Int. J. Electrochem. Sci., 14 **(2019)** 905 – 918, doi: 10.20964/2019.01.77

International Journal of ELECTROCHEMICAL SCIENCE
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The Influence of Electric Parameters on the Manipulation of Biological Cells in a Microfluidic System Using Optically Induced Dielectrophoresis

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Received: 7 October 2018 / *Accepted:* 11 November 2018 / *Published:* 30 November 2018

The integration of optically induced dielectrophoresis (ODEP)-based cell manipulation in a microfluidic system has been actively proposed for various biomedical researches. To realize ODEP-based cell manipulation for a special application, the selection of an appropriate ODEP operating condition is critical. The magnitude and frequency of the exerted electric voltage in an ODEP system are the two commonly-used working parameters. Although several studies had explored the influence of electric conditions on the cell manipulation using ODEP, few of them further considers the other co-existing phenomena which might affect the intended application of ODEP-based cell manipulation. To address this issue, this study has investigated the influence of electric parameters [i.e., magnitude and frequency of electric voltage: 1 - 10 Vpp and 1 kHz - 10MHz, respectively] on the ODEP-based cell manipulation. Results revealed that cell lysis could occur under low electric frequency (e.g., 1 kHz). Moreover, most of the cells were not effectively manipulated when the electric frequency was lower than 100 kHz due to cell adhesion on substrate surface. Conversely, most of the cells were effectively manipulated when the electric frequency reached 200 - 300 kHz. However, these manipulated cells tended to aggregate. This phenomenon could be prevented when the electric frequency was higher than 1 MHz. At a given electric frequency of 1 MHz, furthermore, the magnitude increase of electric voltage (2 - 8 Vpp) did not cause the unwanted phenomena aforementioned but increase the maximum velocity of a dynamic light image that can manipulate cells. At a given magnitude of electric voltage, conversely, further increase (higher than 1 MHz) of electric frequency might compromise the maximum velocity of a dynamic light image that can manipulate cells. Overall, all these information is important for scientists to select appropriate electric parameters for the cell manipulation using ODEP.

Keywords: Optically induced dielectrophoresis (ODEP), Microfluidic technology, Cell manipulation, Electric parameters

1. INTRODUCTION

The manipulations (e.g., transportation, sorting, isolation, or immobilization) of biological cells are the fundamental operations in conventional biomedical studies [1, 2]. Due to the technical progress in bio-microelectromechanical systems (bioMEMS) and microfluidic technology, these operations can be realized within micro-scale devices which provides several advantageous features (e.g., higher working performances) over the conventional macro-scale counterparts [3, 4]. For this purpose, several novel techniques [mainly fluidic- [5], magnetic- [6], acoustophoresis- [7] dielectrophoresis (DEP)- [8], or optically induced dielectrophoresis (ODEP)- [9] based methods] have been proposed to integrate with microfluidic systems for various cell manipulation operations (e.g., transportation [10], sorting [11], isolation [9], patterning [12], or immobilization [13]).

Among these techniques, the utilization of DEP force for cell manipulation in microfluidic systems has attracted most scientists' interest. This is primarily because that DEP-based technique is capable of providing precise cell manipulation in a microfluidic system [14]. The utilization of DEP for microparticle manipulation was first proposed by Herbert A. Pohl in 1951[15]. Briefly, when dielectric microparticles (e.g., biological cells) are suspended in solution under a given electric field, charges can be electrically polarized on the microparticle surface. The interaction between induced charges on the microparticles and the applied non-uniform electric field can generate a so-called DEP force [14]. In practices, one can control the non-uniform electric field created by the microelectrodes within a DEP system to manipulate the dielectric microparticles in a controllable manner. Although the utilization of DEP force-based technique for cell manipulation in microfluidic systems has been successfully demonstrated for various applications [8, 14, 16], such a technique generally requires a time-consuming and costly microfabrication process to create a unique microelectrode layout that is specific to the application [4, 17].

To address the abovementioned technical issue, and to realize more flexible cell manipulation, the cell manipulation using ODEP is found particularly promising. The use of ODEP mechanism for microparticle manipulation was originally proposed by Pei Yu Chiou in 2005 [17]. Its working principle is similar to that of DEP mechanism as aforementioned, in which, however, the concrete microelectrodes are replaced by the light images, serving as virtual microelectrodes. Briefly, an electrical voltage is applied between two indium-tin-oxide (ITO) glass substrates in an ODEP microfluidic system to generate a uniform electric field in the solution layer between the two glass substrates. In this situation, the dielectric microparticles (e.g., biological cells) suspending in the solution layer are electrically polarized. When the photoconductive material on the bottom ITO glass substrate is illuminated with light, it can cause the electrical voltage to drop across the solution layer within the light-illuminated area. This can therefore create a non-uniform electric field within the ODEP system. In the cell manipulation using ODEP, the interaction between the electrically polarized cells and the generated non-uniform electric field is utilized to manipulate the cells [17, 18]. In practice, the scientists can simply control of movement of light images that is illuminated onto an ODEP system to manipulate cells in a controllable

manner, which not only greatly simplifies the microfabrication process but also contributes to more flexible and user-friendly cell manipulation operations [4, 17, 18].

