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Emerging Applications of Droplet Microfluidics in Plant Diagnostics and Pest Management

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abstract

Droplet-based microfluidic platforms has witnessed significant progress in the recent past. In droplet microfluidics, single and discrete droplets are operated on a flat, hydrophobic platform by on-chip electrical forces. The droplet operations can be in various configurations designed to implement algorithms and process flows for the droplet generation, transport, mixing, dispensing, and splitting on the same platform. The discrete droplets provide a versatile compartment to encapsulate microscale particles and organisms while exposing them to a multitude of chemicals or biological reagents. There are a range of new experiments in plant pathology and integrated pest management that benefit from the several advantages of droplet microfluidics, including high throughput screening, automated manipulation of droplets, and real-time imaging of chemical processes at microscale. Within agriculture, droplet microfluidics has proven to be a flexible and versatile platform for behavioral phenotyping of microorgansims, understanding host-pathogen relationships, and discovering new chemicals and natural pesticides for pest control in a sustainable manner. In this review, we cover the abovementioned topics pertaining to the existing and emerging applications of droplet microfluidics within the field of agriculture.

Introduction

Droplet-based platforms have emerged as a microfluidics testbed to generate, manipulate, transport, splot, merge, and split microscale and macroscale droplets within microchannels or open platforms [1-5]. Droplet microfluidics enables forming discrete droplets using different fluidic methods and a variety of channel configurations. In one common configuration, an aqueous and oil phase fluid are brought together in a T-shaped channel at very high speed and controlled fluid flow, thereby generating droplets of aqueous fluid. Flow-based channels pinch off a stream of fluid into discrete droplets moving within a different fluid which is typically oil. After generation of droplets, these droplets can encapsulate a variety of experimental particles to carry over a set of defined operations such as merging of two droplets, splitting of one droplet into multiple droplets, sorting of droplets according to color or fluorescence change, and trapping of specific droplets using electrical forces. These droplet operations employ fluidic forces or electrical forces, such as surface tension, electrophoresis, and dielectrophoresis to allow careful positioning and movement of the droplets over the droplet platform [1-10].

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Droplet microfluidics has been developed to tackle a number of problems in bioengineering and chemical engineering [7-20]. As an example, we can carry out chemical reactions in discrete droplet on the platform by mixing droplets having different reagents at microscale to observe the reaction kinetics with greater detail. In medical diagnostics, droplet microfluidics can be used for testing new drug delivery applications for biological assays, including high throughput screening of new drugs, drug efficacy testing, single cell imaging and analysis, and monitoring microorganisms in micro-environments. There is engineered accuracy and fluidic control over the size, content, and delivery of every droplet. There is also minimal consumption of reagents which are often expensive and costly to purchase in large quantities. Every droplet in the platform plays the role of its own mini-reaction chamber which provides access to high-resolution imaging of reactions and avoids contamination between two different droplet chambers.

Digital Microfluidics

Digital based microfluidics is one version of droplet microfluidics where high electric fields are used to manipulate and operate droplets. The external electric fields are generated by on-chip electrodes using electrical signals generated by a signal generator off-chip [11-20]. There is no need for fabricating microchannels in PDMS through which fluid flow is established and that is usually employed in conventional microfluidics. Digital microfluidics can employ some options for generating and using electrical forces, typically by using electrowetting of surfaces or dielectrophoresis, which are easier to operate with and provide added flexibility of performing various droplet operations for a number of research and other applications.

Digital Microfluidics: Methods of Droplet Operations

Digital microfluidics can be categorized into two types based on the nature of forces being created on the platform. In electrowetting, the surface tension between the droplet and the surface is manipulated which helps to immobilize or move the droplets in a controlled fashion. A series of interdigitated electrodes can be fabricated on-chip to control the surface tension on the platform by addressable electrodes and appropriate electric fields [2, 17]. Apart from electrowetting, dielectrophoresis is an alternate method where alternating or non-constant electric fields are creating on-chip to trap and release particles that are inherently charged, such as biological cells and other biological specimens.

Digital microfluidics consists of an array of electrodes that are fabricated in a solid substrate such as glass [17-22]. The electrodes are patterned in an interdigitated manner that can individually addressed using an external signal source. The solid surface is coated with ahydrophobic material such as Teflon or paralyne to avoid stiction and allow free flow of droplets without frictional forces. A dielectric layer is placed between the electrode array and the droplets to protect the array of electrodes while providing the desired electric field for droplet operations. There is often a top plate to cover and encapsulate the droplet platform to avoid evaporation of the droplets over time and to avoid any contamination. The entire assembly is made robust and reliable to withstand mechanical pressure.

