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REVIEW

Microfluidic approaches for synthetic gene circuits' construction and analysis

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***Background*: Microfluidic systems have advantages such as a high throughput, small reaction volume, and precise control of the cellular position and environment. These advantages have allowed microfluidics to be widely used in several fields of synthetic biology in recent years.**
***Results*: In this article, we reviewed the microfluidic-based methods for synthetic biology from two aspects: the construction of synthetic gene circuits and the analysis of synthetic gene systems. We used some examples to illuminate the progresses and challenges in the steps of synthetic gene circuits construction and approaches of gene expression analysis with microfluidic systems.**
***Conclusion*: Comparing to traditional methods, microfluidic tools promise great advantages in the synthetic genetic circuit building and analysis process. Moreover, new microfluidic systems together with the mathematical modeling of synthetic circuits or consortiums are desirable to perform complex genetic circuit construction and understand the natural gene regulation in cells and population interactions better.**

Keywords: microfluidics; synthetic gene circuit; analysis

Author summary: Microfluidics-based methods for synthetic biology include the construction of synthetic gene circuits and the analysis of synthetic gene systems. In the former, the high-throughput, automated control of reaction media and the mini reaction systems of microfluidic systems for gene circuit synthesis can substantially improve efficiency, which leads to a significant cost reduction. In the latter, the precise control of cellular growth directions and environments combined with time-lapse microscopy makes the description of cell behavior or gene expression easier and more accurate. Accordingly, there is a great opportunity for microfluidics to be applied in synthetic biology research in the future.

INTRODUCTION

In recent decades, synthetic biology has developed very quickly, aiming to create new artificial and predictable "bio-factories" via molecular technologies and quantitative theories. Additionally, the use of synthetic gene circuits can quantitatively verify the design principle of natural circuits, which are also widely used in quantitative biology. Among these studies, whether in industrial design or biological research, there are common requirements for synthetic biology, which include a high throughput, precise control and low cost. In quantitative biological research in particular, there is a high requirement for reaction condition control and experiment phenomenon description to reproduce biological reactions better and obtain more accurate quantitative data.

Microfluidics systems developed in the last twenty years are the right tools to meet these requirements. In

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traditional experimental methods, manual pipetting methods are widely used, and the volume consumed is usually on a milliliter scale. However, microfluidic experiments only require microliters [1], which reduces the cost substantially. On the other hand, with the increasing exploitation of synthetic biology elements, the high-throughput construction and analysis of synthetic gene circuits is necessary for choosing better designs of combined elements. A high throughput is just one of the strengths of microfluidics [2,3]. Traditional synthetic biology experiments are processed in perforated plates or tubes, which have a limited throughput. In comparison, tens to thousands of experiments can be designed on one small chip to realize the high-throughput requirements of microfluidic systems. Finally, the analysis of synthetic gene circuits in cells using microfluidic systems presents unique advantages compared to traditional methods. Traditionally, it is difficult to maintain the environment for long time when cells are grown in a tube or on a plate. However, microfluidic systems can continually renew the environment with flowing culture medium [4] while keeping the cells confined within a designed position suitable to record the cells' high-spatial-temporal-resolution behaviors with time-lapse microscopy.

Due to the particular advantages of microfluidic systems, many traditional synthetic biology experiments have been optimized, and some special experiments have been enabled in microfluidic systems, such as the those requiring mixed/pulse stimulation [5,6] or unconventional stimulation methods [7–9]. This review focuses on the improvement of the applications of microfluidic systems in synthetic biology in recent years, introducing the new developments in related fields from the aspects of synthetic gene circuit construction and synthetic gene circuit expression analysis. We hope that this work will suggest proper methods and microfluidic designs for researchers to accomplish related synthetic biology studies.

MICROFLUIDIC APPROACHES FOR SYNTHETIC GENE CIRCUIT CONSTRUCTION

As a tool with a powerful design ability, microfluidic systems can easily achieve complex reactions and high-throughput experiments, which are needed in synthetic gene circuit construction. In this section, we discuss several applications of microfluidic methods used in aspects of synthetic gene circuit construction, including gene assembly, chassis assembly and integrated assembly (Fig. 1).

Gene assembly in microfluidic systems

In gene assembly, there are some microfluidic approaches used in both gene/oligo synthesis and gene fragment assembly. Generally, gene/oligo synthesis includes synthesis based on columns and synthesis based on arrays [10]. Besides some attempts at achieving the distribution of the

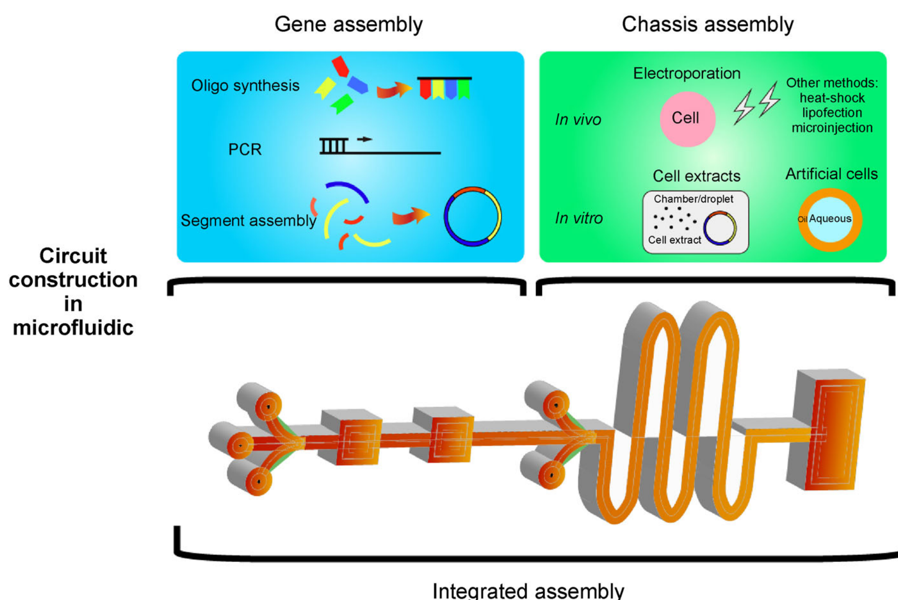

Figure 1. Molecular and cell biology steps involved in synthetic gene circuit construction with microfluidic systems. Two key steps are gene assembly, which includes oligo synthesis and segment assembly; and chassis assembly, which includes multiple methods of transformation/transfection, can both be realized in microfluidic systems. During development, microfluidic systems can complete the whole construction process, which is defined as integrated assembly.

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columns in microfluidic systems in earlier years [11], "next-generation" synthesis methods based on array received more attention in these years. Microarrays, which can also be considered a kind of microfluidic chip, brought the throughput of oligo synthesis to a very high level. On the other hand, some research focused on decreasing the error rate of synthesis was carried out using microfluidic methods in recent years. Yuliya *et al.* provided new methods for gene synthesis and error correction with a droplet actuator [12]. In this system, a pool of synthesized DNA strands was generated by oligonucleotide hybridization and PCR cycling and subsequently enriched for the correct gene sequence. The use of microfluidics allowed these steps to be processed smoothly.

