Pharmaceutical BIOPROCESSING

Is microfluidic perfusion culture the future for large-scale screening of human-induced pluripotent stem cells?

Keywords: cell-culture environment • cost reduction • drug-screening process • high-throughput screening • human pluripotent stem cell • microfluidic culture device • perfusion culture

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Recent developments with human pluripotent stem cells (PSCs), including both human embryonic stem cells [1] and human-induced PSCs (hiPSCs) [2], may revolutionize the field in terms of applications such as regenerative medicine and drug discovery. Human PSCs are promising platforms for basic research and practical application in these fields owing to their almost unlimited capacity for proliferation (self-renewal) and differentiation into all types of cells (pluripotency). Of particular interest is that, since hiPSCs are generated by reprogramming somatic cells, these cells contain the donor's genetic information [2]. Thus, autologous or specific HLA haplotypes hiPSCs may provide immune rejection-free transplantation for regenerative medicine [3]. A pilot study to assess the safety and feasibility of the therapeutic use of iPSC-derived cells is currently underway at RIKEN, Japan [4]. Meanwhile, a variety of hiPSCs, including disease-specific cells, can be easily produced to provide new methods for drug discovery, and there have been an increasing number of studies employing hiPSCs with disease-specific phenotypes [5,6]. The process of utilizing hiPSCs, including differentiated cells and disease-specific cells, for medical applications generally involves deriving hiPSCs from somatic cells, culturing them to prepare a sufficient number of cells, differentiating the cells into specific cell types *in vitro* and then transplanting cells or testing medical drugs. To eliminate disparities in treatment and to realize industrial application of drug screening, the hiPSC culture process must be low cost and allow precise control of the cell culture environment. For drug screening application, the hiPSC culture process should be applicable to high-throughput assay. In addition, for therapeutic application, the issue of safety must be addressed before clinical study. To meet each requirement, microfluidic devices have many favorable properties.

In this study, first we focused on cost reduction in drug screening utilizing hiPSCs by applying microfluidic devices. Culturing hiPSCs is cost prohibitive. For example, Dulbecco's modified Eagle's medium with 10% fetal bovine serum, which is a standard medium for culturing conventional cell lines such as HeLa and NIH3T3 cells, costs approximately Japanese ¥2500–505,000 per 500 ml (¥1000 = €7.25 = US$9.37, September 2014). However, the standard culture medium for culturing hiPSCs with serum replacement [2,7] or with serum-free supplements such as mTeSR2 (STEMCELL Technologies, BC, Canada) costs approximately ¥10,000–60,000 per 500 ml. Moreover, the many growth factors, small molecules and extracellular matrix (ECM) proteins required to differentiate hiPSCs into specific types of cells are also generally expensive. For example, activin A, which is used for inducing mesoendodermal cells in the range of 50 to 100 ng/ml [8], costs approximately ¥50,000–150,000 per 500 ml of culture medium. Thus, reducing the volume of required culture medium is directly linked to cost reduction in drug screening.

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Recently, microfluidic devices have been recognized as potentially advantageous research tools to reduce costs for cell-based assays because of their small culturing volume [9]. Previous studies have demonstrated that microfluidic devices can perform parallel on-chip cell-based assays [10]. In our own previous study, we developed a pressure-driven perfusion culture microchamber array chip in which multiple solutions including various drug candidates can be simultaneously delivered into a microchamber array by applied pressure [11]. The culture area in our microchamber is small (~1.5 mm^2) and takes up approximately 5 and 40% of the culture area compared with a well on a 96-well plate and 1536-well plate, respectively. The volume of each microchamber is extremely low (~0.4 µl) and an array of 8 × 5 microchambers is arranged on a slide-glass-sized chip. In the perfusion culture, 2 ml/day of culture medium is used in total. The pressure-driven perfusion culture microchamber array chip can reduce the consumption of a culture medium compared with a conventional microplate. In addition, our pressure-driven system needs only one air pressure tube and does not require any external medium pumps (e.g., syringe or peristaltic pumps) or robotics, therefore the initial cost is also low. Thus, the microfluidic device can be used in cost-effective cell-based assays.

