2019 ASEE Midwest Section Conference
(Wichita State University-Wichita, KS)

Microfluidics-based Learning and Analysis for Plant Cell Studies

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Abstract

Microfluidics is a new field of technology and science, which brings all the disciplines together, such as physics, chemistry, biochemistry, engineering, nanotechnology, and biotechnology. It manipulates the fluids between microliters (10^{-6}) and picoliters (10^{-12}) in the channels through dimensions from tens to hundreds of micrometers. The attributes of a microfluidic system, such as extensive surface-to-volume proportions, laminar stream, surface tension and capillary effects at the micrometer scale are significant and make the gadgets proficient for the processes as well as investigating complex biological examples. Microfluidic devices are emerging as innovative systems, which can significantly alter sustenance, agriculture, and biosystems enterprises. The standards of electrokinetics, electro-hydrodynamics, and thermo-capillarity of microfluidic systems help take care of significant logical issues that are difficult by regular systems and innovations. The discrete advantages of microfluidic technology over the conventional fluidic systems are low-cost fabrication, development of analytical performance, low power and chemical requirement, and better control and biocompatibility. The benefits offered by miniaturization are quick response, short reaction times, low sample and reagent volumes, reduction of the size of equipment, possibility of portable devices, and parallel tasks for numerous examinations. This paper highlights the manufacturing processes, characteristics of microfluidics, their applications in plant cells, as well as educational use of these devices and technologies in engineering fields.

Keywords: Microfluidics, Plants, Microorganisms, Cell Growth, STEM Education.

1. Introduction

Microfluidics is a system which allows manipulation of fluids in microscale devices by using either external or internal forces. It usually called "labs on a chip." Microfluidic devices analyze and monitor the system which consists of networks of valves, pumps, and channels on a very small-scale to test chemical or biological samples. Also, these devices have the ability to produce high-resolution images that may not be possible with traditional biological instruments. It was listed as one of the "10 Emerging Technologies That will Change the World" by the Massachusetts Institute of Technology Review in 2001. Research and development activities of microfluidics have been growing about 16-20% annually, which is mainly in 35 countries around the globe (Neethirajan et al., 2011).

Traditional plant cell examination techniques depend on culturing the seeds, growing the plant cells in soil pots or agarose plates, trailed by screening the plant phenotypes in conventional nurseries, development chambers or agarose plates. These materials are typically expensive, need enormous analyses, and have potential data loss during the observation of plant phenotypic

changes because of low sequential resolution. The numerous advantages of microfluidics are basically a) easy control and manipulation; b) low and precise sample volume; c) availability of *in-situ* analysis; d) elimination of composition change in biological systems and time lapse between sampling and analyzing; and e) flexibility and mobility to be used anywhere and at any time (Thomas, 2019). This review includes the current research, applications, and future directions of microfluidics systems on plant cells and plant biology.

2. Current Plant Applications of Microfluidics

There are many indications that the microfluidic technology will reshape the plant science and agro-industry for mainly plants diseases treatment, and agricultural modification to suit the change in environmental condition, water shortages, and increasing demands for food in the world's developing countries (Neethirajan, and Lin, 2010). Here we reviewed some of the most advanced research studies in the past 10 years in the field of plant cells and agriculture associated with microfluidics.

Current microfluidics designs for plant cell studies were given in Figure 1. In the RootChip design, seeds were positioned inside the plastic tips and the roots extend through the agar-filled cylinders. The cylinders were monitored inside the growth chamber. The RootArray design uses for examining spatiotemporal gene expression dynamics in the plant cells. In the RootArray system, the roots and shoots were grown in liquid chamber and then gaseous chamber. Both of the chambers were monitored in two-axes. PlantChip system studies the high-throughput plant phenotyping. In PlantChip system, seeds were hydrodynamically fixed at the entry of the funnels and it allows roots and shoots to be monitored at any position. In the TipChip system, successively arranged pollen tubes were fixed at the entry of the microchannels where the growth of pollen tubes can be monitored in the horizontal plane (Nezhad, 2014).