Regarding gene fragment assembly, with the improvement of assembly methods, many advanced methods, such as Golden Gate assembly and Gibson assembly [13], now do not require intermediate steps such as purification or elution, so they are suitable for microfluidic systems. Through earlier studies, considerable progress in gene fragment assembly in microfluidic systems was made [14]. Shih *et al.* presented an innovative Digital Micro-Fluidic (DMF) platform that can accomplish DNA fragment assembly and plasmid transformation steps (Fig. 2A). There are obvious advantages in DNA fragment assembly in this system, such as the smaller volumes needed, automatic processing and the possibility of processing associated with different assembly methods (such as Golden Gate assembly and Gibson assembly) with the same chip [15]. Another design, the ring-mixer of Kong *et al.*, allowed the combination and automatic mixing of up to three different liquid reagents (Fig. 2B). The mixer can be employed not only in different gene assembly methods but also in a wide range of other biochemical reactions [16].

From the works above, it seems that the technological means for gene fragment assembly have matured. It is now time to focus on the normalization and commercialization of these systems.

Chassis assembly in microfluidic systems

The chassis is the platform for synthetic gene circuit expression. Generally, the chassis for synthetic biology consist mostly of living cells, including bacteria [17], yeast [18] and mammalian cells [19]. Thus the most commonly used method for chassis assembly is transformation/transfection. Besides electroporation, which was thoroughly researched in microfluidic systems in earlier years, other methods, such as lipofection and heat-shock transformation, were tested to achieve to higher efficiency in recent years.

Kristina *et al.* created a microfluidic chip that cultures

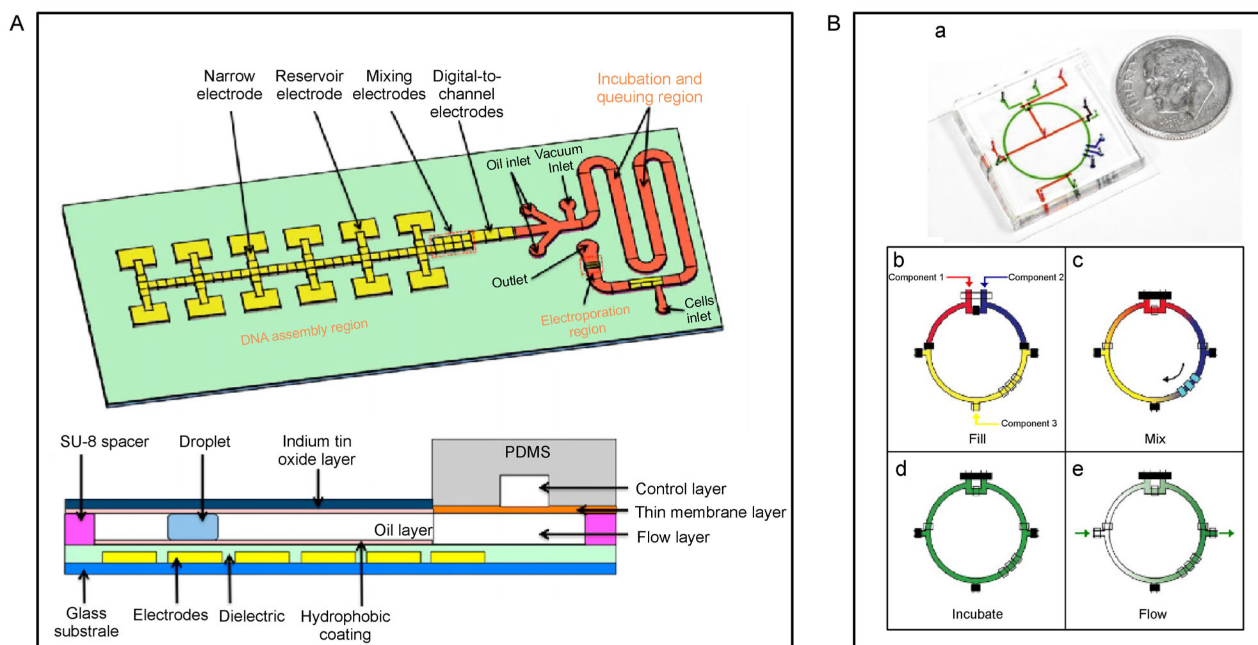

Figure 2. Examples of microfluidic devices for gene assembly. (A) Digital microfluidic device for gene assembly (yellow part) and transformation (red part). Reagents are mixed in droplets, which is controlled by electrodes. Thus the reaction time can be controlled accurately. Reprinted with permission from Ref. [15]. Copyright © 2015 American Chemical Society. (B) Ring-mixer microfluidic device for gene assembly. Reagents are injected into the annular channel and mixed by a peristaltic pump. Reprinted with permission from Ref. [16]. Copyright © 2017 Springer Nature.

cells and implements 280 independent transfections at up to 99% efficiency [20]. Furthermore, the chip can perform co-transfection and can be easily integrated with a high-content microscope, which makes it a useful tool for mammalian cell engineering (Fig. 3A). Zhang *et al.* developed the super hydrophobic micro-well array chip (SMAR-chip) to perform high-throughput chemical transfection, virus packaging, and transduction [21]. A total of 169 chemical transfections were successfully performed at 65% efficiency, which is comparable to the traditional transfection in multi-well plates but with largely reduced manual operations. High-throughput virus packing and transduction were also performed at > 70% efficiency, which proved that SMAR-chip based high-throughput gene delivery is efficient and versatile (Fig. 3B). Philip *et al.* looked at another transformation method, heat-shock. They used a hybrid digital microfluidic/channel-based droplet chip integrated with a temperature controller to complete automated plasmid addition, heat-shock transformation, selection medium addition, culture, and protein expression [23]. The platform can be used in multiple host organisms including bacteria, yeast and fungi, and can realize a significant reduction in consumption (100-fold) and long-term (about 5 days) cell culture. These works indicate the direction of development in this area: automation, cost and time reductions, adaptability and high throughput.

