Colloids and Surfaces B: Biointerfaces 110 (2013) 81–87

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Electroformation and electrofusion of giant vesicles in a microfluidic device

Zhenyu Wang[a], Ning Hu[a,b,*], Li-Hsien Yeh[c], Xiaolin Zheng[a], Jun Yang[a,**], Sang W. Joo[b,***], Shizhi Qian[b,d,****]

[a] Key Laboratory of Biorheological Science and Technology (Chongqing University), Ministry of Education, and Key Lab of Visual Damage and Regeneration & Restoration of Chongqing, Chongqing, Bioengineering College, Chongqing University, Chongqing 400030, China
[b] School of Mechanical Engineering, Yeungnam University, Gyongsan 712-749, South Korea
[c] Department of Chemical & Materials Engineering, National Yunlin University of Science and Technology, Yunlin 64002, Taiwan
[d] Institute of Micro/Nanotechnology, Old Dominion University, Norfolk, VA 23529, USA

ARTICLE INFO

Article history:
Received 10 January 2013
Received in revised form 10 April 2013
Accepted 16 April 2013
Available online 30 April 2013

Keywords:
Giant vesicle
Electroformation
Electrofusion
Drug delivery
Microfluidics

ABSTRACT

Electroformation and electrofusion of giant vesicles with diameters of 10–20 μm have been performed in a microfluidic device with high-density microelectrodes forming the sidewalls of the microchannel. Electroformation of giant vesicles by a solution mixture of phosphatidylcholine (PC) and cholesterol (Chol) with different concentrations under AC electric field was investigated. Under the conditions of 0.5–12 mg/mL PC and 0.1–2.4 mg/mL Chol, vesicles were electroformed by the AC electric field imposed. About 60% electroformed vesicles were giant (unilamellar) vesicles with diameters 10–20 μm. The eletroformed vesicles were collected from the chip, re-suspended in fresh buffer, and then separated by centrifugation to segregate the ones with desired diameters (10–20 μm). Electrofusion of the giant vesicles was conducted in the same chip. Vesicles were aligned to form pairs under AC electric field due to positive dielectrophoresis, and the paired vesicles were subsequently fused upon the application of high strength electrical pulses. The alignment and fusion efficiencies were, respectively, about 50% and 20%.
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1. Introduction

Giant lipid membrane vesicles (giant vesicles) with diameters larger than 10 μm have obtained widespread attentions for their potential applications, including drug delivery [1–4], microreactors [5–8], and modeling cytomembrane systems [9]. Since the electroformation method of giant vesicles was first developed by Angelova and Dimitrov [10] in 1986, a growing number of devices have been implemented to improve this technique [11–24]. Owing to relatively easy fabrication, chemically inert nature to various organic solvents, and adjustable distance between two parallel electrodes, the device with a pair of parallel platinum electrodes apart has been widely adopted for the electroformation of giant vesicles [11–15]. In such a device, Dimitrov and Angelova [11] investigated some key factors, such as the lipid swelling and the charge and frequency of the imposed electric field, on the electroformation process of giant vesicles, and proposed a possible mechanism [12] for the electroformation process. Giant vesicles were also prepared by Angelova et al. [13] to investigate the enzyme-mediated vesicle transformation by microinjecting reagents. Bucher et al. [14] further electroformed giant vesicles as biochemical compartments, while Okumura et al. [15] electroformed giant vesicles on a non-electroconductive substrate that helped further examination and development of the electroformation by allowing various unconventional setups and substrate surfaces. In these devices, the distance between the two parallel electrodes was 0.5–4 mm, and the diameter of the electrodes was 0.48–0.5 mm, resulting in a non-uniform distribution of the electric field [25]. To induce a sufficiently high electric field for electroforming giant vesicles, an electric potential bias (0.01–17 V) is applied [10–15]. However, the shape of electrodes is typically cylindrical [10–15], making the droplets of lipid solution on the electrodes distributed unevenly and affecting the formation of lipid membranes and vesicles. In addition, the traditional device is not transparent,