In the practical operations of ODEP-based cell manipulation, there are several working conditions (mainly the magnitude and frequency of the applied electric voltage, and property of the surrounding solution [18, 19]) that might influence the working performances (e.g., the maximum velocity of a dynamic light image which can manipulate a cell [11, 19, 20]) of ODEP-based cell manipulation. Particularly, in terms of the magnitude and frequency of the exerted alternating current (AC) electric voltage in an ODEP microfluidic system, they could also influence the property of the cells manipulated that might in turn lead to cell aggregation, cell adhesion on substrate, or cell death [12, 18, 19]. As a result, it is important to comprehensively consider the impact of electric conditions used on the ODEP-based cell manipulation for the selection of the appropriate operating conditions for a specific application. For example, the prevention of cell death or cell aggregation during ODEP-based cell manipulation is critical for some specific applications (e.g., cells' gene expression assays [11] or size-based cell sorting, isolation, and purification [9, 11, 19, 21], respectively). In this situation, the selection of an appropriate electric condition for avoiding the unwanted phenomenon as abovementioned is important for the intended application.

Reports in literature have successfully demonstrated the incorporation of ODEP-based cell manipulation in microfluidic systems for a wide variety of applications such as the isolation of circulating tumor cells (CTCs) [11, 19] and CTC clusters [9], sorting of reproductive cells [22, 23], and discrimination of live and dead cells [17, 22, 24]. In these studies, however, the electric conditions adopted for a specific application were mainly determined empirically. Although there are several theoretical studies [11, 20, 22, 25, 26] describing the effect of different operating conditions on the cell manipulation using ODEP, few of them further consider the phenomena (e.g., cell aggregation, cell adhesion on substrate surface, or cell damage) which might co-occur in the real situation. To address this issue, this study aimed to investigate the influence of electric parameters [i.e., magnitude of AC electric voltage: 1 - 10 Vpp; frequency of AC electric voltage: 1 kHz - 10 MHz] on the cell manipulation using ODEP in terms of cell aggregation, cell death, cell adhesion on substrate surface, and the maximum velocity of a dynamic light image which can manipulate cells. Results revealed that the phenomenon of cell lysis could occur under low electric frequency condition (e.g., 1 kHz). Moreover, it was also observed that most of the cells were not effectively manipulated, and thus successfully delivered when the set frequency of electric voltage was lower than 100 kHz. This phenomenon was mainly due to cell adhesion on the substrate surface. Conversely, most of the cells were significantly manipulated, and effectively transported when the set frequency of electric voltage reached 200 - 300 kHz. However, these cells manipulated and collected in the cell collection zone tended to aggregate. Fortunately, this technical problem could be prevented when the set frequency of electric voltage was higher than 1 MHz. At a given electric frequency of 1 MHz, furthermore, the magnitude increase of electric voltage (2 - 8 Vpp) did not cause the unwanted phenomena aforementioned but increase the maximum velocity of a dynamic light image which can manipulate cells. At a given magnitude of electric voltage (e.g., 10 Vpp), conversely, further increase (i.e., higher than 1 MHz) of electric frequency might compromise the performance of the cell manipulation using ODEP in terms of the maximum velocity of a dynamic light

image which can manipulate cells. As a whole, this study has investigated the influence of different electric conditions on the cell manipulation using ODEP in term of the phenomena of cell lysis, cell adhesion on substrate surface, and cell aggregation as well as the maximum velocity of a dynamic light image which can manipulate cells. All these information is both fundamental and important for scientists to select an appropriate electric condition of ODEP-based cell manipulation for a specific application.

2. EXPERIMENTAL

2.1 The microfluidic chip for ODEP-based cell manipulation

In this study, a simple microfluidic chip was designed for ODEP-based cell manipulation. The top-view layout of the microfluidic chip was schematically illustrated in Fig. 1 (a).

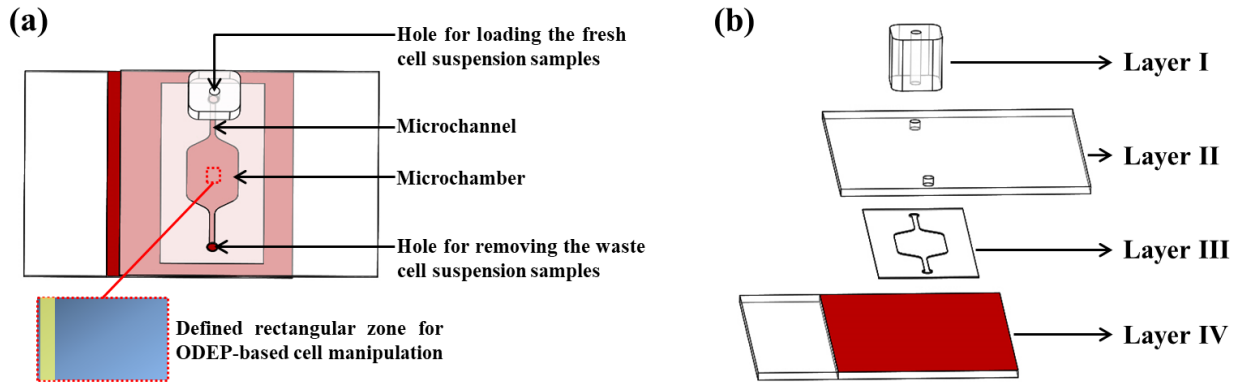

Figure 1. Schematic illustration of the (a) top-view layout and (b) assembly of the ODEP microfluidic chip (Layer I: PDMS connector; Layer II: ITO glass substrate; Layer III: Double-sided adhesive tape with microfabricated microchamber and microchannels; Layer IV: ITO glass substrate coated with photoconductive material).

