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Controllable cell electroporation using microcavity electrodes

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ABSTRACT

Cell electro-fusion includes four steps, cell alignment, cell electroporation, reconstruction of cytomembrane and cytoplasm exchange. The cell alignment and electroporation steps are highly related to the intensity and distribution of the electric field, which depend on the applied voltage as well as the microelectrode structure. The microelectrode structures were first evaluated based on the numerical analysis of the electric field and the transmembrane potential induced on biological cells when the cell electroporation and electro-fusion were performed based on different designs of microelectrodes. Microelectrodes in a micro-cavity geometry can induce electroporation around the contact area of the paired cells for high-yield electro-fusion. Microfluidic chips with co-planar microelectrodes and microelectrodes within micro-cavities have been fabricated and tested for electro-fusion of Myoblast cells, and the experimental results confirmed the numerical analysis.

1. Introduction

When cells are subject to an external electric field, a voltage difference is induced across the cell membrane, which is termed as the transmembrane potential (TMP). If TMP exceeds the membrane breakdown voltage, which is about 1 V for most cells, nanopores are created on the cell membrane, which is called electroporation or electropermeabilization [1,2]. The created nanopores can be reversible or irreversible, decided by the duration and the intensity of the external electric field. For the irreversible electroporation, the TMP required is around twice as high as the reported reversible electroporation potential [3]. One of the most important applications of the cell reversible electroporation is cell electro-fusion, which uses a high-strength electric field to induce cell alignment/pairing, membrane permeabilization, and fusion of paired cells to form hybrid cells [4]. Since the formed hybrid cells contain the genetic materials from parent cells, cell electro-fusion provides a feasible technology and approach to understand and study genetics [5,6], immunology [7,8], hybridization, and crossbreeding [9,10]. Compared with the virus- [11] and PEG-induced cell fusion techniques, electro-fusion attracts high attention due to its non-toxicity, wide adaptability to cell types, easy implementation and high reproducibility [4,12,13]. In addition, the electro-fusion efficiency is much higher than the other methods. Generally, the electro-fusion process can be divided into four sequential steps: cell alignment (pairing), reversible electroporation on cell membrane, reconstruction of cytomembrane, and cytoplasm exchange between two cells [4]. Cell alignment and reversible electroporation are the most important two steps, which highly depend on the local electric field intensity as well as its gradient. Typically, cell alignment is achieved under a low-amplitude (around 100–300 V cm^{-1}) and high-frequency (around 1–3 MHz) sinusoidal alternating current (AC) field, while a series of short-duration (around 10–50 μs) and high-strength (around 1–10 kV cm^{-1}) electric pulses is applied to induce reversible electroporation [14].

For a successful cell electro-fusion, the reversible electroporation should take place only at the contact area of the paired cells, which requires that the TMP distribution should be well controlled to induce electroporation at the contact area. The TMP distribution is controlled by the electric field around the cell, which depends on the geometry of the microelectrodes as well as the microfluidic channel. In the last decade, although many kinds of microelectrode/microfluidic structures have been developed for cell electroporation [15–18] and electro-fusion [19–22], there are few studies focused on the controllable electroporation.

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Techaumnat and Washizu [23] conducted a numerical analysis of the TMP on biological cells with the effects of an orifice plate in electroporation and electro-fusion. Electric field is constricted at the orifice, where the magnitude and localization of the TMP can be controlled, and yields high electro-fusion efficiency. Rems [24] reported that nanosecond pulses can induce selective electroporation at the contact areas of paired cells, regardless of the cells' sizes. In the nanosecond range, cell membranes are still in the charging phase, and electric field at the contact area reaches the highest value, yielding selective contact electroporation. Previously we developed and tested continuous protruding microelectrode array [25–27] for cell electrofusion. As the most important process of cell electrofusion, the location and intensity of reversible electroporation are highly dependent on the electric field distribution around the cells. To obtain desirable cell alignment and reversible electroporation efficiency, two kinds of discrete microelectrode arrays, 3D thin film microelectrode [28] and discrete co-planer vertical sidewall microelectrode [29], have been developed to optimize the electric field distribution. Although the cell electrofusion efficiency is improved by this structure, we found that irreversible electroporation occurring at the contact point between discrete microelectrodes and their adjacent cells. Therefore, the existing microelectrode structures still need to be optimized to achieve controllable electroporation.

