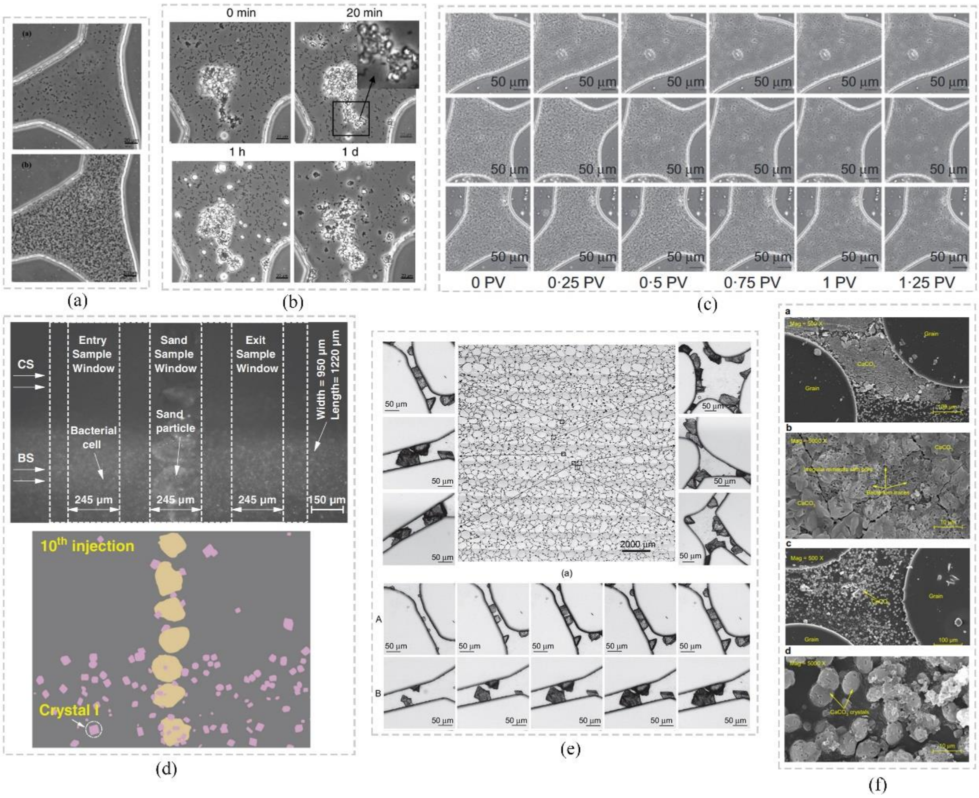

Figure 2. Mechanisms of bacterial behavior and calcium carbonate precipitation revealed by using microfluidic chips: (a) Bacteria continue to grow within 24 h after injection into a microfluidic chip [43]; (b) bacteria aggregation in a microfluidic chip [48]; (c) detachment of bacteria with the injection of cementation solution [18]; (d) distribution characteristics of bacteria and crystals in a microfluidic chip [35];(e) crystal growth with the injection of cementation solution [18]; (f) crystal morphology and bacterial traces in the microfluidic chip under scanning electron microscope [38].

4.Challenges related to MICP study by using microfluidics

Microfluidics holds tremendous potential for advancing our understanding of MICP mechanisms. However, researchers must navigate various challenges related to microbial compatibility, device design, scaling up, heterogeneity, data interpretation, environmental realism, and long-term studies. Addressing these challenges is essential for harnessing the full potential of MICP in geotechnical and environmental applications, offering innovative solutions for soil stabilization, groundwater remediation, and more.

While microfluidic studies provide valuable insights into MICP mechanisms, one of the primary challenges is the significant scale difference between microfluidic devices and the natural environment where MICP occurs. Microfluidic channels are typically on the order of micrometers to millimeters, whereas MICP processes in the field take place at a much larger scale, often involving soil volumes measured in cubic meters [49,50]. This scale discrepancy can result in differences in fluid dynamics, transport phenomena, and reaction rates [51]. For example, the large-scale distribution of bacteria is affected by transportation distance, time, and complex geological structures, which cannot be easily simulated using microfluidic chip experiments (Figure 3). Bridging the gap between microscale experiments and field-scale implementation necessitates careful consideration of factors such as material compatibility, microbial growth, and environmental conditions.