On the other hand, because of the complex interaction between cell growth and synthetic gene circuit expression, some people choose to employ *in vitro* techniques for simplified gene circuit studies [24]. The general method is to introduce plasmids into a mixture of enzymes or a cell extract, allowing the plasmids to be expressed *in vitro*. In this respect, a microfluidic system has the advantage of controlling the reaction volume precisely, which is suitable for these studies [25]. A recent

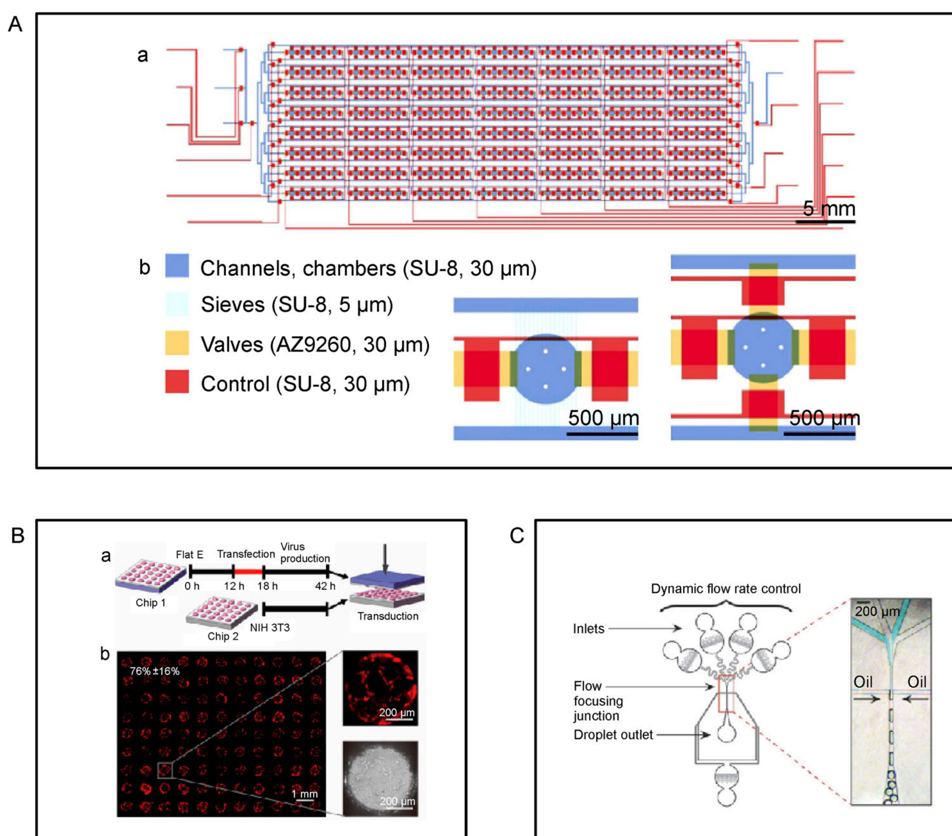

Figure 3. Examples of microfluidic devices for chassis assembly. (A) High-throughput transformation microfluidic system. Two hundred and eighty chambers can be controlled with valves and each 5 chambers can complete a transformation condition. Reprinted with permission from Ref. [20]. Copyright © 2016 Springer Nature. (B) High-throughput transfection micro-well array chip. Chemical transfection, virus packaging and transduction can all be accomplished with high efficiency. Reprinted with permission from Ref. [21]. Copyright © 2019 by the authors. Licensee MDPI, Basel, Switzerland. (C) Droplet generator used as *in vitro* chassis. Different components can be combined and packed into droplets owing to multiple inlets. Synthetic gene circuits can be expressed in a volume with adjustable environmental conditions and similar size as *in vivo*. Reprinted with permission from Ref. [22]. Copyright © The Royal Society of Chemistry 2017.

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example is the work of Yutaka *et al*. [22]. They used a cell-free protein expression system to densely scan a transcription-translation bio-circuit space in a droplet based microfluidic system (Fig. 3C). The system can assay millions of parameter combinations per hour, which is much faster than *in vivo* methods because of bypassing the laborious and time-consuming cloning process.

On the other hand, in order to create systems more similar to *in vivo* conditions, researchers have developed different kinds of artificial cells. Weiss *et al*. created a high-throughput droplet-based microfluidic method to generate stable, defined-size lipid-based giant unilamellar vesicles (GUVs) [26]. Babara *et al*. designed a simple, off-the-shelf approach for the on-demand creation of GUVs with a microfluidic trapping device [27]. These works both increased the application scope of GUVs as a synthetic biology chassis as well as that of other kinds of artificial cells.

Integrated assembly in microfluidic systems

With the expanding application of microfluidic systems in synthetic biology, they are now not restricted to the implementation of a single function but are designed to implement multiple functions on one chip [15,16,28]. Here, we use the concept of integrated assembly to describe these designs. In this case, integrated assembly means the process for accomplishing all or several different steps, such as gene synthesis, gene fragment assembly, transformation/transfection and cell duplication, in only one set of microfluidic systems consecutively or independently. Microfluidic systems designed for integrated assembly always have multiple different integrated assembly modules or versatile reagent inlets and reaction chambers.

Some works mentioned above are just integrated assembly systems, such as Shih's system, which accomplished the process from segment assembly to transformation[15], while Philip's system integrated the functions of the transformation, culture and expression of recombinant proteins [23]. These works are representative examples of integrating different modules. Regarding versatile inlets, Kong's microfluidic ring-mixer device is a good example, which was also mentioned above [16]. Although the innovations of these integrating attempts are largely at a concept level, they point the way forward, toward automated and versatile integrated systems. Based on this, normalization and commercialization will be realized step by step. Another example of a more broadly defined integrated assembly is the work of Tangen *et al*. They performed the work of integrated DNA-library assembly by integrated microfluidic systems and a

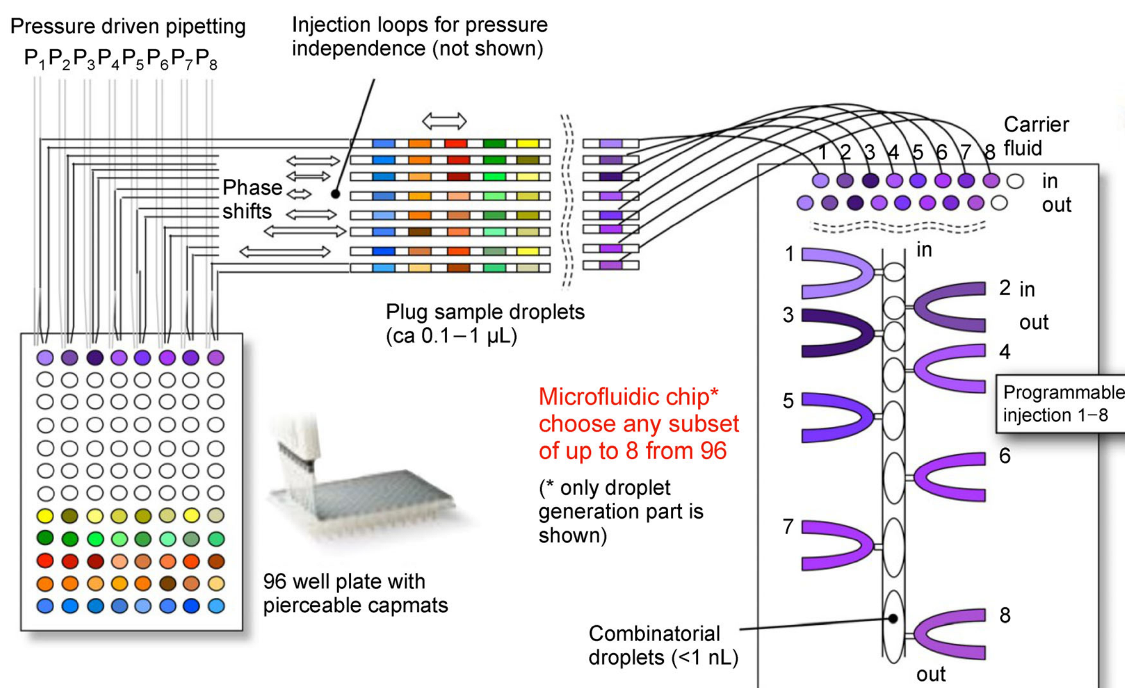

Figure 4. DNA library construction with a core step using a microfluidic chip. Components in microwell plates are pipetted and injected into the droplet on-demand (DoD) system. A sequence of droplets are generated and mixed programmatically. Mixed droplets are exported from the device and finally stored as a DNA library. Reprinted with permission from Ref. [29]. Copyright © 2015 AIP Publishing LLC.

customizable nanoliter-scale pipetting setup [29] (Fig. 4). Through the assembly process, not only the combinatorial generation of a DNA library, but also the Gibson assembly process and fluorescence verification were completed on one chip.