In addition to cell electrofusion, electroporation has been widely used in other applications such as cell lysis [30] and gene transfection [31]. Depending on the application, the desirable position for cell electroporation is different. In addition, most previous studies related to cell electroporation focused on experimental investigation using different structures of microelectrodes and/or microchannel, and there is no appropriate design tool for the design and optimization of the device for achieving selective cell electroporation at the desirable position. This study develops a mathematical model for the electroporation process, and is further validated by experimental results. Based on the distribution of the formed nanopore density predicted from the validated model, one can easily modify and optimize the design for controlling the position of cell electroporation, which would improve the efficiency of the electroporation applications.

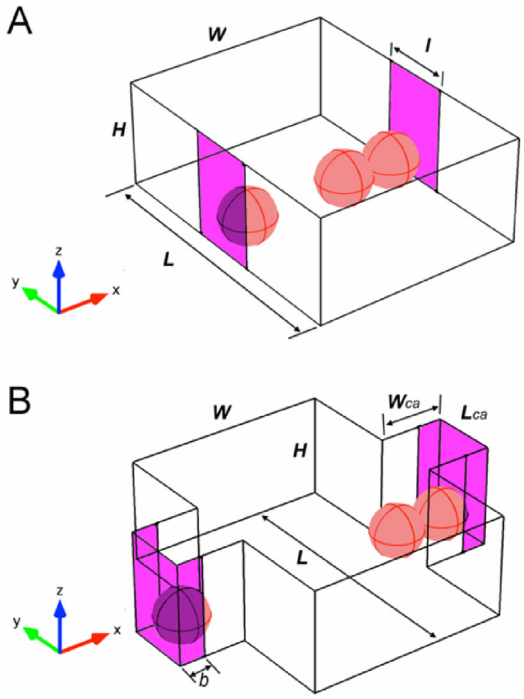

Fig. 1. Configuration for the discrete co-planar microelectrode array (A) and the micro-cavity structure (B).

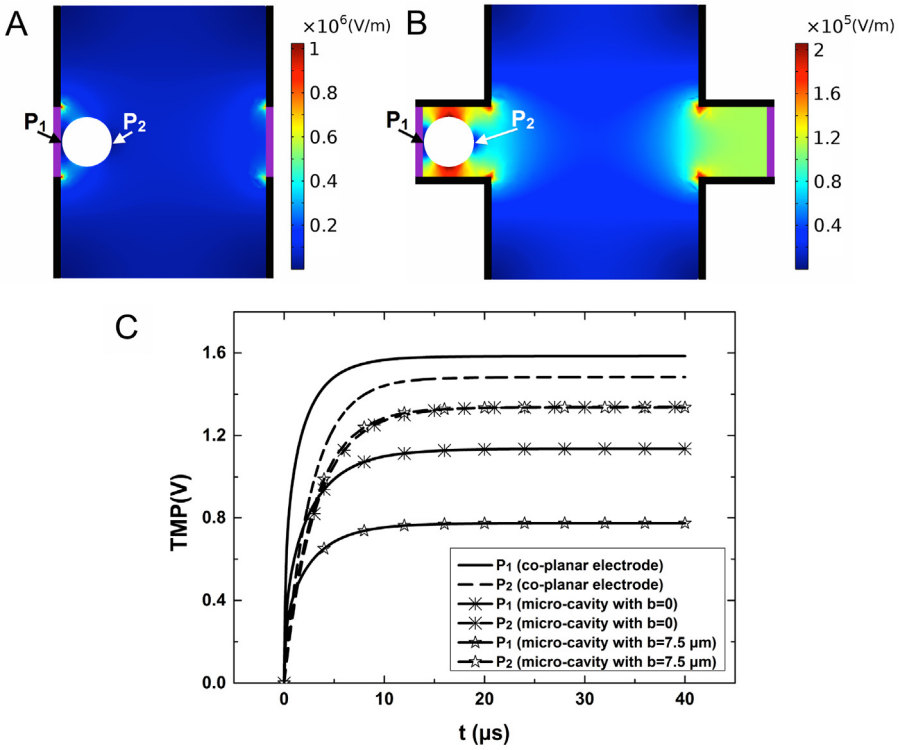

Fig. 2. (A) Spatial distribution of the electric field strength in the discrete co-planar microelectrode array with one cell, (B) Spatial distribution of the electric field strength in the micro-cavity microfluidic channel with one cell, (C) The absolute value of TMP of P_1 and P_2 for the case of single cell electroporation in the discrete co-planar microelectrode and in the micro-cavity structure.