In this work, a microchamber connecting 2 microchannels (L= 4 mm, W= 0.5 mm, and H= 50 μm) was designed. Two through-holes (D= 1 mm) for tubing connections were designed for loading and removing cell suspension sample, respectively. In the microchamber, the cell manipulation using ODEP was carried out within the defined rectangular zone (L= 1.7 mm, and W= 1.2 mm). The assembly of the microfluidic chip is schematically illustrated in Fig. 1 (b). Briefly, the microfluidic chip comprised a top fabricated polydimethylsiloxane (PDMS) connector (Layer I), an indium-tin-oxide (ITO) glass substrate (Layer II), double-sided adhesive tape with fabricated microchamber and microchannels (Layer III; thickness: 50 μm), and a bottom ITO glass substrate with a coating layer of photoconductive material (Layer IV; encompassing a 20-nm-thick n-type hydrogenated amorphous silicon layer and a 500-nm-thick hydrogenated amorphous silicon layer).

2.2. Microfabrication and overall experimental setup

The overall fabrication process was based on computer-numerical-controlled (CNC) machining, a metal mold-punching fabrication process, PDMS replica molding, a thin-film technology using *sputtering*, high-density *plasma chemical vapor deposition* (*HDPCVD*), and a plasma oxidation-aided bonding process as described previously [9, 11, 20]. Briefly, the PDMS connector (Layer I) [Fig. 1 (b)] was fabricated by a combination of CNC machining for poly(methyl methacrylate) (PMMA) mold making, and PDMS replica molding as described previously [9, 11, 20]. For the ITO glass substrate (Layer II), the two through-holes were mechanically drilled in ITO glass (7 Ω, 0.7 mm; Ritek, Taiwan) using a drill (rotational speed: 26,000 rpm). For Layer III, a custom-made metal mold was used to create the hollow structure of the microchamber and microchannels in double-sided adhesive tape (L298, Sun-yieh, Taiwan) through manual punch operation. For the bottom substrate (Layer IV), a 70 nm-thick ITO layer was first sputtered onto cleaned dummy glass, followed by a thermal annealing process (240 °C, 60 min). A 20-nm-thick n-type hydrogenated amorphous silicon (n-type a-Si:H) layer was deposited onto the treated ITO glass through a HDPCVD process. Next, a 500-nm-thick hydrogenated amorphous silicon (a-Si:H) layer was deposited onto the treated ITO glass through the HDPCVD process [17].

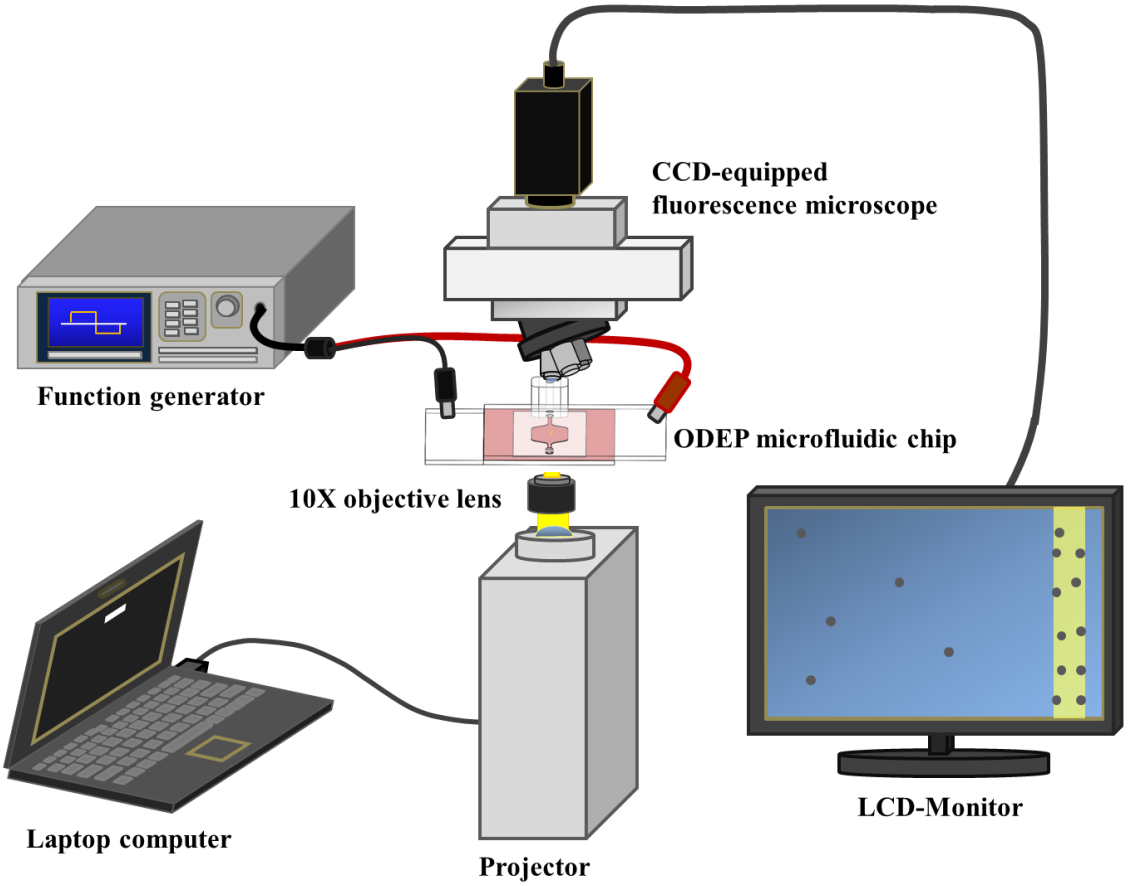

Figure 2. Schematic illustration of the overall experimental setup.