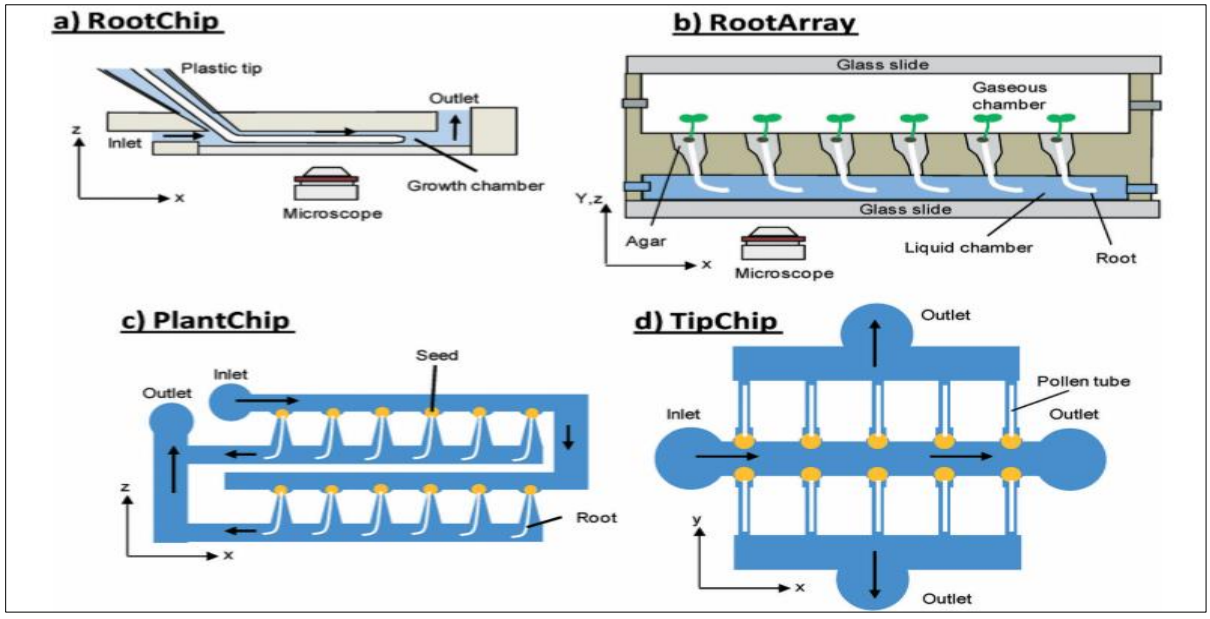

Fig. 1 Microfluidics designs for plant cells studies: a) RootChip, b) RootArray, c) PlantChip, and d) TipChip design.

Plant roots play an important role in manipulating the rhizosphere in the earth wherein cooperation with assorted microorganisms happens. Due to the opaque nature of the soil, it is difficult to analyze the plant root system and rhizosphere simultaneously. Tracing the root-

organism communications at high spatial resolution is necessary, so the methodological multifaceted design can be used to enable analyze the root systems in microfluidic devices (Massalha et al., 2017). Table 1 shows currently used root-chip systems and their applications.

Many research and experiments have been conducted to monitor plant growth inside a polydimethylsiloxane (PDMS) microfluidic chamber to capture high-resolution imaging over a long period of time. Bascom et al., 2016 used microfluidics and high-resolution imaging for root development of moss (*hyscomitrella patens)* to overcome the problem of imaging in solid media. Protonemata (moss filaments) with a thin film of agar medium was used for several days, and then live cell imaging was performed. The significance of this research was to understand the simple body/root development and observe cellular process differences between mature chloronemal and caulonemal cells at high resolution. The microfluidic devices were designed with a large central port opening into a chamber that is 45 µm from the coverslip. By gently applying pressure with a syringe pump, a liquid medium could push the tissue into the narrower portion of the devices, a 30 µm deep chamber. Barriers were positioned radially to help support the roof of the chamber and prevent tissue from clogging the flow through channels. The entire device then completely submerged in the lightning liquid medium (Bascom et al., 2016).

Reusable and mechanically interlocking Lego-based platform (8x8x6µm size) was designed by Lin et al., 2014 for analyzing plant root growth in a heterogeneous environment. Lego-based setting can structurally be tailored based on the environmental conditions and the size of the organism, allowing real-time monitoring of root systems and fully controlled environment (e.g., air pockets, solid barriers, chemical and soil biota gradients) in a homogeneous growing medium. Figure 2 shows the platform which can construct different biological experiments for different scale organisms. In the platform each heterogeneity showed different dyes, such as green for potassium, yellow for potassium nitrate, red for calcium chloride and blue for magnesium sulfate (Lin et al., 2014).

