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A REVIEW PAPER ON MICROFLUIDIC TECHNOLOGY IN SYNTHETIC BIOLOGY: ACOMPUTATIONAL APPROACH

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ABSTRACT

The analysis of biological and biochemical samples is made easy by Microfluidic technologies compared to the conventional, macro scale instruments. Synthetic biology is an emerging field of biology and has drawn much attraction due to its potential to create novel, functional biological parts and systems for special purposes. Since it is believed that the development of synthetic biology can be accelerated through the use of microfluidic technology, in this review work the discussion on the latest microfluidic technologies that can provide unprecedented means whole-cell analysis.

Keywords: microfluidics; synthetic biology; genetic circuits; metabolite detection; whole-cell analysis.

I. INTRODUCTION

i) Synthetic Biology: The development of bioinformatics and functional genomics has enabled not only the ability to understand or modify existing biological systems but also to create new biological systems for special purposes. The natural outcome of such an advance is synthetic biology, which deals with the design and assembly of predictable and robust biological parts/systems and systems biology, which aims at system-level understanding of biological systems. These well-characterized and novel biological parts/systems would in turn provide useful drugs, green fuels, or other high value biomaterials [1,2]. Challenges associated with the progress in synthetic biology and systems biology will be the focus of this review. The two main challenges that limit the progress of synthetic biology are the complexity of the biological systems and the physical variations in biological behaviour. These limitations lead to an uncertain probability of success of the engineered biological systems and an inability to fully predict even a simple component [3]. Automated, multiplex, and parallel reactions are mandatory in order to gain a deeper insight into the complex biological systems. Microarrays, micro plate readers [4], flow cytometers, and fluorescence microscopes [5] are currently being used for high-throughput screening.

ii) Microfluidics: Microfluidics is an analytical system enabling the processing and manipulation of small amounts of fluids. A single chip enables high-throughput continuous and batch processing of multiple samples both in series and in parallel. Therefore, it is believed that microfluidics can provide unprecedented approaches for synthetic biology. Microfluidic tools are especially useful in biological studies for analysing a large number of samples simultaneously and providing dynamic and controlled micro-environmental conditions. Time-lapse

experiments are also made possible with the advent of microfluidics [6]. The cost of micro scale multiplex experiments is several fold lesser than that of macro scale multiplexing. Microfluidics can increase the number of samples that can be analysed: as many as 1.5 million samples can be analysed simultaneously [7].

iii) Blending Microfluidics with Synthetic Biology: Microfluidic devices help in integrating the two major analytical techniques (sampling and assaying) on a single chip, which can reduce the time needed for biological assay and favour real-time monitoring [8]. They also offer room for immobilization and controlled transport of cells. Since microfluidic devices are small, they favour accumulation of nutrients and hence form a stable microenvironment around the cell. Continuous-liquid-flow-type microfluidic devices can be used for long-term culture of cells, as the waste is removed and nutrients are replenished continuously. Optical tweezers facilitate the analysis of a single cell at high resolution [9]. Complex microfluidic devices with an array of cells, each controlled individually by valves, may help perform several parallel experiments [10]. Flow-switching valves can be used to manipulate the environment of the cell with time and hence can help understand the dynamics of gene regulation. Microfluidic devices that can produce a spatial gradient of chemicals can be a tool in understanding the mechanism of chemotaxis and quorum sensing, where the concentration of the signalling molecule determines the fate of the cell [11]. Slipchips are recently emerging as a novel tool showing a high potential for high-throughput parallel screening of various parameters on a sample and for multiplexed applications such as nanoliter PCR arrays on a chip [12]. Microfluidic devices coupled with optical tweezers have been designed to perform whole-cell assays and to study the mechanism of chemotaxis in *Escherichia coli* [13,14].

