Enhanced discrimination of normal oocytes using optically induced pulling-up dielectrophoretic force

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We present a method to discriminate normal oocytes in an optoelectrofluidic platform based on the optically induced positive dielectrophoresis (DEP) for *in vitro* fertilization. By combining the gravity with a pulling-up DEP force that is induced by dynamic image projected from a liquid crystal display, the discrimination performance could be enhanced due to the reduction in friction force acting on the oocytes that are relatively large and heavy cells being affected by the gravity field. The voltage condition of 10 V bias at 1 MHz was applied for moving normal oocytes. The increased difference of moving velocity between normal and starved abnormal oocytes allows us to discriminate the normal ones spontaneously under the moving image pattern. This approach can be useful to develop an automatic and interactive selection tool of fertilizable oocytes. © *2009 American Institute of Physics.* [DOI: 10.1063/1.3086600]

I. INTRODUCTION

Assisted reproductive technologies such as *in vitro* fertilization (IVF) have attracted much attention in veterinary science.[1] Although the selection of fertilizable oocyte is one of the most important issues in IVF process,[2] it has been manually conducted by a skillful expert with a labor-intensive and time-consuming process, wherein an observation using a microscope and a direct-contact manipulation using pipettes are required.

As lab-on-a-chip and microfluidic technologies have been widely applied to chemical and biological studies, several kinds of microdevices have been introduced into embryo manipulation.[2–11] However, although the oocyte selection is essential for successful IVF,[9] only a few attempts have been tried in a microfluidic system.[12] Recently, we have reported a new oocyte selection method for IVF using dielectrophoresis (DEP) in a microfabricated electrode system.[13] The results of previous study show that DEP can be a useful criterion for discriminating normal oocytes. However, the labor-intensive manual processes have still been required for injection, alignment, and collection of the oocyte samples.

To develop a fully automated system for selecting normal oocytes using noncontact interactive manipulation of them, we herein used an optoelectrofluidic platform, which allows the programmable cell manipulation based on the optically induced DEP and the image-driven virtual electrodes. The optoelectrofluidic platform utilizes a photoconductive layer on a plate electrode instead of patterned metal electrodes for inducing nonuniform electric field in the medium.[14–16] When a dynamic image is projected from a display device including a digital micromirror device[14] and a liquid crystal display[15,16] (LCD) onto the photoconductive material such as amorphous silicon, the partially illuminated area becomes much more conductive than the other dark area,

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becoming a virtual electrode to form a nonuniform electric field. Consequently, we can freely manipulate the objects in the medium by several ac electrokinetic mechanisms such as DEP[14–16] and ac electro-osmosis.[17,18]

In this paper, we applied an LCD-based optoelectrofluidic platform[16] for programmable manipulation of porcine oocytes. To enhance the performance of the fertilizable oocyte discrimination, we developed a new scheme for the optoelectrofluidic particle manipulation considering the gravity and the surface interaction, which act more dominantly in the case of large, heavy, and sticky cells such as oocytes. By using this novel method for oocyte discrimination, the automatic and interactive selection of oocytes for IVF would be possible without direct contacts and exposure to external environments that interfere with the success rate of the fertilization.

II. PRINCIPLES AND THEORY

A. DEP characteristics of oocyte

In the optoelectrofluidic platforms, several mechanisms including optically induced ac electrokinetics[14–18] and electrostatic interactions[19] affect the particle behaviors. Among those mechanisms, the optically induced DEP force was utilized as a driving force for oocyte discrimination in this work. The DEP force acting on a spherical particle is defined as follows:[20]

$$F_{\mathrm{DEP}} = 2\pi R^3 \varepsilon_m \,\mathrm{Re}[f_{\mathrm{CM}}]\,\nabla\,|E|^2,$$

where R is the particle radius, E is the applied electric field, and $\varepsilon_m = \varepsilon_0 \varepsilon_r$ is the permittivity of the fluid, where ε_r is the relative permittivity of the fluid and ε_0 is the permittivity of free space. The Clausius–Mossotti (CM) factor $f_{\mathrm{CM}} = (\varepsilon_p^* - \varepsilon_m^*)/(\varepsilon_p^* + 2\varepsilon_m^*)$, where ε_p^* and ε_m^* are the complex permittivities of the particle and the fluid, respectively. The real part of CM factor ($\mathrm{Re}[f_{\mathrm{CM}}]$), which depends on the applied ac frequency and the dielectric properties of the particle and the fluid, can have a value between +1.0 and −0.5 and determine the direction of the DEP force. When $\mathrm{Re}[f_{\mathrm{CM}}]$ is positive, where the particle is more polarizable than the medium, the particle moves toward the strong electric field region by positive DEP. On the contrary, the negative $\mathrm{Re}[f_{\mathrm{CM}}]$, if the medium is more polarizable than the particle, induces negative DEP; thus the particle moves toward the weak electric field region.

