SMOOTH-TIP DIELECTROPHORESIS BASED TWEESERS FOR SINGLE LIPOSOME HANDLING

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ABSTRACT

In prior work, we reported an efficient methodology for cell-sized liposome manipulation using DEP-based tweezers [1]. In this work, we improved the tweezers that was added further precision in the liposome handling. This devise consists of a smooth-tip glass needle and a pair of thin Au-film electrodes. The smooth tip, which is fabricated by melting a finely-pulled glass needle, is able to concentrate the DEP effect. Using this tweezers, we succeeded in capturing, manipulating and positioning single giant-liposomes of various sizes. This device will become an efficient tool for handling single biological particles such as liposome-based artificial cells.

KEYWORDS

Liposome, Single-particle Handling, Dielectrophoresis

INTRODUCTION

Liposome is a spherical shape of a bilayer lipid membrane which is a basic component of cell membranes and contains an aqueous solution in the inside of the membrane. In nature, living organisms use this structural aspect to wrap up the cell itself, to encapsulate biological substances for intercellular transports, to store enzymes, and to create isolated chemical reaction chambers. In addition, the bilayer form of the membrane is able to keep membrane proteins which play significant roles in living cells. Because of these advantages in the structure and the characteristics, artificially formed liposomes are widely used in biological research such as drug delivery systems [2] and membrane protein studies [3].

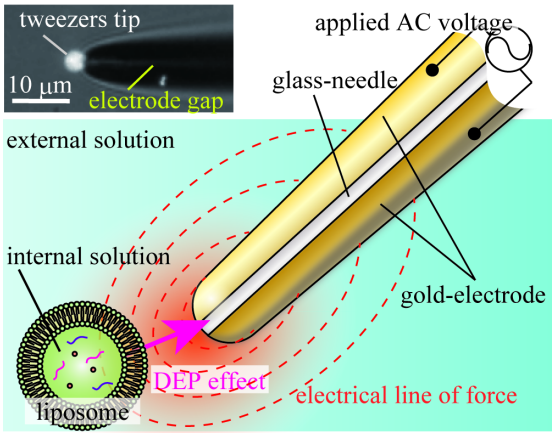

Fig. 1 Conceptual diagram of the smooth-tip DEP-based tweezers for giant-liposome handling. A pair of Au-electrodes is coated on the both sides of a fine glass-needle whose tip is smoothly melted. When an AC potential is applied, the electrical field concentrates near by the tip and able to capture a single liposome.

In recent years, cell-sized liposomes have been also focused as minimal bio-reactors or artificial cell models since the size is suited for robust containers of pL volumes as well as for observation with fluorescence microscopy [4-5]. For these cell-sized liposome applications, an efficient tweezing methodology is required for precise operation with a single liposome level in order to observe and analyze the individual liposomes. However, the conventional methodologies of particle handling, such as the pipet-based tweezers and the optical tweezers, are not perfectly matched for the liposomes because the liposome core is mainly composed by an aqueous solution (unlike cells) as well as the lipid membrane easily deforms. The pipet-based methodology attracts and holds a target particle on the thin capillary tip by applying a negative pressure inside of the capillary. This methodology is very powerful to manipulate a single cell since it can easily switch the holding/releasing modes but the strong flow by the pressure induces the deformation of the cell-sized liposome and suction into the capillary [6]. On the other hand, the optical tweezers hold the target particle by using a laser beam which is exposed from an objective lens of a microscope. The applied laser is refracted in the target particle suspended in a solution, which generates a momentum change of the laser beam that holds the particle. In order to generate a sufficiently strong trapping force, the major difference of "refraction index" is required between the target substance and the medium [7]. However, the liposome core often contains an aqueous solution similar to the external solution, unlike the cellular cytoplasm. Hence, the force of the optical tweezers only influences to the thin lipid membrane, which is insufficient for the liposome handling.

