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Review

Cell electrofusion in microfluidic devices: A review

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ABSTRACT

Cell electrofusion in microfluidic devices attracted great attention in recent years due to its widespread applications potential in cell-based studies. In these microfluidic devices, many manipulation methods, such as chemical conjugation, electric field induced dielectrophoresis, and microfluidic controlling based on microstructure, are used to improve the pairing precision of cells, especially heterogeneous cells. High-strength electric field can produce minipores on cell membrane and induce cell fusion. It can be generated by a constricting electric field with microstructures or two microelectrodes. In comparison with the traditional electrofusion or other cell-fusion methods, microfluidic cell-electrofusion method has many advantages such as precise manipulation, high efficiency in cell pairing and fusion, higher cell viability, lower sample contamination and smaller Joule heating effect. In this article, the development of various microfluidic cell-electrofusion methods is reviewed. Some important parameters affecting the cell electrofusion are discussed in detail. Techniques that can be integrated on microfluidic devices for high-efficiency cell electrofusion, such as on-chip cell separation and culture, are also discussed comprehensively.

Contents

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1. Introduction

Recently, nucleus transfer [1], hybridoma [2], production of cloned offspring [3–6], and the epigenetic reprogramming of somatic cells [7–10] attract many attentions. Cell fusion is one of the most important methods that can produce new intercellular genetic materials, where mediating and culturing methods are applied to merge two or more cells into a hybrid cell in an asexual way [11]. The hybrid cell obtains genetic materials from two parent cells. In addition to aforementioned applications, it also can be used in genetics [12,13], immunology [14–20], developmental biology [21], drug/gene delivery [22,23], hybridization/crossbreeding studies [6,24–28], among others [29,30]. Compared with biological [31] and chemical fusion methods, in which some hazardous exogenous materials such as inactivated virus [32,33] or polyethylene glycol (PEG) [34] are introduced, the electrofusion has considerable advantages, including easy operation, low toxicity and widespread adaptability. In addition, the efficiency of electrofusion is usually much higher than the PEG-based approach [26,35–40].

The traditional cell-electrofusion process, which was firstly developed by Zimmermann [35], can be divided into four consecutive steps: (i) cell alignment/pairing by positive dielectrophoresis (the electric field strength: 100–300 V/cm) or other methods, such as laser-based single cell manipulation [41–43], (ii) reversible electroporation on cell membrane under high-strength direct current (DC) pulses (1–10 kV cm^{-1}), (iii) membrane reconstruction and cytoplasm exchange between two cells, and (iv) nucleus fusion and hybrid cell formation. In the cell electrofusion process, cell pairing and reversible electroporation play important roles for the formation of final products. Original cell electrofusion system used a fusion chamber with two parallel wire electrodes of 0.1–0.2 mm spacing as reactors. In addition, helical chamber and fusion chamber with two wide-distance parallel electrodes (0.1–20 mm) are also developed for large-scale electrofusion [44]. Therefore, a high-voltage power supply is required to generate electric field strong enough for reversible electroporation on the cell membrane. Moreover, it is difficult to avoid the formation of multi-cell fusion in traditional electrofusion systems because similar membrane potential at each cell junction point within a long cell chain results equal probability of reversible electroporation and fusion [45]. It is also difficult to separate the hybrids from multi-cell fusion by existing cell separation methods, including fluorescence activated cell sorter (FACS), magnetic-activated cell sorting (MACS), and hypoxanthine–aminopterin–thymidine (HAT) screening. Moreover, large-scale electric field in traditional cell-electrofusion systems cannot precisely manipulate cells, and so high-precision cell pairing and high-efficiency cell electrofusion cannot be achieved.

With the development of microfabrication techniques, different types of microelectrodes and microstructures are integrated on microfluidic chips to solve the aforementioned problems by shortening the distance between the two microelectrodes or by constricting the electric field. Most existing studies focus on the two steps of the cell-electrofusion process, *i.e.* the cell pairing and cell electroporation [46,47]. Different methods used to improve these two processes are schematically shown in Fig. 1. In general, three methods, namely chemical conjugation, dielectrophoretic force (field modification/enhancement by microelectrode geometry or microstructure between electrodes) and field-free microstructures trapping (such as micro-traps, flow control *etc.*), have been used in cell pairing, whereas cell electroporation is induced by controlling the geometry of the microelectrodes or microstructures within the microfluidic devices. In the chemical conjugation method of cell pairing, two cells can be chemically conjugated by lectin or biotin–streptavidin interaction with high throughput [48,49]. However, this method lacks the ability of pairing unmodified cells. In addition, the random conjugation may induce undesired pairing. To overcome these difficulties, dielectrophoretic force, generated by optimized microelectrodes, is applied to enhance the cell-pairing efficiency. However, the dielectrophoretic force by itself cannot realize precise pairing of two heterogeneous cells like A–B type with high efficiency. The combination of microstructures, like micro-orifice/micro-trap/micropit, with other controlling methods, such as hydrodynamic pressure/gravity/dielectrophoretic force, is developed for manipulating the pairing process of heterogeneous cells. Due to the short distance between the microelectrodes or the electric field constriction, a low voltage is sufficient to achieve cell electrofusion in microfluidic cell-electrofusion devices, and thus can reduce the cost of high-voltage power generators as well as the negative effect of Joule heating present in traditional cell-electrofusion systems. Besides the cell pairing and electrofusion, the cell separation and cell culture are also indispensable manipulations for the cell-fusion research, but are not considered in most microfluidic electrofusion devices.

Since the cell pairing and cell electroporation methods are considered to be the two most important steps for electrofusion, a comprehensive review of the existing processes and current trends in these fields warrants considerable attention. And therefore, in this article, we have presented a detailed review of the latest achievements of the chip-based cell electrofusion as follows:

- Firstly, cell pairing in microfluidic devices is discussed in detail. Different methods used to obtain high heterogeneous cell pairing efficiency, such as electric field manipulation, chemical conjugation and microstructure trapping, are discussed in terms of improved cell pairing processes.
- Secondly, reversible electroporation on microfluidic devices is presented in detail. Various methods used to generate high-strength localized electric field in terms of optimization of the geometry of microelectrodes/microstructures *via* electric field constriction effect are discussed.

In order to understand the functionality and performance of these microfluidic devices better, various methods used for cell pairing and cell electroporation are compared as well. Additionally, some novel microfluidic cell electrofusion devices using suitable combination of aforementioned cell pairing and cell reversible electroporation methods are described. In addition, the influences of some important factors on the overall electrofusion process, such as the material and shape of the electrodes, cell types, osmolarity of buffer solution *etc.* are also discussed in detail. Finally, the shortcomings of the existing microfluidic cell electrofusion are summarized, and some trends and guidelines are proposed for future works.

2. Cell pairing

It is well known that stable pairing of cells is the basis for electrofusion. In order to obtain high fusion efficiency, high efficiency in cell pairing is required. In conventional research, cell fusion is accomplished in a fusion chamber by high speed centrifugation [50], chemical induction [51,52] and dielectrophoresis to perform cell pairing [38,53–56]. However, these methods are in general based on a random cell contact, and cannot control the number of cell chains. It results in low pairing efficiency, especially for the two-cell pairing and the pairing of heterogeneous cells. The paired cells include the types of AA, BB, and AB, among which only AB is the desirable type for cell fusion. Some complex detection or separation techniques, such as FACS, MACS, HAT screening, are developed for isolating AB cells from the cell suspension. Moreover, high-ratio alignment/pairing of multiple cells will produce many undesirable