In the assembly process, the PDMS connector (Layer I) [Fig. 1 (b)] containing one through-hole

was bonded with the Layer II through O_2 plasma surface treatment. This was followed by assembly with Layer IV via the fabricated double-sided adhesive tape (Layer III). In practical operations, the loaded cell suspension sample was delivered in the microchamber and microchannels manually using a pipetman. For realizing the cell manipulation using ODEP, a function generator was used to apply an AC electric voltage between the two ITO glass substrates [i.e., Layer II and IV; Fig. 1 (b)] of the microfluidic chip. A commercial digital projector (PLC-XU350, SANYO, Japan) coupled with a computer was used to display light images onto the photoconductive material (Layer IV) to generate ODEP force on the manipulated cells. In addition, a CCD-equipped fluorescence microscope (Zoom 160, OPTEM, USA) was utilized to observe the cell manipulation process in the ODEP system. The overall experimental setup was schematically illustrated in Fig. 2.

2.3 Working conditions that can influence ODEP-based cell manipulation

The principle of ODEP-based microparticle manipulation has been described in the introduction section (also well described elsewhere [11, 17, 18, 26]). In theory, the ODEP force generated on a cell can be expressed by the equation (1) that describes DEP force [14, 18, 26]:

$$F_{DEP} = 2\pi r^3 \varepsilon_0 \varepsilon_m \mathrm{Re}[f_{CM}] \nabla |E|^2 \quad (1)$$

where r, ε_0, ε_m, $\nabla|E|^2$, and $\mathrm{Re}[f_{CM}]$ represent the cellular radius, vacuum permittivity, relative permittivity of the surrounding solution, gradient of the applied electrical field squared, and real part of the Clausius–Mossotti factor (abbreviated as f_{CM}; relevant to the angular frequency of the electric field, the conductivity of the medium, the internal conductivity of the cells, and the membrane capacitance of the cells [26]), respectively. Regarding the ODEP operating conditions, overall, the ODEP force generated on a specific cell is influenced by the magnitude and frequency of the electric voltage applied under a given solution conditions.

2.4 Evaluation of the ODEP manipulation force generated on a biological cell

In this study, the ODEP force generated on a biological cell was experimentally assessed according to the method described previously [11, 19, 20]. In order to simplify the complicacy of the external forces working on a manipulated cell occurred in a real condition, the ODEP manipulation force, a net force between the ODEP force and friction force, generated on the manipulated cell was evaluated [11, 19, 20]. In a steady state, briefly, the ODEP manipulation force of a biological cell is balanced by its viscous drag of surrounding fluid. As a result, the hydrodynamic drag force of a moving cell was used to assess the ODEP manipulation force of a cell based on Stokes' law [11, 19, 20]. Stokes' law [equation (2)] describes the drag force (F) acting on a spherical particle in a continuous flow condition.

$$F = 6\pi r \eta v \quad (2)$$

where r, η, and v represent the radius of a cell, the viscosity of the fluid, and the terminal velocity of a cell, respectively. Under a given solution property and size of the manipulated cells, therefore, the ODEP manipulation force generated on a manipulated cell can then be experimentally evaluated via the measurement of the maximum velocity of a dynamic light image which can manipulate such a cell [11,

19, 20]. In this study, such a velocity was used an index for the evaluation of the ODEP manipulation force exerting on a specific cell.