MICROFLUIDIC APPROACHES FOR SYNTHETIC GENE CIRCUIT EXPRESSION ANALYSIS

Synthetic biology aims to build designed circuits in cells or cell-free systems to understand natural genetic network functions or achieve desired artificial biofunctions. With the development of microfluidic technology, microfluidic devices have been widely used in synthetic gene circuit expression analysis, from single-cell gene expression dynamics tests to synthetic cell consortium interaction characterization.

For single-cell gene expression dynamics, specific cell traps have been designed for different cells, such as *E. coli* [30–35], yeast [36–41], mammalian cells or cancer cells [42–46], and precise environment control [47–50] can be achieved in microfluidic systems (Fig. 5). Compared with traditional synthetic biology analysis methods such as fluorescence-activated cell sorting (FACS), which generally can only use population gene expression data at one set time, a microfluidic system combined with a microscopy system and photo-processing software can realize gene expression or circuit dynamic studies at the single-cell level with a higher spatial-temporal resolution.

For synthetic consortium interaction analysis, a complicated environment can be designed to make it closer to the natural environment, helping us to understand more

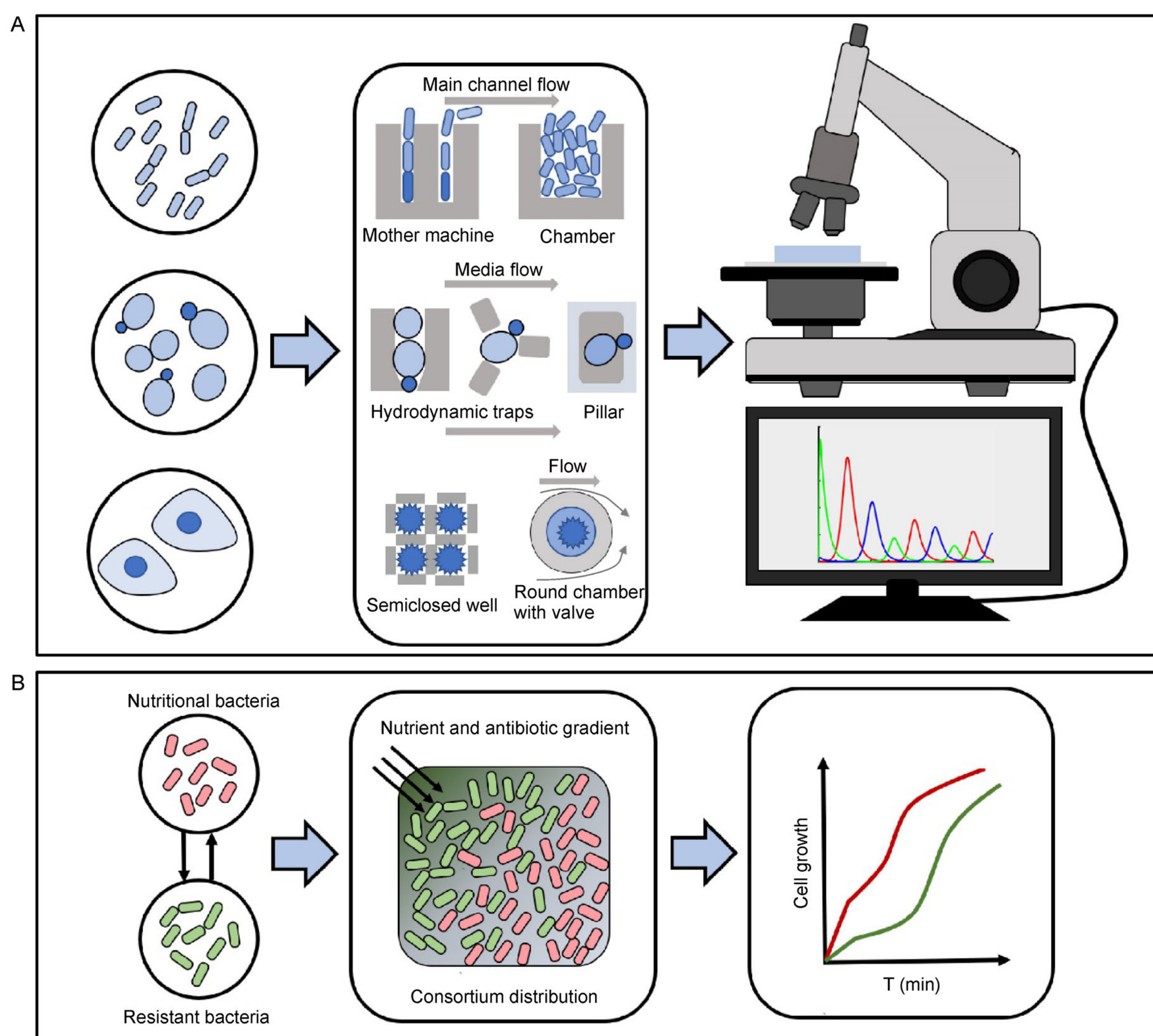

Figure 5. Microfluidics for single-cell gene expression analysis and synthetic consortium property characterization. (A) Illustration of microfluidic traps for different cell types in single-cell gene expression study devices. (B) Illustration of experiment of synthetic consortium with microfluidic device.

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about population interactions in the natural world. Antibiotics, nutrients, pH, and gene expression-inducing chemical gradients can be achieved by different microfluidic constructions, like hydrogel systems [51] or a Christmas tree-type gradient generator [50]. Compared to conventional methods such as plate-based approaches, the quantitative information of a synthetic consortium is easier to obtain, such as the ratio of interacting populations, the dynamic pattern of the consortium.

Approaches for gene circuit dynamics at the single-cell level

Trap structures of different cell types

Mean gene expression dynamics at the population level may introduce misleading dynamic information of the genes in the gene circuits [52,53]. In this case, single-cell-resolution information is needed, and microfluidic device is a suitable platform to study single-cell gene expression dynamics. For different cell types, corresponding cell traps have been designed to satisfy conditions of cell immobilization, nutrient supplies, low cell mechanical force and normal cell growth. *E. coli* and yeast are the most commonly used synthetic model organisms, although mammalian cells such as cancer cells and leukocytes have also been widely studied in recent years.