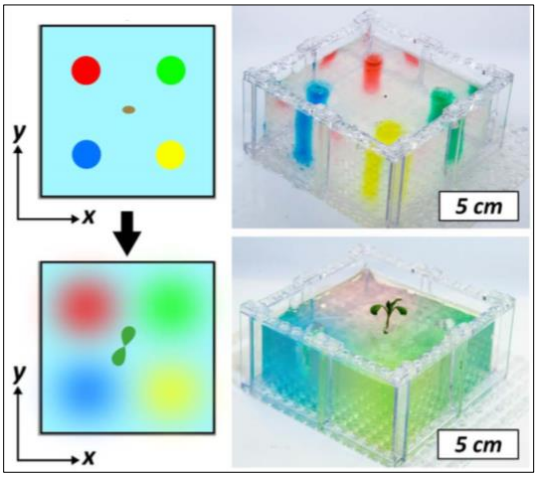

Fig. 2 Schematic view of controlled heterogeneities in plant growth environments.

Another study analyzed the interaction of six Arabidopsis strains' root systems with fluorescently labeled *Bacillus subtilis* to enhance understanding of root– bacteria interactions (see Fig. 3) (Massalha et al., 2017). Result of this study showed the discrete chemotactic behavior of *B. subtilis* in the root elongation zone, followed by rapid colonization over the first 6 hrs of root–bacteria interaction. Furthermore, the double-channel tracking root– bacteria interactions system allows real-time tracking and monitoring of two root systems in one chamber with a bacterial preference between root genotypes. The use of microfluidic chip platform called "RootChip," makes the process easy to study the physiology of root growth and plant development in the cellular and subcellular level (Unger et al., 2000). Here microfluidic system allows live-cell imaging of growth and metabolism of *Arabidopsis thaliana* roots under the controlled environmental conditions. The RootChip system was fabricated with polydimethylsiloxane (PDMS) that had the genetically encoded fluorescence sensor. This system monitored time-resolved growth and detected cytosolic sugar levels at subcellular resolution in plants (Grossmann

et al., 2011). Fig. 4 shows the RootChip with eight mounted live plants which have control and flow channel systems.

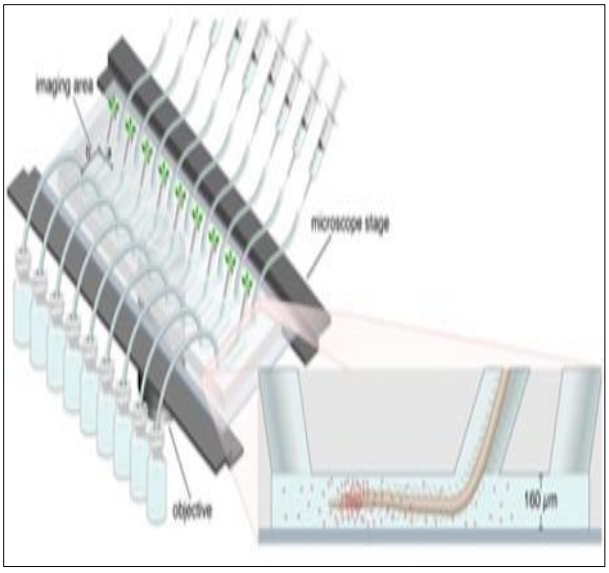

Fig. 3 Schematic illustration of a microfluidics device for tracking root-bacteria interaction.