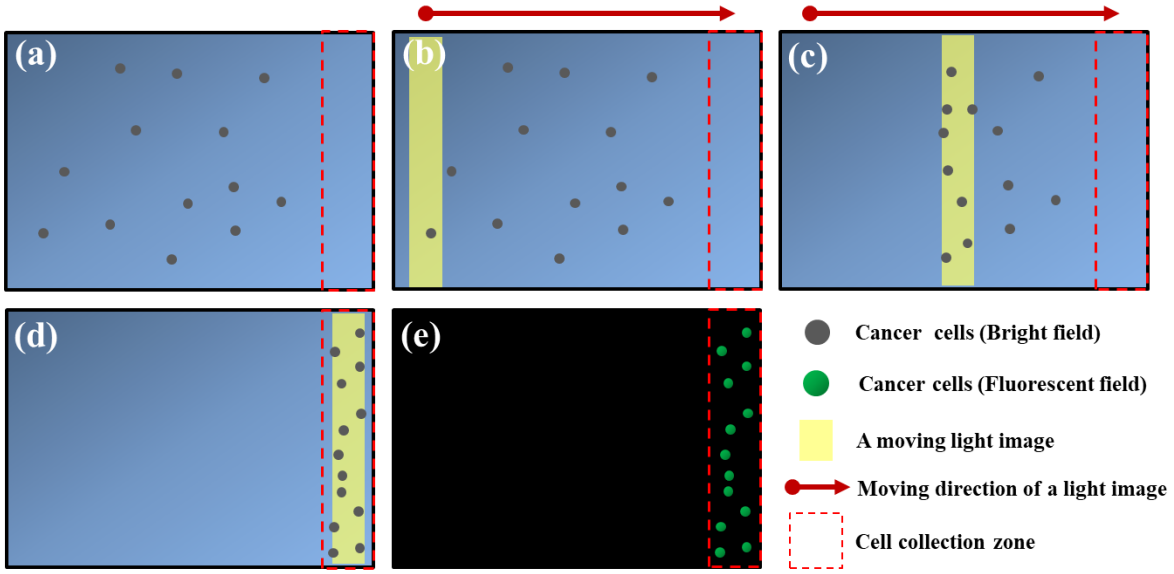

Figure 3. Schematic illustration of the working scheme for the evaluation of effect of electric conditions on the ODEP-based cell manipulation in terms of cell aggregation, cell death, cell adhesion on substrate surface, and the maximum velocity of a dynamic light image which can manipulate cells; (a) the fluorescent dye-stained cancer cells loaded into the microchamber of ODEP microfluidic chip were distributed within the defined ODEP-based cell manipulation zone, (b) - (d) A moving rectangular light bar with a wide velocity range (from high to low velocity; maximum velocity: 500 μm s^{-1}, set decrement: 25 μm s^{-1}, minimal velocity: 25 μm s^{-1}) was used to "screen" the cells within the defined cell manipulation zone. Once most cells were able to be manipulated by a moving light image at particular moving velocity, such a moving velocity of light image was defined as the maximum velocity of a dynamic light image which can manipulate cells, (e) fluorescence microscopy observation was subsequently performed to evaluate the status of the manipulated cells (e.g., cell death, cell aggregation, or cell adhesion on substrate surface).

In this study, experimental works were carried out to investigate the influence of electric parameters [i.e., magnitude of AC electric voltage: 1 - 10 Vpp; frequency of AC electric voltage: 1 kHz - 10 MHz] on the cell manipulation using ODEP in terms of cell aggregation, cell death, cell adhesion on substrate surface, and the maximum velocity of a dynamic light image which can manipulate cells. In practical operations, the cancer cells (MES-SA cancer cell line) stained with a fluorescent dye were loaded into the microchamber of microfluidic chip [Fig. 1 (a)]. This was followed by conducting ODEP-based cell manipulation to distribute the cells within the defined ODEP-based cell manipulation zone as illustrated in Fig. 3 (a). In the subsequent steps for the evaluation of the maximum velocity of a dynamic light image which can manipulate the cells, a rectangular light image (L: 1.3 mm, W: 100 μm) with a wide velocity range (from high to low velocity) to "screen" the cells within the defined cell manipulation zone. During operation, a rectangular light image with a high moving velocity (e.g., 500 μm s^{-1} in this

work) was first used to manipulate the cells (i.e., screen the cells). If no cell was manipulated, the moving velocity used for the next round of screening was gradually decreased in a set decrement of 25 μm s^{-1} (set minimal velocity: 25 μm s^{-1}). Once most cells were able to be manipulated and delivered to cell collection zone by a rectangular light image at particular moving velocity as schematically illustrated in Fig. 3 (b) - (d), such a moving velocity of a rectangular light image was defined as the maximum velocity of a dynamic light image that can manipulate cells. After the processes described above, fluorescence microscope observation was subsequently performed to evaluate the status of cells (e.g., cell death, cell aggregation, or cell adhesion on substrate surface) [Fig. 3 (e)].

2.5. Cell staining

In this study, MES-SA cancer cells (a cancer cell line) were used as the model cells for the testing. Before loading the cell suspension sample into the proposed microfluidic chip, the MES-SA cancer cells were stained with the Calcein-AM fluorescent dye (LIVE/DEAD1 Viability/Cytotoxicity Kit L-3224, Molecular Probes) to identify the live cells (green fluorescence staining) [24, 27]. The assay was performed according to the manufacturer's instructions. This was followed by microscopic observation using a fluorescence microscope (Zoom 160, OPTEM, USA).

3. RESULTS AND DISCUSSION

In the cell manipulation using ODEP, there are several conditions such as the inherent property of the cells (e.g., size, conductivity, permittivity, or membrane capacitance), the property of working solution, and electric conditions that might influence ODEP-based cell manipulation in terms of the magnitude of ODEP manipulation force acting on a cell (i.e., the maximum velocity of a dynamic light image which can manipulate a cell) [11, 19, 20, 24], or the direction of ODEP force exerting on a cell (e.g., attractive or repulsive ODEP force) [11, 18, 24]. In terms of operating conditions, therefore, the ODEP manipulation force generated on a specific cell is dependent on the magnitude and frequency of the electric voltage applied at the given solution conditions. The influence of abovementioned electric conditions on the cell manipulation using ODEP has been described previously [11, 20, 22, 25, 26]. However, few of these studies explored the real influence of these electric conditions on the cell aggregation, cell adhesion on a substrate surface, and cell death during ODEP-based cell manipulation process. These phenomena could further affect the performances of such cell manipulation as well as the application intended to achieve. For example, if cell aggregation occurs during ODEP-based cell manipulation process, such a phenomenon might affect not just the manipulation velocity of cells (i.e., the size of microparticle plays an important role on the maximum velocity of a dynamic light image wich can effectively manipulate such a microparticle according to equation (1) [9, 11, 19, 21]), but also the cell manipulation using ODEP for the intended applications of size-dependent cell sorting, isolation, and purification as reported previously [9, 11, 19, 21]. For the occurrence of cell adhesion on a substrate surface during ODEP-based cell manipulation, for example, it undoubtedly could significantly impair

the cell manipulation process, and in turn might affect the applications wanted to achieve such as cell transportation [10], cell sorting, [10, 28, 29], or cell isolation and purification [11, 19]. Regarding the cell death caused by the cell manipulation using ODEP, this phenomenon could be an issue if one intends to harvest the ODEP-manipulated cells for further bioassays in which the live cells are required (e.g., gene expression analysis [10, 11]). To find out the influence of electric conditions (e.g., magnitude of AC electric voltage: 1 - 10 Vpp; frequency of AC electric voltage: 1 kHz - 10 MHz), commonly used in ODEP-based microparticle manipulation [12, 18], on the ODEP-based cell manipulation in terms of cell aggregation, cell death, cell adhesion on substrate surface, and the maximum velocity of a dynamic light image that can manipulate cells, experimental works were carried out in this study.