In this research, we took advantage of the dielectrophoresis (DEP) effect to hold a single cell-sized liposome. DEP is the phenomenon arising from a non-uniform electric field and has been applied in microfluidic systems to trapping, sorting, and patterning target particles in a fluidic solution with electrodes, in which the devices were fabricated by using a basic MEMS technique. DEP force (F_{DEP}) depends on the electrical characteristics between the target particle and the surrounding medium, and can be described as

$$F_{DEP} = 2\pi a^3 \varepsilon_m Re(K(\omega))\nabla E^2$$

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

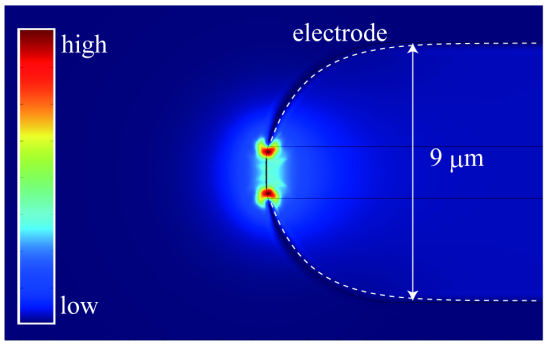

Fig. 2 The simulated result of the electrical field intensity square at the tweezers tip model. An electrical potential (100 mV_{pp}) was applied between the two electrodes. The smooth shape concentrates the electrical field intensity within the two narrow regions.

, where a is the particle radius, ε_m is the permittivity of the medium, E is the electric field intensity, and $K(\omega) = (\sigma_p^* - \sigma_m^*)/(\sigma_p^* + 2\sigma_m^*)$ is the Clausius-Mossitti function; the dimensionless factor (*) represents the frequency-dependent electrical properties of the particle ($\sigma_p^* = \sigma_p + i\omega\varepsilon_p$) and the medium ($\sigma_m^* = \sigma_m + i\omega\varepsilon_m$) [8-9].

Previously, several DEP-based tweezers were reported as cell handling devices. One type of the devices consists of a fine-tipped glass capillary embedding a metal wire, and the other type coats the capillary with metal films as the electrodes [10-14]. The tweezers with the metal wire succeeded in precise handling of living cells, but the fabrication procedure was difficult to reproduce. The other type of the device with metal films was easy to fabricate but difficult to handle a single cell one by one. Therefore, we aimed at developing the tweezers for single liposome handling with an easy fabrication process. The DEP-based tweezers consist of a glass needle with a pair of metal film electrodes as described in Fig. 1. The shape of the tweezers tip is smooth hemisphere, which enables the concentration of the electrical field near by the tip, and to handle a single cell-sized liposome. With the application of an AC potential, a non-uniform electrical field is generated at the tweezers tip and the target liposome is attracted by DEP effect. This tweezers can be fabricated by a robust and very easy step, which may be applied to bulk production.

EXPERIMENT

Cell-sized liposomes were formed by the common electroformation process [15]. A lipid solution (0.5 mg/ml of DOPC with 5% rhodamine-conjugated DOPE in chloroform and methanol (2:1 in volume)) was dried in vacuum and hydrated with the electroformation. By using the electroformation method, various sizes of liposomes were prepared (1~30 μm). The interior and exterior solutions were replaced with 10 mM NaCl/sucrose and glucose, respectively (adjusted at 100 mOsm).

The smooth-tip DEP-base tweezers was fabricated by the following process. Round shaped glass rod (diameter: 1 mm, length: 90 mm) purchased from NARISIGE (Tokyo, Japan) was stretched to a fine tip by using a glass puller. The tip was then melted smoothly by a thermal polishing process with a microforge, and cleaned by oxygen plasma. The cleaned glass needles were sandwiched between the PMMA double-side shadow-masks, which consist of two glass-needle holders with narrow slits [1]. Thin Cr/Au films were sequentially deposited on one side of the glass needle through the slits with a high-vacuum thermal evaporator, and then the PMMA mask was flipped and metals were subsequently deposited on the other side. After the electrode depositions, the smooth-tip DEP-based tweezers was wired with electrically conductive tapes to connect to an AC potential generator for the liposome handling experiments.