* Corresponding author at: Key Laboratory of Biorheological Science and Technology (Chongqing University), Ministry of Education, and Key Lab of Visual Damage and Regeneration & Restoration of Chongqing, Chongqing, Bioengineering College, Chongqing University, Chongqing 400030, China. Tel.: +86 23 65111931; fax: +86 23 65111931.
** Corresponding author. Tel.: +86 23 65111931; fax: +86 23 65111931.
*** Corresponding author. Tel.: +82 53 810 2568; fax: +82 53 810 2062.
**** Corresponding author at: Institute of Micro/Nanotechnology, Old Dominion University, Norfolk, VA 23529, USA. Tel.: +1 757 683 3304; fax: +1 757 683 3200.
E-mail addresses: Huning0214@hotmail.com (N. Hu), bioyangjun@cqu.edu.cn (J. Yang), swjoo@yu.ac.kr (S.W. Joo), sqian@odu.edu (S. Qian).

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http://dx.doi.org/10.1016/j.colsurfb.2013.04.042

so that it is difficult to observe the vesicles electroformed near two electrodes, especially those under the cylindrical electrode.

In order to observe giant vesicles, Angelova et al. [16] used two transparent indium tin oxide (ITO) glass electrodes 0.3 mm apart each other to electroform giant vesicles. To further reduce the distance between the two parallel ITO glass electrodes, Kuribayashi et al. [21] developed a chip consisted of several microfluidic channels sandwiched between two ITO glass electrodes to produce various types of giant vesicles. Estes and Mayer [18–20] electroformed giant vesicles by first spin coating lipids on an ITO glass slide. The electroformation of giant vesicles can be observed in these transparent devices. The electric field strength between two ITO glass electrodes is higher than that between two parallel platinum wires, and the ITO glass electrodes are planar and have larger surface area than the parallel platinum electrodes, resulting in higher electroformation efficiency. In addition, the uniform distribution of the electric field between two ITO glass electrodes also produces a constant electrical field for electroformation, improving the performance of that device. However, precise control of the vesicle formation on ITO glass is challenging because it is not easy to pattern lipid film on ITO glass due to its complex surface properties [22]. In addition, the two parallel flat ITO glass electrodes are placed at different levels, resulting in vesicle chains stacked on top of each other along the direction of the electric field. Therefore, in these transparent devices it is still challenging to observe the electroformation process of individual giant vesicles, and thus hard to enlighten the mechanisms involved.

With the rapid development of microfabrication technology, precise control of the vesicle formation with a desired size has attracted considerable attention. Microchips for the electroformation of giant vesicles have been developed. Taylor et al. [17] electroformed giant vesicles with a narrow size distribution on an ITO glass substrate, which had patterned lipid film by using polydimethylsiloxane (PDMS) stamp technique. The size of electroformed vesicles can be controlled by the size of the lipid film pattern. Using a micropatterned silicon dioxide layer with circular holes arranged in a hexagonal array on a Si substrate and an ITO glass electrode, separated by a 1 mm silicone rubber spacer, a microchip was developed by Le Berre et al. [22], and giant vesicles with a narrow size distribution were successfully obtained. Diguet et al. [23] fabricated a microdevice composed of a microstructured silicon wafers (with hexagonal arrays of holes) and an ITO glass slide as a counter electrode separated by 4 mm silicon rubber spacer, and giant vesicles with narrow size distribution were electroformed by applying an AC electric field between the two electrodes. Takeuchi and Kuribayashi [24] designed a microchip with a microaperture array to electroform dome-shaped organic-solvent-free artificial lipid membranes. This device overcomes some disadvantages of the ITO glass based devices, and has high yield to produce giant vesicles with narrow size distribution. However, in these devices, at least one ITO glass slide is used as one electrode, and it is separated from the other electrode by a micro-spacer. Furthermore, in these devices, lipid film must be formed at some given positions and manipulation is complicated [17,22–24].