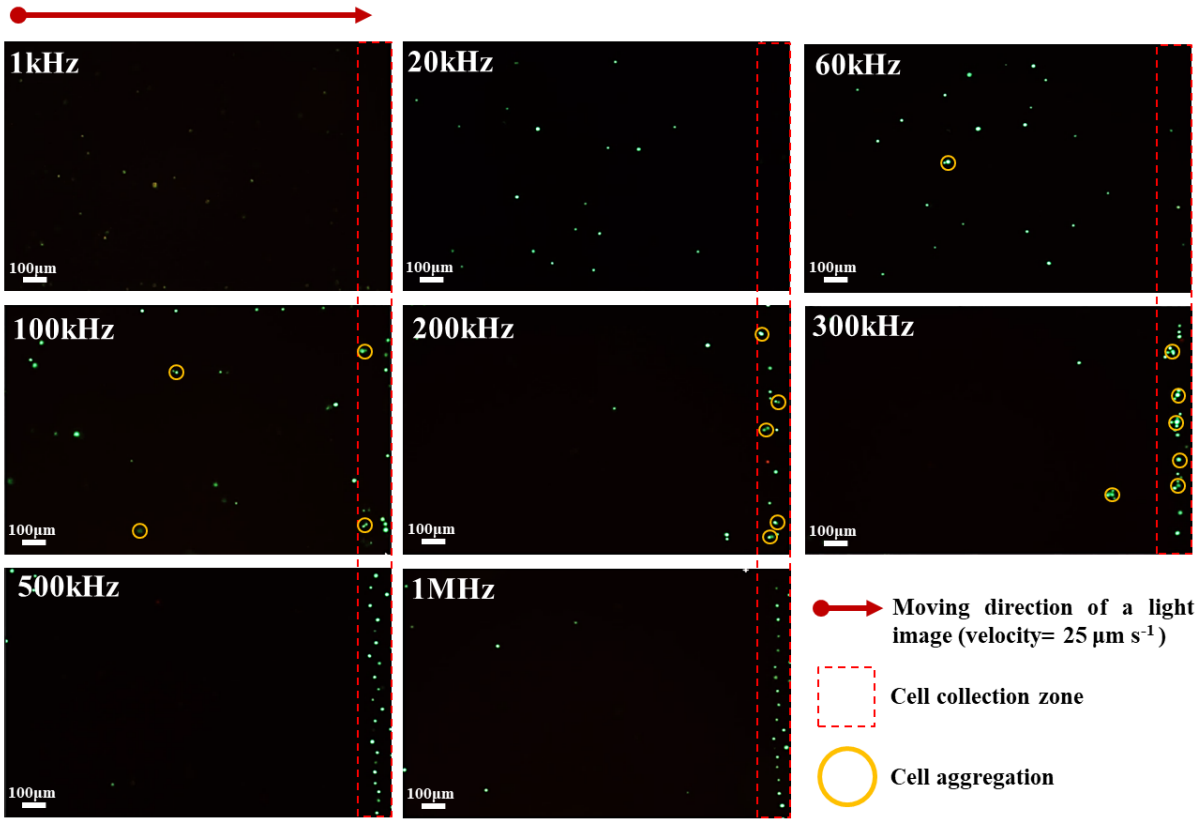

Figure 4. The fluorescent microscopic images of the cancer cells within the defined ODEP cell manipulation and cell collection zones after the cell manipulation using ODEP (Constant electric voltage: 5 Vpp; Electric frequency: 1 kHz - 1 MHz; Moving velocity of a light image: 25 μm s^{-1}) based on the working scheme illustrated in Fig. 3.

In this work, the frequency effect of electric condition on the manipulated cells during ODEP-based cell manipulation was first explored. In this study, the cancer cells stained with Calcein-AM fluorescent dye were first loaded in the defined ODEP cell manipulation zone in the microchamber of a microfluidic chip [Fig. 1 (a)]. This was followed by ODEP-based cell manipulation under the electric conditions explored (i.e., 5 Vpp, and 1 kHz - 1 MHz) using a rectangular light image with a moving velocity of 25 μm s^{-1} to manipulate the cancer cells as schematically illustrated in Fig. 3. In this work,

the low moving velocity (i.e., 25 μm s^{-1}) of a light image was used ensuring the cells can be effectively manipulated under the electric conditions explored in this study. Fig. 4 showed the fluorescent microscopic images of the cells within the defined ODEP cell manipulation zone after the ODEP-based cell manipulation using the working scheme illustrated in Fig. 3. It can be clearly found from Fig. 4 that the fluorescent cell dot images either disappeared or became weak when the frequency of electric voltage was set at 1 kHz.