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Digital microfluidics gives the advantage of precise and flexible control over droplet manipulations where the platform can handle a range of droplet compositions and operations [15-22]. The pattern of electrodes and droplet process flow sequence can be altered based on accompanying software which for adaptable, flexible, and digitally programmable operations for improved capability and reconfigurability. As is the case for conventional microfluidics testbed, digital microfluidics uses small volume of bio-reagents, which reduces experimental costs and laboratory waste. The on-chip integration of interdigitated electrodes on the dielectric eliminates the requirement of actual microscale fluidic channels which streamlines the micro device fabrication and reduces the chances for clogging or cross-contamination.

Digital Microfluidics and Droplet Operations

Digital microfluidics have found a variety of applications [22-26]. In biological assays, they can be used to perform DNA amplification, cell sorting, and other biochemical assays.In chemical assays, they can be used to conduct precise, small-scale chemical reactions. In diagnostics, they can be used to develop point-of-care diagnostic tools and lab-on-a-chip devices for blood analysis or genetic manipulations.

Digital microfluidics enables a range of droplet operations, including droplet generation, droplet movement, merging of droplets, splitting of one droplet, and mixing of two droplets. In droplet movement and translocation, discrete droplets are transported across an electrode array by carefully activating the array of electrodes beneath them. Droplet merging involves bringing two or more droplets together by controlling the voltage of array of electrodes which results in the formation of a bigger droplet which can be transported later. Droplet splitting occurs when a single, larger droplet is split or physically divided into two or more smaller droplets using a high energy. For droplet mixing operations, droplets are brought together, merged, and mixing by repeatedly and recursively merging and splitting them or by moving them between two locations over the interdigitated electrodes.

Digital microfluidics has found applications in a variety of scientific fields. In biology, digital microfluidics have been widely used for genomics studies, DNA amplification, PCR studies, single cell analysis, and other biomedical applications. In the field of chemistry, digital microfluidics allows us to observe chemical reactions at a much smaller scale to observe and discover new chemical kinetics and process techniques that were hard to observe in macroscale experiments. In diagnostics, digital microfluidics have been attempted for a number of lab-on-chip experiments to detect and diagnose biomarkers of diseases from blood, saliva or urine samples.

Droplet microfluidics for Agricultural Pest Management and Plant Pathology

Droplet microfluidics offers significant advantages for plant pathology applications, including high throughput, precision, and low reagent consumption. These capabilities enable researchers and practitioners in plant pathology to conduct more precise, efficient, and comprehensive analyses, enhancing the understanding, diagnosis, and management of plant diseases.

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Droplet microfluidics has several key applications in plant pathology, including chemical screening, real-time imaging of host and pathogen interactions, sensing of various chemicals, and genomics. It enables the faster screening of plant specimens for agricultural pathogens where each discrete droplet acts as an isolated micro-chamber for faster screening. Bio-techniques used in genomics, such as polymerase chain reaction (PCR) and real-time quantitative PCR, can be conducted within discrete droplets, that allow for higher sensitive and more specific detection and identification of the pathogen's genetic material (DNA, protein or mRNA). Fluorescent probes can be developed to be placed within droplets to signal the presence or absence of specific pathogens and provide real-time detection and diagnostics. Additionally, the integration with electro- and microchemical on-field sensors allows for the detection and identification of pathogen linked proteonomic or metabolic material that is specific to the pathogen. In single cell genomics for biomedical research, droplet microfluidics can be used to segregate and isolate individual cells from plant or animal tissues for subsequent genomic, proteonomic and metabolonomic analysis, which helps in the investigations of host and pathogen interactions, identification of resistance pathways, mutagenesis, and tests of natural pest control methods [15-28].

Droplet microfluidics facilitates the study of host-pathogen interactions by creating controlled microenvironments, enabling researchers to observe infection mechanisms and defense responses in real-time. In chemical screening, this technology can be used to identify pathways, toxins, or pesticides that impact plant health. Its high-throughput capabilities allow for the screening of numerous chemical compounds, aiding in the discovery of new treatments for plant diseases. Additionally, droplet microfluidics can profile microbial communities associated with healthy and diseased plants, offering insights into the microbiome's role in plant health and disease. For environmental monitoring, portable droplet microfluidic devices can be used for on-site detection of plant pathogens in agricultural fields. The technology is also applicable in water and soil quality testing to prevent disease outbreaks and ensure healthy crop growth.