Second, we focused on the control of the culture environment during drug screening by microfluidic perfusion culture. In conventional culture, it is difficult to determine whether exogenous factors act directly or through paracrine-dependent mechanisms because of poor spatiotemporal control of the microenvironment. Therefore, screening of differentiation conditions by a conventional culture is intrinsically limited [12,13]. Generally, cellular kinetics depends on the culture microenvironment, including the ECM, soluble factors and cell–cell interactions. We developed a 'microenvironment array' in which cells are cultured within the microchamber array under 16 different controlled microenvironmental conditions, such as combinations of four different soluble factor conditions and four different ECM conditions [14]. Using the microenvironment array, the cell culture microenvironment can be accurately defined in discrete microchambers. More recently, we applied our microfluidic perfusion culture technique for culturing hiPSCs under fully defined culture conditions [7]. We found that medium perfusion is important to maintain the growth rate of hiPSCs in the microchamber with small volume. We also demonstrated that the undifferentiated and differentiated states of hiPSCs can be controlled by the microfluidic perfusion system [7].

More importantly, microenvironment control of cells enables the study of physiologically relevant phenomena. Using microfluidic perfusion culture, autocrine and paracrine processes, which are generally hidden in conventional static culture systems, can be uncovered, resulting in finding the minimal amount of soluble factors required to drive cells toward a desired fate [15,16]. Thus, the microfluidic system has many advantages for controlling the microenvironment, which is important for culturing iPSCs for therapeutic and drug screening applications.

Finally, we focused on the improvement of throughput in drug screening by microfluidic devices. In a conventional dose–response assay in drug screening, the preparation of a large number of drug solutions (e.g., Minami *et al.* tested 10,000 chemicals to find a chemical promoting cardiac differentiation [17]) with different concentrations (spanning up to six orders of magnitude) sometimes causes an amassment of dilution errors by the tedious and time-consuming serial dilution process. For efficient drug screening, high-throughput screening (HTS) systems composed of integrated dispensing robots and disposable multiwell plates have been used in the field of drug screening. However, the installation of HTS is limited owing to the high cost, and alternatives are therefore needed.

Microfluidic devices can automatically and simultaneously manipulate multiple small-volume liquids by a microfluidic network. Microfluidic networks are capable of generating concentration profiles semi-automatically for cell-based assays [18]. Recently, we applied a serial dilution microfluidic perfusion network to on-chip cell-based assays using the pressure-driven perfusion culture microchamber array chip [11]. More recently, we developed a microplate-sized integrated perfusion culture microchamber array chip for a high-throughput cell-based assay [11]. The integrated perfusion culture microchamber array chip was composed of an array of 384 microchambers and a serial dilution microfluidic network. Dose–response curves spanning four orders of concentrations of 12 drugs can be evaluated based on cellular viability by scanning the microchamber array with a commercial microplate reader. Thus, microfluidics is applicable to cell-based HTS systems.

In this paper, we describe the importance of a small volume, well-controlled and high-throughput culturing method using microfluidic devices for applications of hiPSCs to regenerative medicine and drug discovery. We also describe the current advances in microfluidic perfusion culture systems for these applications. We focused on three issues: cost, microenvironment control and throughput. Microfluidic perfusion culture systems can reduce the volume of required culture medium, which is directly linked to reducing costs, allows for controlling the microenvironment in terms of the culture medium, ECM and cell–cell interactions

and can integrate a large number of microchambers in a small area. Thus, microfluidic perfusion culture might be advantageous for the future use of hiPSCs in regenerative medicine and drug discovery. As the next step, we need to evaluate the effectiveness of the microfluidic perfusion culture system for practical cell therapy and drug screening applications and improve the remaining problems of the present microfluidic perfusion culture system. For the application to regenerative medicine, the microfluidic perfusion culture system should be automated to avoid contamination of other normal cells, pathogens. We believe our serum-free culture system for hiPSCs in a closed microchamber [7] is advantageous for reducing contamination risk. As for the application to drug discovery, we have to design a user-friendly interface, improve the versatility of the system and achieve mass production. We intend to apply this microfluidic perfusion culture system to the cultivation of hiPSCs for future regenerative medicine and drug discovery applications.

Financial & competing interests disclosure

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