E. coli

For *E. coli*, small channels of nearly the same size as the bacteria, called a mother machine [30,31] (Fig. 6A), have been widely used to trace single-cell linkage and gene expression dynamics. In these structures, mother cells

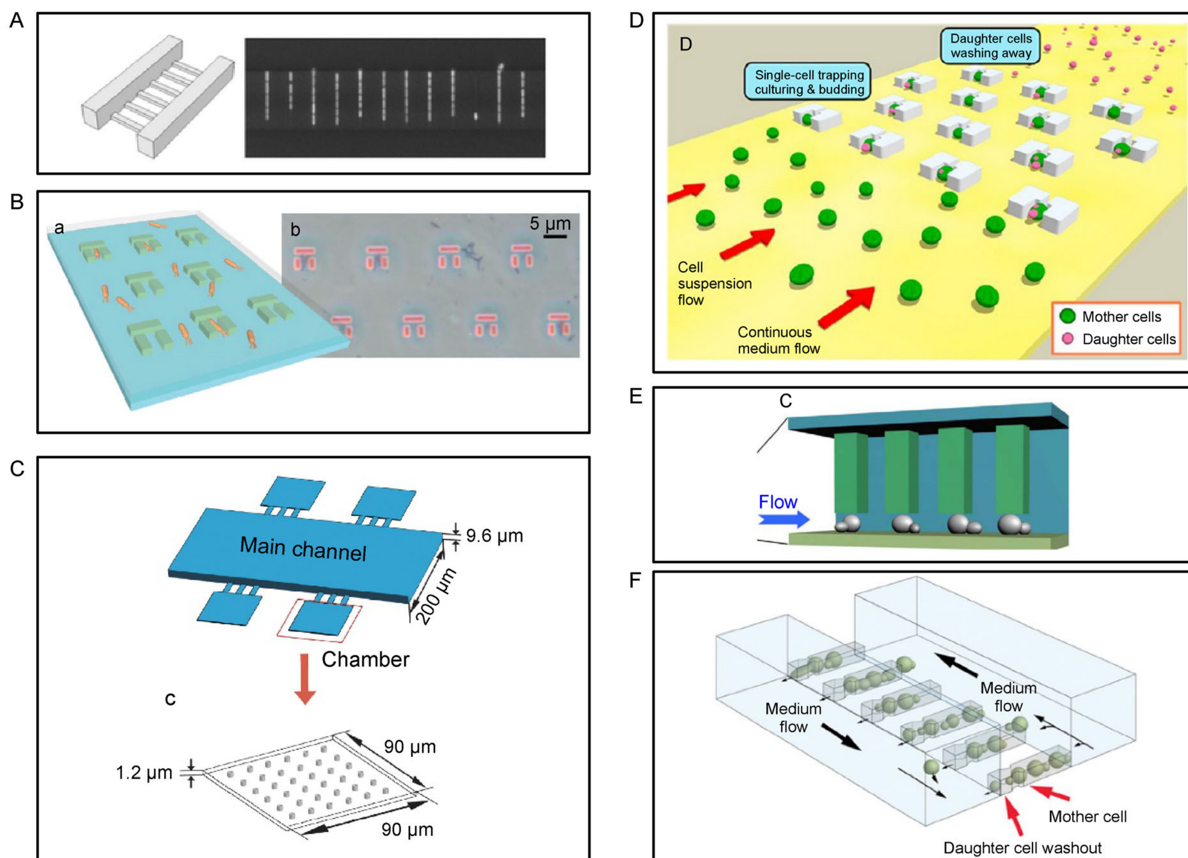

Figure 6. Examples of microfluidic traps for *E. coli* or yeast. (A) Mother machine for *E. coli* single-cell tracking. Reprinted with permission from Ref. [31]. Copyright © The Royal Society of Chemistry 2013. (B) Hydrodynamic traps for *E. coli*. Reprinted with permission from Ref. [33]. Copyright © The Royal Society of Chemistry 2019. (C) Chambers to trap *E. coli* in monolayers. Reprinted with permission from Ref. [32]. Copyright © 2015 Wang *et al*. PLoS ONE. (D) Hydrodynamic trap for yeast single-cell study. Reprinted with permission from Ref. [36]. Copyright © 2015 Proceedings of the National Academy of Sciences. (E) Pillars to trap yeast on the bottom. Reprinted with permission from Ref. [38]. Copyright © 2012 Zhang *et al*. PLoS ONE (F) Mother machine-like device to trap yeast for long term single-cell study. Reprinted with permission from Ref. [40]. Copyright © 2017 Proceedings of the National Academy of Sciences.

stay at the end of the closed channel perpendicular to the main flow channel, while daughter cells will be flushed away with the waste. A sufficient and uniform nutrient supply and precise single-cell tracking can be achieved in this device. However the one end closed channel can only track one cell, and getting enough statistical data requires a large number of channels.

Hydrodynamic traps [33] (Fig. 6B) have also been used for single-cell studies in *E. coli* but are not common because the throughput may be limited in some circumstances due to the restricted cell number trapped in a finite area. According to the cited study, non-motile strains are hard to trap because the fluidic resistance is always much higher than the surrounding regions and non-motile strains will simply follow fluidic streamlines, in contrast to swimming organisms which may move across streamlines increasing the probability of encountering a trap. Single-cell motility can be reflected by average pixel intensity variations over time, and the presence of bacteria can cause abrupt changes in the intensity across the trap and the motions of cells will result in increased fluctuations. In addition to cell motility, other characteristics can be tested simultaneously, such as morphology and gene expression. For the study of single-cell multiple characteristics like motility, morphology and gene responses, it is a very useful platform because of its short temporal resolution and non-intrinsic averaging procedure.

Chambers [32,35,54] (Fig. 6C) are another construction used to study single-cell dynamics, but compared to a mother machine, a long-duration single-cell track is more difficult to apply, all the cells change their positions during growth, and only several (less than ten) generations can be grown within the chamber before the cells expand beyond the chamber. On the other hand, in some nutrient-limiting conditions, cells in different positions may grow differently because of nutrient non-uniformity.

Yeast

For yeast, different kinds of structures such as hydrodynamic traps, pillars, chambers, and mother machines are used in different kinds of biology process studies, such as gene circuit studies and aging process studies.

Hydrodynamic traps [36,37] (Fig. 6D), pillars (Fig. 6E) and mother machine structures (Fig. 6F) are widely used for single-cell gene expression studies, especially during aging. In hydrodynamic traps (Fig. 6D), yeast mother cells can be kept in the traps for a very long time while new daughter cells can be flushed away in time, but the cell number on one chip is limited because one trap can only catch one cell, and there is a probability of loss of cells during culture. By making the gap between the pillar and the bottom slide smaller than the diameter of cells, the yeast cells can be trapped under the pillar but normally grow; this approach has been widely used in gene circuit studies and aging studies [38] (Fig. 6E). More cells can be tracked using pillars compared with hydrodynamic traps during cell growth, but higher mechanistic force may be introduced. The mother machine structure for yeast is slightly different than that used for *E. coli*. Additionally, the size of the mother machine structure for yeast is designed to fit the yeast cell (approximately 5–6 μm) [40] (Fig. 6F). However, there is a narrow neck designed for the original closed end of the channel. Yeast cells may bud around the mother cell, so some daughter cells growing in the direction of the narrow neck may be flushed away by passing the narrow neck to avoid pushing the mother cell out of the mother machine.