We simulated the 2-dimensional electrical field intensity square (E^2) at the tweezers tip under the application of an AC potential using COSMOL Multiphysics to confirm the holding point of the developed tweezers. Fig. 2 shows the result applied an electrical potential of 100 mV between the two metal-film electrodes. The red and blue colors represent the intensity of E^2 from a positive potential to zero. As shown in Fig. 2, E^2 concentrates at the edges of the two electrodes and covers the tweezers tip. Therefore, the captured targets by the DEP force would be attracted to these points.

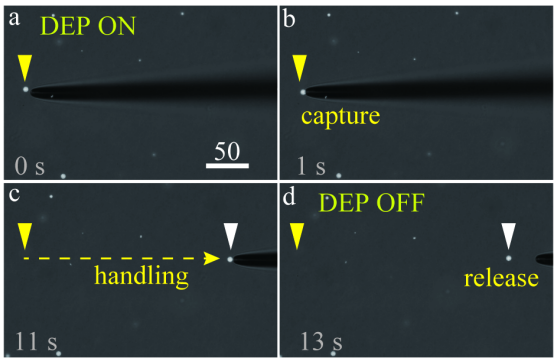

Fig. 3 Liposome handling by the DEP-based tweezers (Applied AC field:1 MHz, 200 mV_{pp}). (a) The tweezers tip was positioned by a liposome and an AC potential was applied. (b) The tweezers captured the liposome. (c) DEP force allowed to manipulate the liposome. (d) By turning off the AC field, the liposome was released and positioned.

RESULTS & DISCUSSION

The performance of the tweezers is presented in Fig. 3. The tweezers tip was set near by a target liposome and an AC potential (200 mV, 1 MHz) was applied between the two electrodes by a function generator (Fig. 3a). The liposome was immediately attracted toward the tweezers tip and captured (Fig. 3b). With the lateral motion of the tweezers by a micromanipulator, the captured liposome followed (Fig. 3c). When the applied AC potential was turned off, the liposome was released from the tip and stayed still (Fig. 3d). Due to the focused DEP force at the smooth tip, a single liposome with 2 μm in diameter was successfully captured, manipulated and positioned.

Fig. 4 shows the handling capability of the tweezers

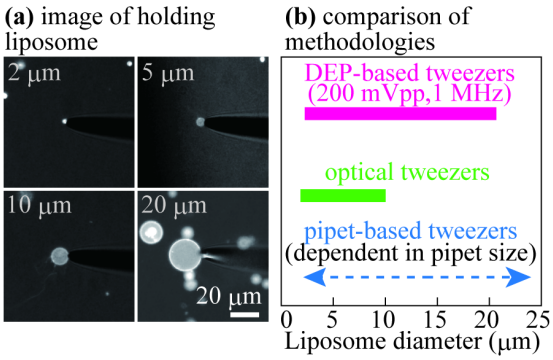

Fig. 4 Handling capability of the DEP-base tweezers on the liposome size (a) Microscopic images of the manipulated liposomes with various sizes. (Applied AC field: 1 MHz, 200mV). (b) Comparison of the handling performance between the different types of tweezing methods. The pipet size determines the feasible liposome sizes for the pipet-based tweezers, but the size range is small.

against the liposome size. Comparing to the other handling methods, the developed smooth-tip DEP-based tweezers was able to handle single liposomes with a wide range of sizes by the one device design [7,16].

CONCLUSION

In this study, we succeeded in handling cell-sized liposomes by using the smooth-tip DEP-based tweezers. The smooth-tip design enabled the concentration of electric field at narrow space, allowing capturing, manipulation, and positioning of a single liposome one by one. This device showed a wider size range of the handling capability compared to the other methods. The developed DEP-based device will become an efficient tool for handling single biological particles such as liposome-based artificial cells.

ACKNOWLEDGMENT

This work was partly supported by JSPS (Grant-in-Aid for Young Scientists B; 23710154), Japan.

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