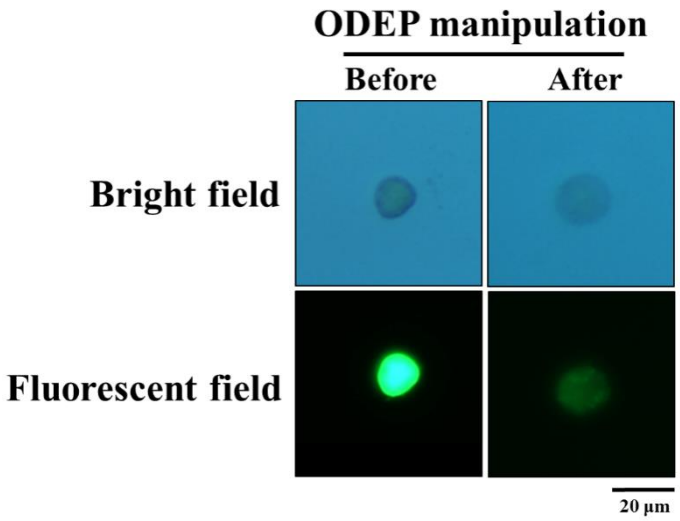

Figure 5. Bright field (Upper) and fluorescent (Lower) microscopic images of a cell before (Left column) and after (Right column) ODEP-based cell manipulation (Electric voltage and frequency: 5 Vpp and 1 kHz, respectively).

This result could be due to the phenomenon of cell lysis as seen in Fig. 5, in which the integrity of cell membrane might be affected under low electric frequency condition, and therefore the Calcein-AM fluorescent dye would no longer be able to stain on it. This phenomenon of cell lysis occurred under the low electric frequency condition could be explained by the fact that the applied electric current might concentrate on a cell surface when the applied electric frequency is lower than 10 kHz. In this situation, such a cell could suffer from high level of transmembrane potential that might in turn cause the cell to lyse when the transmembrane potential is greater than 2.3 kV cm^{-1} (also known as cell electrical lysis) [18, 30, 31]. Moreover, it was also observed that most of the cells in the defined ODEP cell manipulation zone were not effectively manipulated and thus successfully delivered to the cell collection zone when set frequency of electric voltage was lower than 100 kHz. This finding could be due to cell adhesion on the substrate surface as seen in Fig. 4 (e.g., 20, 60, and 100 kHz), in which most of the cells retained in the defined ODEP cell manipulation zone. The finding of cell adhesion on substrate surface as abovementioned might be explained by the phenomena of electrically-induced membrane pore formation, and cell deformability increase under this specific electric condition, which therefore increased the surface roughness of a cell and the contact area between cell and substrate surface. These phenomena could increase the possibility of cell adhesion on a substrate surface [31, 32]. Conversely,

most of the cells were significantly manipulated, and effectively transported to the cell collection zone when the set frequency of electric voltage was higher than 200 kHz (Fig. 4). However, these cells manipulated and collected in the cell collection zone tended to aggregate, which could be due to higher cell density at this zone. This phenomenon became more significant when the set frequency of electric voltage reached 300 kHz (Fig. 4). As discussed earlier, the phenomenon of cell aggregation during ODEP-based cell manipulation process could hinder the applications intended for size-based cell sorting, separation, or isolation using ODEP technique [9, 11, 19, 21]. Nevertheless, this technical problem could be solved out when the set frequency of electric voltage was higher than 1 MHz, by which the cells manipulated and collected in the cell collection zone no longer aggregated (Fig. 4). This phenomenon could be explained by the fact that the phenomenon of mutual DEP could be down-regulated when the electric frequency is greater than 1 MHz [33, 34]. In this situation (i.e., low mutual DEP), phenomenon of cell aggregation might be minimized.

As a whole, the results in Fig. 4 revealed the specific frequency range of AC electric voltage applied that the phenomenon of cell lysis, cell adhesion on substrate surface, or cell aggregation tended to occur. It seemed that the generally optimal frequency of AC electric voltage applied for ODEP-based cell manipulation is when the frequency is higher than 1 MHz, by which the abovementioned unwanted phenomena during ODEP-based cell manipulation could be prevented. Based on this fact, the next attempt of this study was to investigate the magnitude effect of AC electric voltage applied (set electric frequency: 1 MHz; electric voltage: 1 - 10 Vpp) on the cell manipulation using ODEP. Overall, results demonstrated that the phenomena of cell lysis, cell adhesion on substrate surface, and cell aggregation were not observed within the experimental conditions explored, except for the phenomenon of cell lysis which occurred when the electric voltage reached 10 Vpp (image not shown).

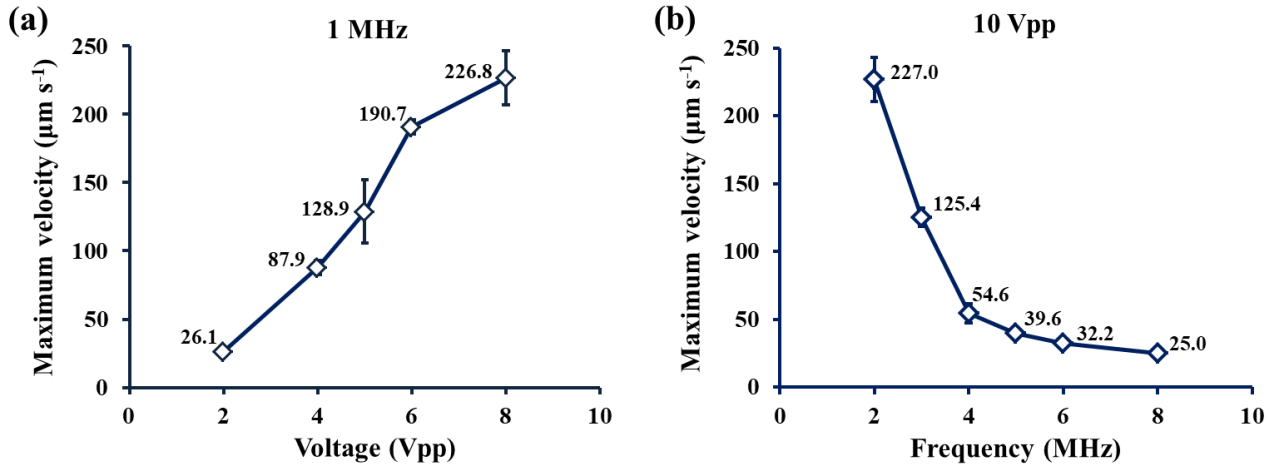

Figure 6. The maximum velocity of a moving light image that can manipulate cells under different electric conditions: (a) Electric frequency and voltage: 1 MHz and 2 - 8 Vpp, respectively; (b) Electric frequency and voltage: 2 - 8 MHz and 10 Vpp, respectively.