A chamber is another construction for single-cell studies in yeast. Compared to the above structures, a chamber [41] is more suitable for performing a high-throughput study for gene circuit analysis, as noted for *E. coli*, but it is only suitable for observation within several generations.

Mammalian cells

For mammalian cells (usually cancer cell lines), various constructions have been designed for gene expression analysis, like chambers [42] (Fig. 7A), droplet arrays [43] (Fig. 7B), microwells [44], DMF (digital microfluidics) [43,45], and other structures [46]. In these studies, microfluidic devices were designed to test natural gene expression levels in cancer cells for detecting drug therapy efficiency or providing the basis of drug design. Microfluidic platforms for the analysis of synthetic gene circuits or gene expression dynamics in mammalian cells are not yet common. In the future, with the development of gene editing manipulation technologies and the further elucidation of gene expression pathways of mammalian cells, microfluidic systems may be a very promising platform with a high throughput and high temporal resolution allowing automated multi-manipulation.

Other functions in microfluidic systems for gene circuit studies in single cells

High throughput

A high throughput is significant for the analysis of various massive synthetic gene units, for the construction of more complicated gene circuits or for understanding gene expression regulation in nature. Many high-throughput microfluidic devices have been designed for different cell types, such as yeast or *E. coli*. For the high-throughput detection of massive strains on one chip, a lack of cross-contamination and a sufficient cell sample should be

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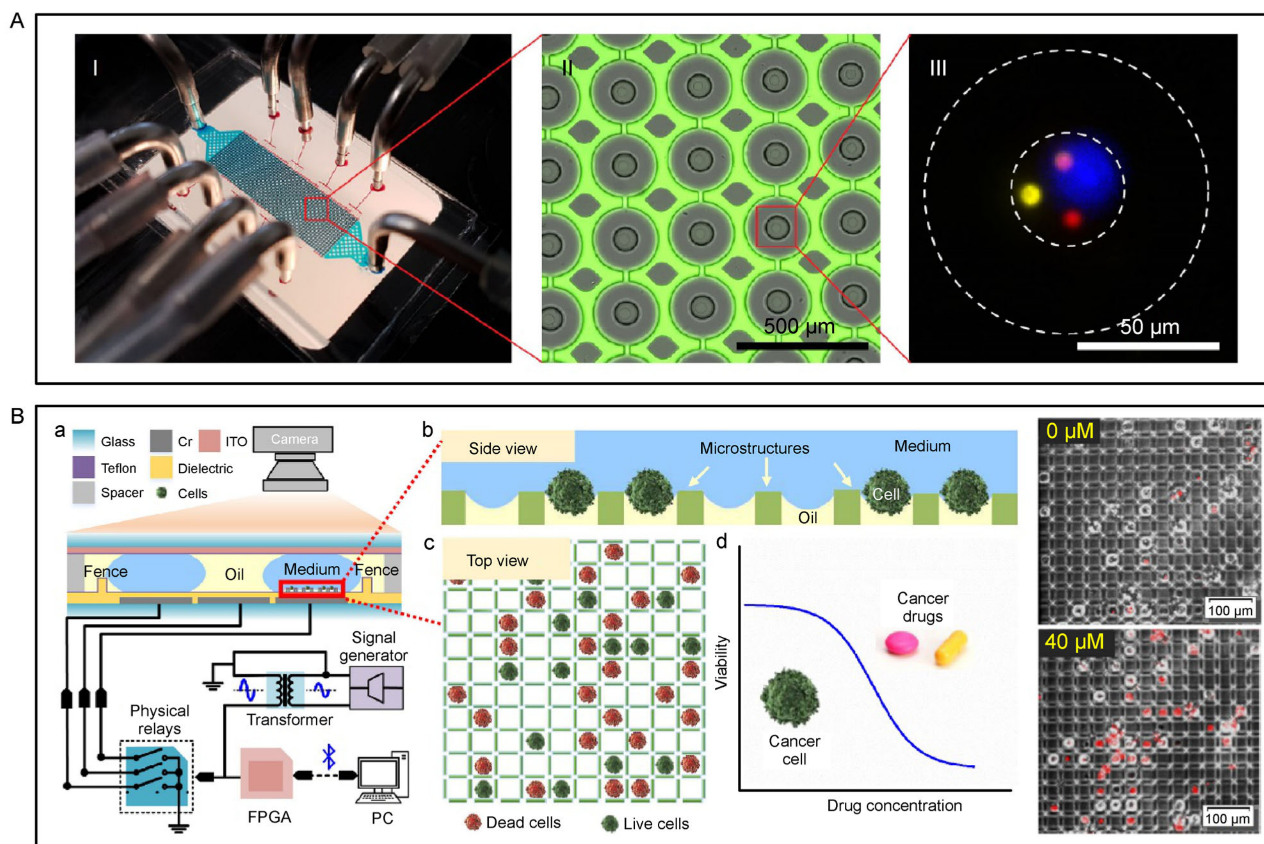

Figure 7. Microfluidic devices for the single-cell study of mammalian cells. (A) A microchamber device for single-cell protein profiling with barcoded beads. Reprinted with permission from Ref. [42]. Copyright © 2019 Springer Nature. (B) A DMF (digital microfluidic) system for single-cell culture and analysis. Reprinted with permission from Ref. [43] . Copyright © 2020 Springer Nature.

ensured, and several strains in different chambers can be viewed in one microscopic field to achieve a better temporal resolution.

Droplet devices [51] can achieve the high-throughput analysis of single-cell gene expression but can only test the gene expression dose and cannot reveal dynamics. It is more suitable to screen production in synthetic cells and extracts of high-productivity cells. Dénervaud *et al*. [55] designed a microchemostat array able to test 1152 yeast strains at the single-cell level in parallel with a temporal resolution of 20 min. A similar device with a throughput of 2176 strains has been developed to study transcriptional dynamics in *E. coli* by Graham *et al*. [54] (Fig. 8A). These two platforms use a chamber to trap cells, have achieved very high-throughput detection of massive strains, and can be extended to other cell types by replacing the trap structure. However different strains need to be arrayed on the coverslip using a DNA array spotter during microfluidic system assembly. In this way, a lack of cross-contamination may be hard to guarantee, and an expensive and professional DNA array spotter is needed when using this microfluidic system. Zhang *et al*. [41] proposed a much simpler high-throughput microfluidic chip to achieve 96 experiments on one chip for yeast gene expression analysis, but the throughput is lower compared to the two devices mentioned above. Microfluidic devices with a higher throughput that are simple to use and guarantee an absence of contamination remain to be developed in the future.