Droplet microfluidics is particularly useful in plant pathology because of its high sensitivity and specificity, which allow for the detection of pathogens even in low abundance [21-29]. This technology requires only small amounts of samples and reagents, minimizing the need for extensive plant tissue and chemical usage in tests. Its flexible design enables rapid and parallel processing, making it ideal for large-scale studies and quick disease management. Moreover, droplet microfluidics reduces the risk of contamination since each sample is contained within individual, isolated droplets, leading to more accurate results compared to traditional microfluidic systems [29-33].

Nematode Behavioral Analysis in Droplet Microfluidics

Droplet platforms have found exciting applications in nematology where nematodes or roundworms are assayed for various biological and chemical experiments [5-19]. Droplet microfluidics offer the benefits of high-throughput screening of microorganisms in discrete droplets in miniaturized compartments which mimics and scales down the traditional agarose plate assays. As nematodes are quite prevalent in biology and agriculture, droplet microfluidics have found

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applications in understanding the neuroscience of microorganisms and developing innovative pest management control techniques that can speed up experiments [22-34].

The detection and diagnostics of nematode pests is an ongoing area of research [11-20]. One topic is identification of relevant nematode to develop control strategies. This requires soil sampling and fast screening of the nematode type using droplet based approaches using PCR. Droplets can serve as reservoirs for DNA sequencing and DNA amplification which has value in genomics. Furthermore, it is possible to identify and characterize the bacteria and viruses within nematodes using droplet technologies. DNA techniques can help understand the role of mutatiosn and resistance offered by nematodes, and eventually develop natural control strategies to ward of the harmful nematodes [28-37].

Behavioral assays can be created with controlled microenvironments within droplets to study nematode behavior, such as movement, feeding, and mating, under different conditions [11-16]. Furthermore, the response of nematodes to chemical stimuli, including pesticides, attractants, and repellents, can be studied in droplets [22-30]. For toxicological studies, there is a need to screen large libraries of chemical compounds for nematicidal activity [30-39]. Each droplet can contain a single nematode exposed to a specific compound, allowing for rapid and parallel toxicity testing. Furthermore, it is worthwhile to investigate the mode of action of potential nematicides by observing their effects on nematode physiology and behavior in droplets. Droplets can be used to mimic soil environments to study nematode interactions with plant roots, including penetration and feeding behavior. Droplets can be used to screen nematode effector proteins (secreted proteins that manipulate plant processes) to understand their role in plant-nematode interactions and plant defense mechanisms. Droplets can be used to screen plant varieties for resistance to nematode infestations using droplet microfluidic systems to test interactions between nematodes and different plant genotypes. Droplets can be used to identify and characterize genes in plants that confer resistance to nematode attacks, aiding in the development of resistant crop varieties. Droplet microfluidics can be used to profile the microbial communities associated with nematodes to understand their role in nematode biology, pathogenicity, and interaction with host plants. This can also further studies on nematode embryogenesis, development, and growth in droplets, providing detailed insights into their life cycle and developmental stages.

Current Challenges and Future Scope for Droplet Microfluidics

Droplet testbeds face several challenges that are addressable with further research [2- 17]. The stability of droplets on platforms is a concern, and it is important to find methods and materials to make droplets stable and stop them from mixing or merging together. There is a need to design new methods and fabrication techniques to perform complex fluid manipulation steps in a robust and reliable manner. There has been several recent developments in artificial intelligence and edge computing that can be leveraged in droplet microfluidics that serve to optimize the process flow of droplet operations, such as mapping the steps of droplet generation, droplet transport, and droplet mixing for on-chip assays. Digital microfluidics employs A.C. and D.C. electric fields to

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manipulate droplets without the need for microfluidic channels. Similarly, complex structure scan be created using 3D printers that were not possible with traditional fabrication techniques.

Conclusion

Droplet microfluidics is a promising technology with possible applications in chemical diagnostics, high throughput drug screening, environmental field deployable monitoring, and synthesis of new materials [26-30]. Its ability to manipulate small fluid volumes in a precise and controlled manner is valuable in research and education. Droplet microfluidic platforms offer a versatile tool for teaching students the art of microfluidics and the skills to design experiments using the basic principles of physics and chemistry. The applications of droplet microfluidics in plant pathology and nematology is emerging, and a number of experiments can now be conducted in droplet microfluidics where high throughput and accuracy is need, along with low cost, design flexibility, and reconfigurability of experiments.

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