iv) Whole-Cell Analysis: It is important to understand natural cell communities before developing an artificial cellular community (e.g., quorum sensing systems). The uncultivable microbial species area major challenge in microbiology. Despite the presence of a large pool of microorganisms that grow in the laboratory, a vast majority of the microorganisms are uncultivable even with a rich medium. The group of uncultivable microbes is of particular interest to the synthetic biologist as they may provide evidence for evolution and are the main source of novel genes.[15] Isolation of a pure culture of uncultivable microbes is impossible without isolation chip (Ichip)-based microfluidics. An Ichip offers a miniaturized diffusion chamber that helped isolate a significant and novel group of microorganisms from environmental samples. The microbial species presented in the Ichip were different from those obtained with a rich medium in a Petri dish [16]. Separation and screening of living cells is an essential preparatory step in not only cell-based biological and physiological studies, but also practical applications such as cell engineering, clinical immunoassay, and drug tests. Whole-cell assay is a pre-requisite in toxicogenomics to study the biological impact of toxic compounds. More elaborate methods have been developed, such as fluorescence-activated cell sorting (FACS) and magnetically activated cell sorting (MACS), that require a large volume of samples and are also labour and time intensive [17] as shown in the Figure 4a & 4b. For miniaturized FACS, Y-shaped junctions are widely utilized for positioning individual cells at the center of a laminar flow controlled by optical tweezers. After detecting a fluorescence signal [18], an EOF [19] can be implemented to switch the position of the cell from the center to one of the edges based on the fluorescence signal. The main advantage of the microfluidic FACS, compared with the conventional FACS, is the ability to sort cells at a faster rate (~100 cells/s)[18]. Also, microfluidic approaches are free from contamination with cells of the previous run as these microchips are disposable, being fabricated from cost-

effective materials. In a similar manner, for miniaturized MACS, sample cells are labelled using magnetic beads with an antibody acting as an anchor between the magnetic beads and the cells [10]. Then, magnetic fields are induced to control the position of the cells for continuous separation and sorting. Compared with a fluorescence signal, magnetic fields can extend over longer distances and manipulate cells simultaneously, resulting in higher throughput (1011 cells in 30 min) [21]. Active sorting mechanisms rely on external forces such as an optical, magnetic, dielectrophoretic, or acoustic force. This approach inherently has no physical or mechanical stress that can cause cell death or mutation. One of the advanced methods has been shown in the figure 4c.