Single-cell manipulation

In some circumstances, single-cell manipulation is needed, for example, to select a mutant of interest or the best gene circuits out of numerous of cells based on single-cell gene expression dynamics.

An optical trap is one of the methods for single-cell manipulation, in which a single cell with better performance can be selected by the optical trap. Luro *et al*. [56] (Fig. 8B) utilized an optical trap to achieve single-cell manipulation in a mother machine to collect cells with better dynamic performance without contamination after

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examining a long-duration single-cell dynamic track. However, using an optical trap to perform single-cell manipulation is still not common because of its cost.

Droplets are another method for single-cell manipulation. Xu *et al*. [57] developed a large-scale 2D monolayer droplet array in which droplets could be further incubated and detected in real time. Cells in droplets with desired performance can be picked out by a droplet-handling robot for further incubation or testing. However, the tracking of gene expression dynamics in droplet devices is difficult because it is difficult to culture cells for a long-duration in the droplets. Therefore, droplet devices are more suitable for gene expression dose comparison, and the use of an optical trap combined with a mother machine is more appropriate for gene expression dynamics comparisons.

In addition to these two methods, another method that can achieve a similar function is microfluidics with programmable microvalves. Kim *et al*. [58] developed a high-throughput microfluidic single-cell screening platform capable of extracting cells of interest selectively by opening and closing a relative trap controlled with microvalves. Compared to the previously mentioned optical trap technology and droplet arrays, the use of microfluidic chips with microvalves for cell manipulation can be cheaper, but it is complicated to design and fabricate the chip, and the throughput can be limited because of the area of chamber and valves. Additionally, single-cell capture is difficult to guarantee when cells are small. In the future, with the development of optical traps or the microvalves, single-cell manipulation may become simpler and less costly.

Accurate environmental stimulus control

One of the advantages of microfluidic platforms is

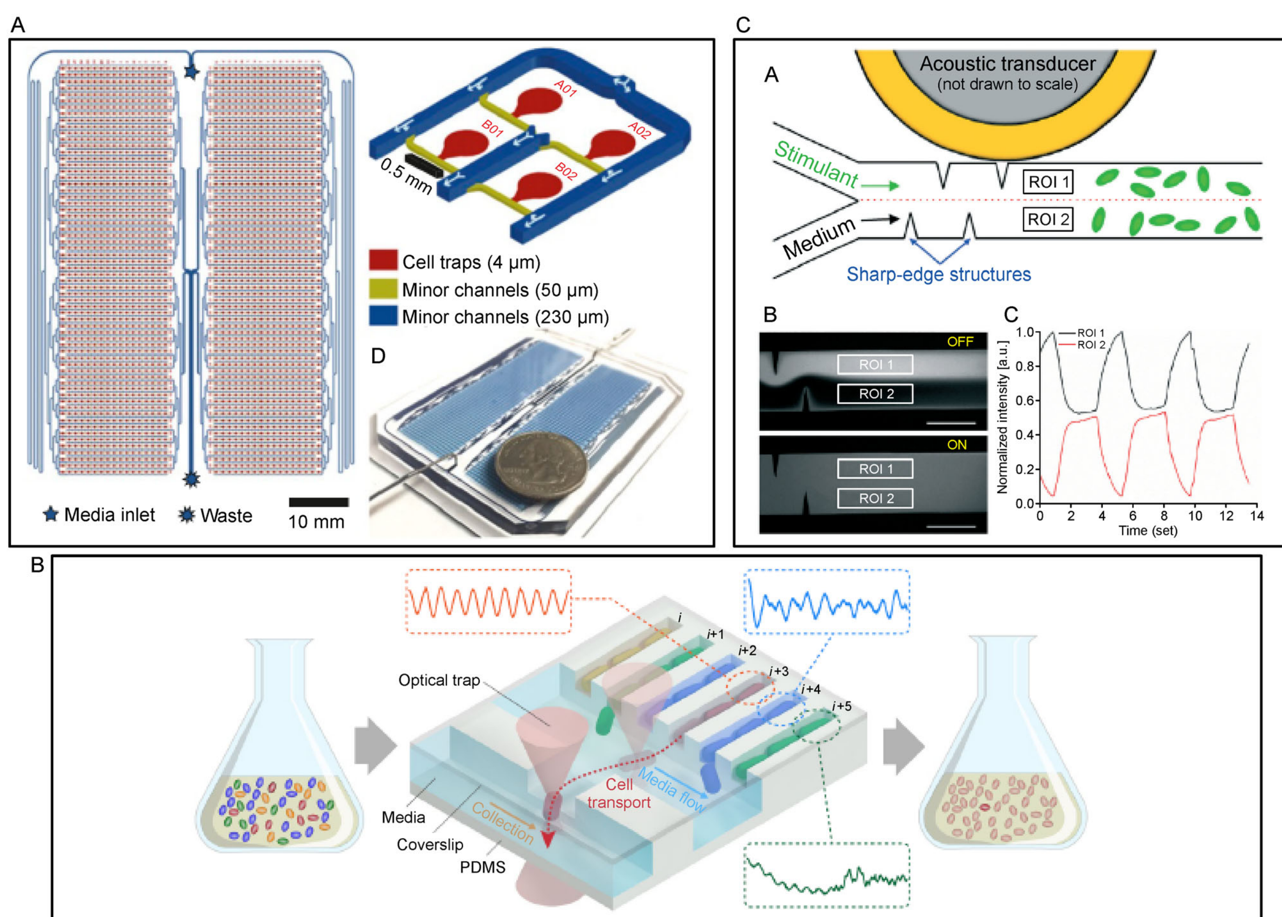

Figure 8. Microfluidic devices with different functions. (A) A high-throughput microfluidic device that can culture 2176 *E. coli* strains in parallel. Reprinted with permission from Ref. [54]. Copyright © 2020 Proceedings of the National Academy of Sciences. (B) A mother machine based microfluidic device with optical trap technology to pick out mutants of interest. Reprinted with permission from Ref. [56]. Copyright © 2019 Springer Nature. (C) An acoustofluidic chemical signal generator that generates single pulse or periodic chemical stimuli. Reprinted with permission from Ref. [47]. Copyright © The Royal Society of Chemistry 2018.

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accurate environment control. Periodic stimuli, chemical environment switches, or gradient generation [49,50] functions achieved by microfluidics have already been widely used in different experiments for specific research programs. To analyze synthetic circuit functions, chemical environment regulation is indispensable. Microfluidic systems can apply chemical stimuli automatically, greatly reducing labor and costs, and achieve complex environment generation.