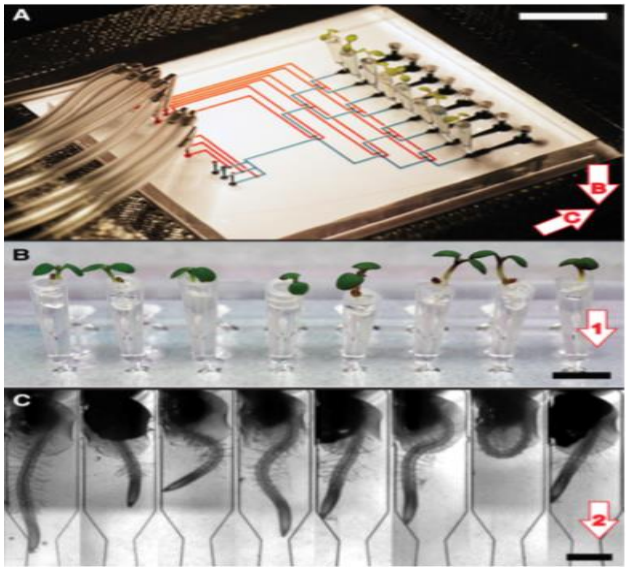

Fig. 4 (a) Control and flow channels of the chip, (b) top view of the plants and (c) Roots of seedlings in microchannels.

Current RootChip designs have been effectively used for analyzing plants phenotypes; however, the system was able to analyze only phenotypes of plant roots. Thus, this system was not feasible for quantifying phenotypes of other organs. The PlantChip overcame some of the challenges by offering quantitative analysis of the complete plant phenome of multiple plants, roots, shoots, hypocotyl, cotyledon, and leaves. Current research on the RootChip has been described in Table 1. The system was made of successive funnel-like microchannels where the seed was located at the narrow side of the funnel via hydrodynamic fluid drive. The roots stretch from seeds through seed holding site were down in the direction of the bottom side of the microchannel. The perception chamber of the PlantChip is situated in a vertical position which empowers continuous observing of an entire living being (Fig. 1).

RootArray was used to study the complexity of gene regulation in a multicellular organism because the dynamics of gene expression requires some special and temporal characteristics. Microfluidic chips allow long term imaging of many specimens to evaluate spatiotemporal gene expression. Current RootArray studies have been analyzed in Table 1. Pollen tube plays an important role in flowering plants since it was the fastest tip growing plant cell. Pollen tube germinates from pollen grain on stigma and capable to sense many extracellular chemical and mechanical changes from its floral surroundings. TipChip was a combined microfluidic system (see Fig. 1) which developed to characterize the growth of plant cells by focusing on the pollen tube structures. In the TipChip system, pollen tubes were fixed at the entry of the microchannels where the growth of pollen tubes were monitored in the horizontal direction. Current examples of TipChip system has been provided in Table1.

Table 1: Applications and advantages of microfluidics for plant cell studies.

Application Focus	Benefits of System over Traditional Systems	References
RootChip system designed for monitoring plant root development.	The use of microfluidic chip platform called "RootChip," makes the process easy to study the physiology of root growth and plant development in the cellular and subcellular level	Grossmann, et al., 2011. Massalha et al., 2017
Designing reusable microfluidic system for the heterogeneous environment to see root development.	The LEGO-based system allows rapid monitoring of root systems in 3D, can be structurally redesigned to change the environment of an organism during its growth and generates precisely controlled heterogeneities in the homogeneous growing medium.	Lind et al., 2014
Plant-in-Chip system was designed to analyze root growth and pathogenic interaction in Arabidopsis.	The transparent microfluidic chip is useful in high-magnification imaging and analysis of root structures.	Parashar, and Pandey, 2011
PlantChip was designed for quantitative analysis and monitoring phenotypes of immutans mutant intended for multiple roots.	Continuing and parallel monitoring of shoots, roots, hypocotyl, cotyledon, and leaves.	Jiang et al., 2013
RootArray was designed for observing spatiotemporal gene expression dynamics.	Real-time monitoring of gene expression, powering the imaging systems restructuring 3D root shape.	Busch et al., 2012
Digital microfluidic chip technology developed for water permeability measurements on single isolated plant protoplasts (e.g., *Arabidopsis thaliana).*	This study is novel in terms of an automation system which was firstly introduced to water potential studies. The digital microfluidic chip was effectively used for single cells analysis of a non-adhering cell type. Also, the output of measurements was significantly increased with this system.	Kumar et al., 2014
TipChip system was developed to analyze high throughput chemical or biological simulation of pollen tubes.	Offering same growth condition for a succession of individual pollen tubes.	Agudelo et al., 2013
Arrayed pollen tubes provide long-term high-resolution imaging of pollen tubes.	Restricted pollen tube growth provides precise growth analysis under the microscope.	Horade et al., 2014
Toxchip was developed as a cell sensor to detect the existence of pollutants in the environment.	The system provides higher accuracy compared with the previous open assays, specifically on the detection of secondary oscillation frequency and its change under chemical treatment.	Nezhad et al., 2013
The microsystem-based assay was developed to mimic the *in vivo* microenvironment of ovule fertilization via pollen tubes in the *Arabidopsis thaliana*.	The system provides a rapid and consistent change of growth medium at a subcellular resolution around growing pollen tubes.	Yetisen et al., 2011 Horade et al., 2012