In this study, a microchip with two types of microelectrode arrays, schematically shown in Fig. 1, is designed, fabricated and tested on a transparent quartz-glass substrate to electroform cell-size giant (unilamellar) vesicles (i.e., 10–20 μm in diameter). In contrast to the traditional devices composed of two large parallel electrodes, such as the ITO glass slide, this microchip consists of two parallel chiasm-shaped microelectrode arrays (serpentine-shaped microchannel sidewall). Each microelectrode array has many silicon microelectrode strips. In the first design each strip has protruding microelectrodes (Fig. 1b), resulting in spatially non-uniform electric field inside the microchannel. The second design does not protrude microelectrodes on each strip (Fig. 1c), and the resulting electric field between two opposite electrodes is uniform. After loading the lipid solution into the microchannel, lipid film forms on the electrodes which are also the sidewalls of the microchannel, after the lipid solution is dried. Owing to the small distance between the opposing electrodes (i.e., the width of the microchannel), a strong electric field can be generated under a low electric voltage. Since the direction of the electric field on this chip is horizontal, the formed vesicles will not stack lengthwise, which is helpful for real-time observations. To further test that the electroformed giant vesicles have the capability to enclose other materials for applications, such as drug delivery and gene transferring, the formed giant vesicles are washed out, resuspended, separated by centrifugation, and reloaded into the same device for electrofusion.

2. Experimental methods

2.1. Materials and instruments

L-α-Phosphatidylcholine (1,2-diacyl-sn-glycero-3-phosphocholine), cholesterol (3β-hydroxy-5-cholestene), and fluorescent dye (DiI) (1,1′-dihexadecyl-3,3,3′,3′-tetramethylindocarbo-cyanie perchlorate, ex/em: 549/564 nm, Molecular Probes) were purchased from the Sigma–Aldrich, Inc. Centrifuge 5417R (Eppendorf, Germany) was used for the separation of giant vesicles by centrifugation. A home-made electrical signal generator was used to generate required electrical signal for the electroformation and electrofusion of giant vesicles, and DMI4000 B phase contrast inverted fluorescence microscope (Leica, Germany) was used for experimental observation.

2.2. Chip design and fabrication

The microdevice consists of a serpentine-shaped microchannel of 42 μm in depth, whose opposite sidewalls are made of two chiasm-shaped microelectrode arrays. Two types of microelectrode arrays, one with protruding microelectrodes to generate spatially non-uniform electric field (Fig. 1b) and the other with planar microelectrodes to generate uniform electric field (Fig. 1c), are designed for testing the effect of the distribution of local electric field on the electroformation of giant vesicles. For the design with protruding microelectrodes, the width of the microchannel is 80 μm. Both the length and width of each protruding microelectrode are 20 μm, and the distance between two adjacent protruding microelectrodes is 60 μm (Fig. 1b). For the design with planar electrodes, the width of the microchannel and the distance between two counter electrodes are 80 μm (Fig. 1c).

The microchip was fabricated by using the MEMS (microelectro-mechanical systems) fabrication techniques [22–24]. The chiasm-shaped microelectrode arrays were fabricated on a 40 μm thick, highly doped silicon wafer, which also worked as the vertical sidewalls of the microchannel. First, the silicon wafer (resistance 7–9 mΩ, crystal orientation ⟨1 0 0⟩) was bonded with a 500 μm thick Corning 7740 glass wafer by using the electrostatic-alloy bonding technique under the condition of 400 °C, 600 V, and 1000 N force in the vacuum. Subsequently, a 50 nm Cr film was sputtered onto the silicon wafer, followed by electroplating 2 μm Au on the surface of the Cr film. The unwanted Cr/Au was etched away by using KI etching solution (28 g KI; 20 g I_2, and 800 mL H_2O). Finally, the microelectrode arrays were etched on the heavily doped silicon by inductively coupled plasma (ICP) etching technology. The chip was then fixed on a printed circuit board (PCB), and the microelectrode arrays were connected to gold weld spots on the PCB by using gold silks of 75 μm in diameter. The ceiling of the microchip was a PDMS layer, which was bonded on the microchip after the organic solvent of the lipid solution evaporated.

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