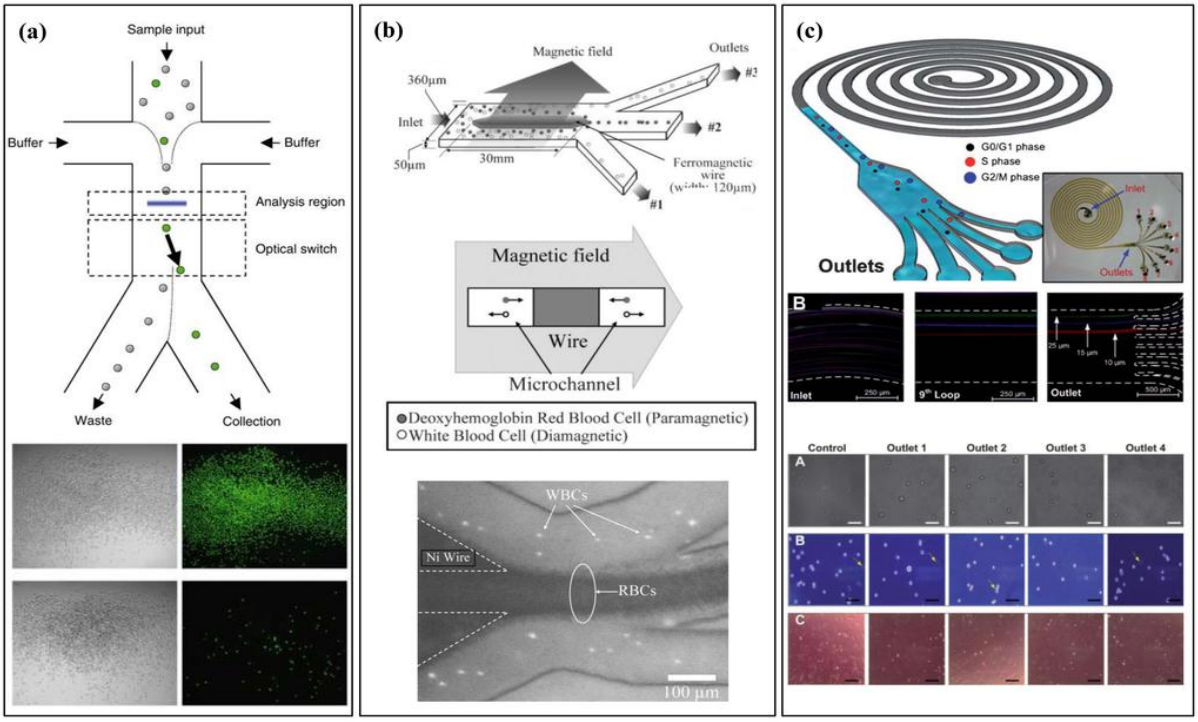

Figure 4. (a) Miniaturized FACS. Cells are analyzed and then sorted on the basis of the detected fluorescence signals. Target cells are directed by the laser to the collection output, whereas all other cells flow to the waste output. The image is reproduced with the permission of Nature Biotechnology . (b) Miniaturized MACS that contain a patterned ferromagnetic wire in the microchannel. Under a magnetic field across the microchannel, paramagnetic-labeled RBCs come close to the central wire, whereas diamagnetic-labeled WBCs experience repulsion from the central wire. The image is reproduced with the permission of the Royal Society of Chemistry . (c) Under the influence of inertial lift forces and Dean drag forces, asynchronous cell populations are size-fractionated to obtain relatively pure populations of cells. The image is reproduced with the permission of the Royal Society of Chemistry . Source: [19(4a),20(4b),224(c)].

II. FUTURE PERSPECTIVES

Microfluidics technology has been proving its potential to offer a means to detect biological response at the single-cell level by automation and multiplexing. Functional screening of drugs with better efficacy would be made simpler with the aid of microfluidics. The field of synthetic biology has been progressing rapidly with the success of rebooting life from a chemically synthesized genome and multiplex automated genome engineering (MAGE) for large-scale programmed evolution of cells in a week's time for an improved phenotype [23].

Microfluidics technology will provide the hope that the synthetic biologists dream of constructing and understanding the machinery of life (like engineers control mechanical devices) is not too far from reality.

III. CONCLUSIONS

Recent progress in genetic engineering and molecular biology has opened a new era of science called synthetic biology. The study intends to build artificial organisms by applying the engineering approach to biology [24]. Synthetic biology is considered to have a huge impact in various industrial fields by providing cheap and readily accessible drugs, developing new anti-cancer drugs, and producing biofuels. One excellent example is artificial artemisinin, an antimalarial drug produced by engineered E. coli [2]. Electronics perfectly controls signal transmission by restricting the signal line, the biological counterparts are disturbed by multiple bypass pathways due to the stochastic behavior of each component [25]. To solve these problems, new techniques that can offer stable and robust tools for precise control of microenvironments and reactions on the cellular level are highly required. Thus, microfluidic techniques are a vital and key technology in synthetic biology [7]. The advantages of microfluidics are obvious. The technology has been successfully applied to many biological problems, especially high-speed, gene sequencing, high-throughput screening and quantitative analysis of multiple or single cells[26]. Microfluidics is one of the best ways to accomplish automation and multiplexing of biological parts. The importance of microfluidics in synthetic biology will be more appreciated if the process of handling the microchips is simplified in order that it could even be managed by a non-expert.

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