Application Focus	Benefits of System over Traditional Systems	References
Plant on a chip microfluidic platform has been designed to identify how to root development is affected by auxin.	Observing the transportation systems of auxin in the roots due to local manipulation at subcellular resolution. The technique allows multi-laminar flow for local chemical stimulation, so *Arabidopsis* root was stimulated by the auxin derivative 2,4-D.	Meier, Lucchetta, and Ismagilov, 2010
Bending-Chip was developed for quantifying the biomechanical properties of *Camellia* pollen tube cell wall.	The capacity of direct bending-based manipulation with respect to pollen tube. Results were autonomous from the properties of the small-scale indenter	Nezhad et al., 2013
Microgap chip was developed for measuring the growth force of pollen tube.	System integrate sensors in subcellular size. The sensor avoids redirection of growth which results in a decisive growth force in comparison with off-chip sensors.	*Nezhad et al., 2013
Directional memory chip designed to investigate the presence of "inner memory" in various plant cells.	Ability of systematic testing on plant cells via offering required subcellular microenvironment.	Bibikova, Zhigilei, and Gilroy,1997. Held, Edwards, and Nicolau, 2011
Cell division studies have been performed by long term monitoring of plant cells division with minimum damage to cells.	Requires fewer volumes of media, smaller cell population size, optimal supplying of nutrients, and highly spatiotemporal control of the microenvironment.	Wu et al., 2011
Electric cell fusion chip was developed with an embedded cell delivery function run by surface tension for analyzing plant cells.	On-chip electrofusion saves the volume of media used, requires a smaller cell population, optimizes nutrients supply, and provides spatiotemporal control of microenvironment. Also, the system provides excellent control on cell manipulation at a single cell level.	Ju et al.,2009

Source: Adapted from Nezhad, 2014.

4. Integrating Microfluidics into STEM Education

The microfluidic systems provide several benefits for the improved properties of fluids, whether liquids or gases, once used in microscale. The level of control offered by microfluidic systems has changed the views of researchers to study the interactions molecular levels. The use of this highly efficient tool is expanding into multiple areas of research, especially the field of plant science and agriculture. The Department of Mechanical Engineering at Wichita State University (WSU) has over 480 undergraduate and 100 graduate students, and some of the students began to work on microfluidics research projects during their studies. Mr. Sattar Ali (Ph.D. Candidate) and Mr. Amanuel Wondimu (undergraduate student) from the Department of Mechanical Engineering at WSU and Mr. A. Bilal Ozturk (Ph.D. Candidate) from Department of Transdisciplinary Science and Engineering, Tokyo Institute of Technology, were involved in the present study, learned many new techniques and gained a lot of new skills and knowledge about microfluidics and their plant and cell applications. The undergraduate student used these research activities for their Engineer 2020 requirements in the College of Engineering at WSU. These students are also co-authors of the present study and made many contributions during the

preparations. We believe that microfluidics training will enhance the knowledge of many engineering students to perform more detail studies in their future education.

5. Conclusions

The microfluidics technology was advanced rapidly in the last few years and gained great attention from various universities, research centers and industrial laboratories working on the plant cells. Using microfluidics will open up new possibilities in the plants and agro-industry, including new microfluidic products and techniques, creating new generations of smart agriculture products, and so on. The main problems that engineers are facing include the primary gaps among the researchers, lab-scale experiments, and manufacturability. This new opportunity will advance the technology in agriculture and other disciplines. Also, there are some needs to develop standards and validation for the microfluidic systems, convincing evidence for manufacturing profitability, and acceptance by consumers. Here, one of the engineering students, also authors of this study reviewed microfluidics for plant cell studies to address the problems and concerns. The undergraduate student has used these research activities for his Engineer 2020 requirements. Overall, these studies greatly benefit undergraduate engineering students for their future academic studies at different institutions.

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