Microfluidic studies of MICP often involve the use of specific microbial strains. Challenges arise in maintaining microbial viability within microchannels because the confined environments may differ significantly from natural habitats. Ensuring compatibility between microbial requirements and microfluidic conditions is crucial for obtaining representative MICP results. Additionally, in natural environments, MICP processes often involve diverse microbial communities with intricate interactions [49,53]. For practical reasons, microfluidic studies may use simplified microbial populations or monocultures, which can neglect the complex interplay among microorganisms, potentially leading to deviations in MICP behavior.

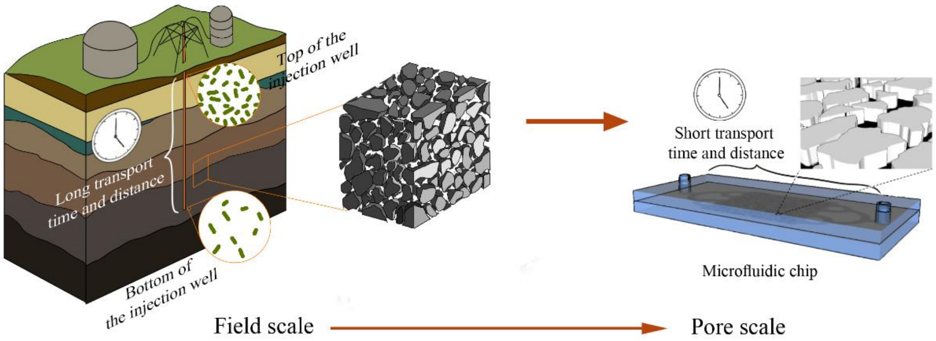

Figure 3. Schematics of MICP in field and pore scales.

Microfluidic devices often simplify [34] the complexity of natural MICP environments. Soil properties, such as grain size distribution, mineral composition, and porosity, can vary widely in natural settings. Achieving realistic heterogeneity in microfluidic setups can be challenging because natural environments exhibit significant variability in factors such as pH, nutrient availability, and microbial populations [54]. Researchers must carefully design microfluidic experiments to capture this heterogeneity realistically. However, microfluidic studies can sometimes lack full environmental compatibility, potentially leading to deviations from real-world MICP processes. For example, natural MICP environments in soil or groundwater exhibit spatial and temporal heterogeneity in parameters such as pH, nutrient concentrations, and microbial populations. In contrast, microfluidic studies may oversimplify these factors, leading to deviations from the dynamic and heterogeneous conditions found in real-world environments. Ensuring that microfluidic experiments accurately replicate the environmental conditions relevant to MICP applications is crucial for their practicality.

Many microfluidic experiments are conducted over relatively short timeframes due to practical constraints. In field MICP processes, MICP can occur over longer periods, potentially spanning months or even years [57,58]. The differences in temporal scales can impact the kinetics and efficiency of MICP and may not accurately represent real-world scenarios.

5.Conclusions

This article highlights the critical role of microfluidics in advancing our understanding of MICP. Microfluidic systems offer precise control over environmental conditions, enabling researchers to investigate how factors like pH, temperature, and nutrient gradients influence MICP. Real-time visualization capabilities within microfluidic channels provide insights into the temporal and spatial dynamics, microbial interactions, and mineral nucleation and growth. Furthermore, microfluidics facilitates the high-throughput screening of microbial strains and environmental conditions, expediting the optimization of MICP parameters for practical applications. Nonetheless, employing microfluidic technology to investigate MICP still presents several challenges. These challenges encompass bridging the substantial scale disparity between microfluidic experiments and natural MICP environments, ensuring the vitality of microorganisms within microchannels, replicating the intricate microbial communities existing in the natural environment within microfluidic chips, and addressing the temporal-scale differences between microfluidic experiments and field-based experiments, among others.

By addressing these challenges and continuing to innovate in microfluidic design and experimentation, researchers can unlock the full potential of MICP as a transformative biotechnological tool for geotechnical and environmental engineering. Designing microfluidic devices that mimic natural MICP environments while offering precise control is a non-trivial task [35,59]. Achieving the right balance between complexity and simplicity, as well as accommodating different types of experiments, requires careful consideration. To address the current challenges, advancements in the fabrication process of microfluidic chips are required to meet the experimental demands of MICP. Meanwhile, it is essential to integrate microscale experiments with macroscale experiments, wherein microscale mechanisms and macroscale responses are closely interconnected and mutually validated. This integration serves as a bridge between on-site MICP experiments and large-scale MICP applications. Despite these challenges, the integration of microfluidics with MICP research holds immense promise. It offers a pathway for enhancing our understanding of MICP mechanisms and optimizing its applications in soil stabilization, concrete crack repair, and other geotechnical and environmental endeavors.

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