Huang *et al*. [47] (Fig. 8C) developed an acoustofluidic chemical generator capable of generating single pulses and periodic chemical signals in a temporally controllable way. The chemical signals generated in this way were all pulses. This device can be used to simulate the signaling dynamics of various physiological processes, but it may be not suitable for a simple quantitative study of synthetic gene circuits in some circumstances that require switches of homogeneous chemical concentrations. Woodruff *et al*. [48] designed a microfluidic module that can produce concentrations with a dynamic range of three orders. Dynamic chemical concentration control was achieved via a pulsed flow created by the opening and closing of specific inlets. This device can generate a wide-ranging profile of chemical concentration dynamics, but the delay when flows pass through serpentine channels and ultimately become homogeneous should be considered when it is applied to experiments.

Compared to traditional methods using chemicals to study gene expression, microfluidic platforms can provide accurate environment regulation, single-cell tracking and high temporal-resolution screening, which are important for the quantitative study of gene expression dynamics and for further synthesizing more complex circuits.

Approaches for synthetic consortium studies

Cooperation is crucial for the survival of microbial ecosystems. It is of great significance to understand how microbes cooperate to defend against environmental stress and how they interact with each other. One typical method to study microbial ecosystems is the use of a bottom-up synthetic consortium [59–66]. Conventional studies of synthetic consortiums are achieved by culturing interacting strains in plates, where one can study the variation of the pattern over time, but to some extent, the nutrient environment changes over time during cell

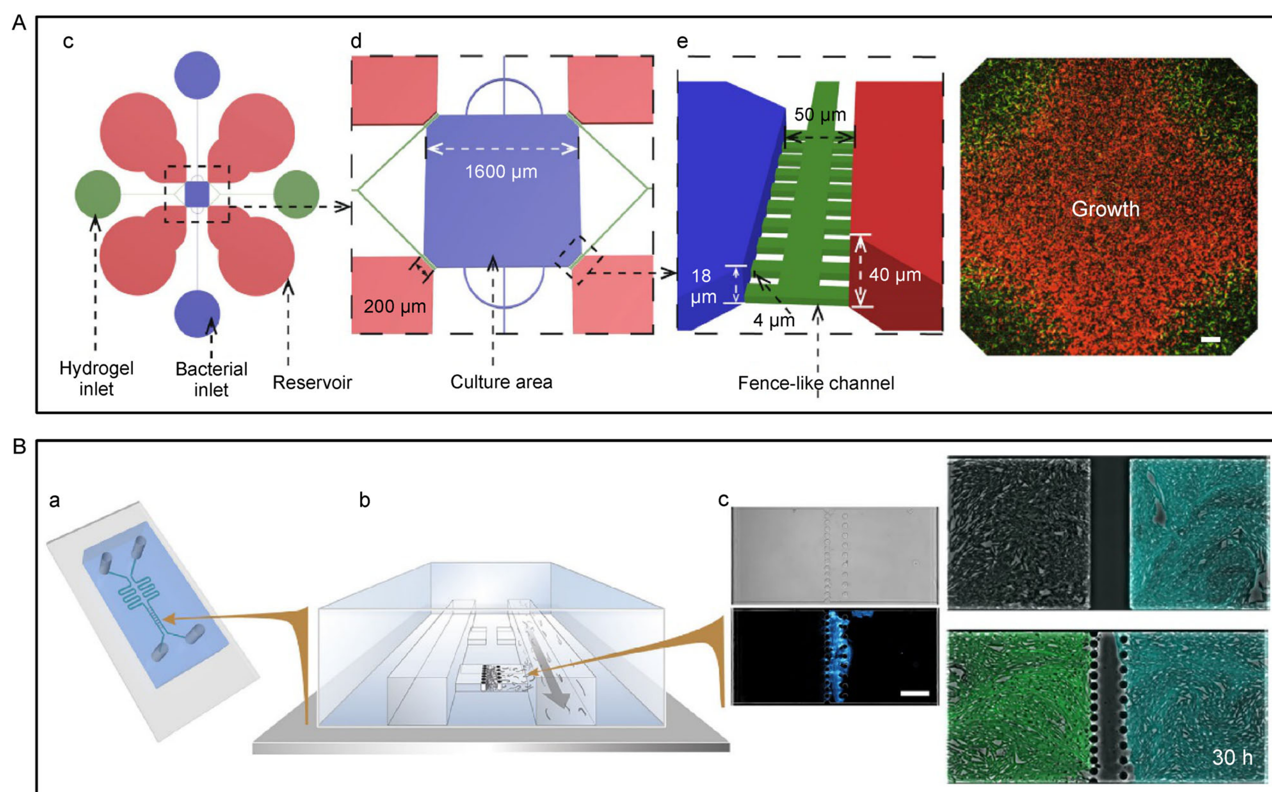

Figure 9. Microfluidic devices in synthetic consortium studies. (A) A microfluidic device that generates a gradient of nutrients and antibiotics in the designed area to study cooperation dynamics between two strains. Reprinted with permission from Ref. [50]. Copyright © 2019 Springer Nature. (B) A microfluidic system allowing communication between two microbial populations through a thin nano-cellulose filter. Reprinted with permission from Ref. [66]. Copyright © 2018 Springer Nature.

growth. To study the evolution of a synthetic consortium quantitatively, microfluidic systems are suitable platforms, which can elucidate the pattern of the consortium and the population numbers of interacting strains quantitatively. Precise environment control, such as maintaining a constant chemical concentration or generating a homogeneous gradient, is simpler to realize using microfluidics than conventional plates.

Microfluidics have already been widely used in studies of synthetic consortiums. Li *et al*. [50] designed a microfluidic device that generates a gradient of nutrients and antibiotics in the designed area (Fig. 9A). They used this device to study the cooperation of two selected strains, a nutrient provider and an antibiotic protector. Osmekhina *et al*. [66] constructed a microfluidic chip in which cells grow confined in a trapping chamber, and two bacterial populations are separated by a filter composed of interwoven PDMS pillar arrays and cellulose nanofibrils (CNFs) (Fig. 9B). Separated bacterial populations communicate through quorum sensing signaling. Droplets [64,65] have also been used to analyze microbial population interactions, but characteristics such as spatial distributions or patterns cannot be reflected using droplets.

With the development of microfluidics, the bottom-up construction of a synthetic consortium can be analyzed more quantitatively, as the environment can be precisely controlled, the growth of populations can be characterized through differences in fluorescence, and variations of two interacting populations can be recorded through automated time-lapse microscopic photography.

SUMMARY AND OUTLOOK

The main goal of synthetic biology is to build new functional genetic circuits in living cells or cell-free systems for scientific studies or biochemical production. In the artificial genetic circuit building and analysis process, microfluidic tools promise great advantages over traditional methods because of technical properties such as a high throughput, small reaction volume, and precise control of the cells' position and environment. This review mainly introduced the application area and new progress in microfluidics in synthetic and quantitative biology in recent years. In the future, microfluidic systems together with the mathematical modeling of synthetic circuits or consortiums will certainly help scientists to better perform complex genetic circuit construction and better understand the natural gene regulation in cells and population interactions.

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COMPLIANCE WITH ETHICS GUIDELINES

The authors Fengyu Zhang, Yanhong Sun, and Chunxiong Luo declare that they have no conflict of interests.

This article is a review article and does not contain any studies with human or animal subjects performed by any of the authors.

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