Moreover, results [Fig. 6 (a)] exhibited that the maximum velocity of a dynamic light image which can manipulate cells increased with the magnitude increase of AC electric voltage (electric voltage

range: 2 – 8 Vpp). This finding was not out of our expectation because, in theory, the ODEP manipulation force (and thus the maximum velocity of a dynamic light image which can manipulate cells) would be proportional to the voltage squared according to the equation (1) [14, 18, 26]. In some ODEP-based cell manipulations (e.g., continuous cell separation and isolation [9, 19, 21]), the manipulation of cells in a high velocity manner would be required. In these cases, the operation conditions abovementioned could be useful, by which the velocity of a dynamic light image that can manipulate cells could reach as high as 226.8 μm s^{-1} when the electric condition of 8 Vpp and 1 MHz was used [Fig. 6 (a)].

As discussed earlier, the unwanted phenomenon such as cell lysis, cell adhesion on substrate surface, and cell aggregation could be technically prevented when the frequency of AC electric voltage applied for ODEP-based cell manipulation reached 1 MHz (Fig. 4). In theory, the unwanted phenomena abovementioned could become less likely to occur when the frequency of AC electric voltage applied was further increased [31, 33]. Nevertheless, further increase of electric frequency could compromise the maximum velocity of a dynamic light image which can manipulate cells as seen in Fig. 6 (b). As a whole, this study has investigated the influence of electric parameters on the cell manipulation using ODEP in term of the phenomena of cell lysis, cell adhesion on substrate surface, cell aggregation, as well as the maximum velocity of a dynamic light image which can manipulate cells. All these information is both fundamental and important for scientists to select the appropriate electric conditions of ODEP-based cell manipulation for a specific application.

4. CONCLUSIONS

In biomedical studies, the manipulation of biological cells is both fundamental and important. Compared with the cell manipulation using conventional macro-scale devices or techniques, it is well-recognized that the manipulations of cells within micro-scale devices could provide several advantageous features. Among the techniques capable of performing cell manipulation in a micro-scale, the cell manipulation using ODEP is found particularly promising mainly due to its ease of fabrication and its ability to realize flexible cell manipulation. To realize ODEP-based cell manipulation for a special application, the selection of an appropriate ODEP operating condition specific to the application is critical.

Among the operating conditions, the magnitude and frequency of the exerted AC electric voltage in an ODEP microfluidic system are the two commonly-used working parameters. Although there are several studies which had explored the effect of different electric conditions on the cell manipulation using ODEP few of them further considers the phenomena (e.g., cell aggregation, cell adhesion on substrate surface, or cell damage) which might co-occur in the real situation. These unwanted phenomena could affect the intended application of ODEP-based cell manipulation. To address this issue, this study has investigated the influence of electric conditions [i.e., magnitude of AC electric voltage: 1 – 10 Vpp; frequency of AC electric voltage: 1 kHz – 10 MHz] on the cell manipulation using ODEP in terms of cell aggregation, cell death, cell adhesion on substrate surface, and the maximum velocity of a dynamic light image which can manipulate cells. Results revealed that the phenomenon of cell lysis

could occur under low electric frequency condition (e.g., 1 kHz). Moreover, it was also observed that most of the cells were not effectively manipulated and thus successfully delivered when the set frequency of electric voltage was lower than 100 kHz. This phenomenon was mainly due to cell adhesion on the substrate surface. Conversely, most of the cells were significantly manipulated and effectively transported when the set frequency of electric voltage reached 200 - 300 kHz. However, these cells manipulated and collected in the cell collection zone tended to aggregate. Fortunately, this technical problem could be prevented when the set frequency of electric voltage was higher than 1 MHz. At a given electric frequency of 1 MHz, furthermore, the magnitude increase of electric voltage (2 - 8 Vpp) did not cause the unwanted phenomena aforementioned but increase the maximum velocity of a moving light image that can manipulate cells. At a given magnitude of electric voltage (e.g., 10 Vpp), conversely, further increase (i.e., higher than 1 MHz) of electric frequency might compromise the performance of the cell manipulation using ODEP in terms of the maximum velocity of a dynamic light image that can manipulate cells. As a whole, this study has investigated the influence of different electric conditions on the cell manipulation using ODEP in term of the phenomena of cell lysis, cell adhesion on substrate surface, and cell aggregation, as well as the maximum velocity of a dynamic light image which can manipulate cells. All these information is both fundamental and important for scientists to select an appropriate electric condition of ODEP-based cell manipulation for a specific application.

ACKNOWLEDGEMENTS
This study was sponsored by the Ministry of Science and Technology, R.O.C. (MOST 105-2221-E-182-028-MY3, MOST 107-2221-E-182-033-MY3 and MOST 107-2811-E-182-001) and Chang Gung Memorial Hospital (CMRPD2E0011-13, CMRPD2G0061-62, and CMRPD2H0121).

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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