To figure out the dielectrophoretic phenomena of oocyte, the protoplast model for mammalian spherical cells can be applied [Fig. 1(a)].[20,21] Protoplasts are very fragile, balloon-like particles prepared by digesting the cell wall. The porcine oocytes, for which it is too difficult to determine very thin cell membrane, are structurally somewhat comparable and exhibit a polarization response similar to protoplasts. We established an expression for the complex cell permittivity as $\varepsilon_p^* = (c_{\mathrm{mb}}^* R \varepsilon_c^*)/(c_{\mathrm{mb}}^* R + \varepsilon_c^*)$, where R is the cell radius, ε_c^* is the complex permittivity of the cytoplasm, and c_{mb}^* is the complex capacitance per unit area of membrane, which is given by $c_{\mathrm{mb}}^* = c_{\mathrm{mb}} - j g_{\mathrm{mb}}/\omega$, where c_{mb} and g_{mb} are the capacitance and the conductance of the cell membrane, respectively. Figure 1(b) shows the variation in polarizability parameter ($\mathrm{Re}[f_{\mathrm{CM}}]$) with the ac frequency based on the model. For the dielectric properties of an oocyte, the following parameters were used: The cell radius R is 60 μm, the dielectric constant and the conductivity of the cytoplasm are 70 and 5 mS/cm, respectively, and the capacitance and the conductance of the membrane are 1.25 $\mu\mathrm{F/cm}^2$ and 400 Ω cm, respectively.[21] The media conductivities are 0.013, 0.13, and 1.3 mS/cm. As a result, proper transportation of the oocyte is expected under the frequency change in the range from 30 kHz to 3 MHz at the experimental condition $\sigma_m = 0.13$ mS/cm. In this condition, the oocytes exhibited a positive DEP since the polarizability parameter has a positive value.

The CM factor, which can determine the dielectrophoretic mobility of the cells, can be affected by the composition of the cytoplasm and nucleus[22] and the conductivity and permittivity of the cytoplasm.[23,24] Therefore, different cytoplasmic contents between normal and abnormal oocytes would induce the differences in their mobility due to the optically induced DEP force. We calculated the CM factor of oocyte by decreasing the conductivity of the cytoplasm from 5 mS/cm, which is a normal value, to 0.2 mS/cm of [Fig. 1(c)]. The other dielectric parameters of oocyte are

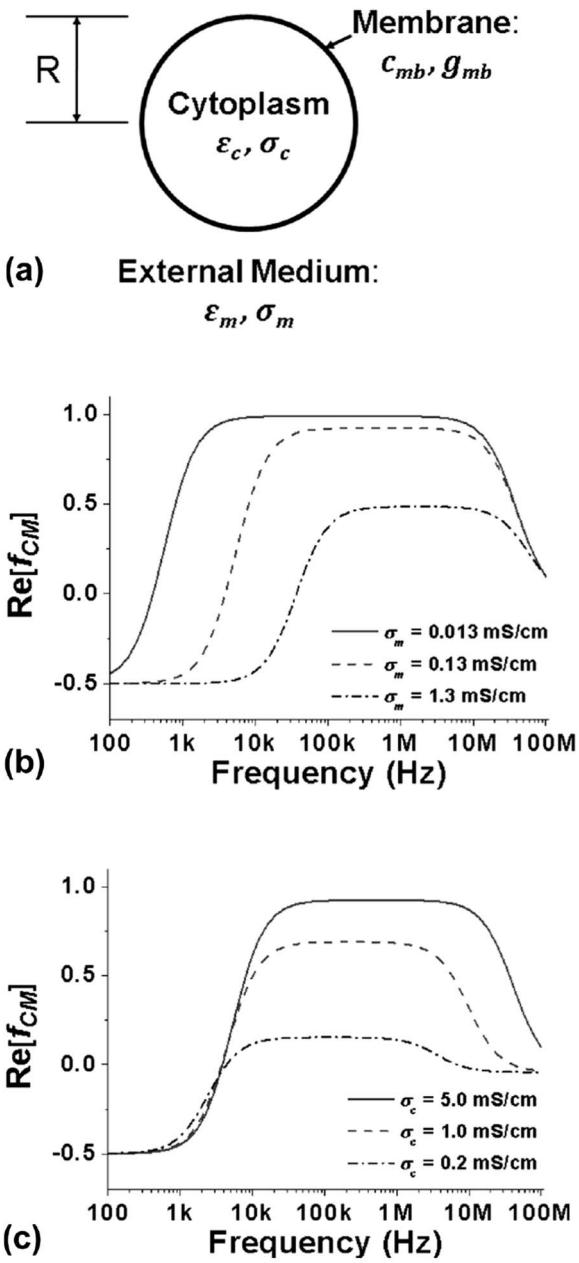

FIG. 1. (a) Dielectric model of protoplast for spherical cell. The dielectric permittivity ε and electric conductivity σ are represented. The subscripts c, m, and mb indicate the cytoplasm, the medium, and the membrane, respectively. Calculated variation in Re[f_{CM}] according to the ac frequency at different conductivities (b) of the external medium (σ_m=0.013, 0.13, and 1.3 mS/cm; σ_c=5 mS/cm) and (c) of the cytoplasm (σ_c=0.2, 1, and 5 mS/cm; σ_m=0.13 mS/cm). The fixed parameters are R=60 μm, capacitance, c_{mb}=1.25 μF/cm^2, g_{mb}=400 Ω cm, ε_c=70ε_0, and ε_m=80ε_0.

the same with the previous calculation represented in Fig. 1(b). As the conductivity of the cytoplasm decreases, the CM factor has a tendency to decrease; thus the decreased velocity of the oocyte would be expected. The cytoplasmic conductivity, of course, may not be a dominant factor inducing the different DEP mobilities of the oocytes. Therefore, further studies about physical and chemical characteristics of fertilizable oocytes should be followed to validate the theoretical hypothesis in our biological viewpoint.

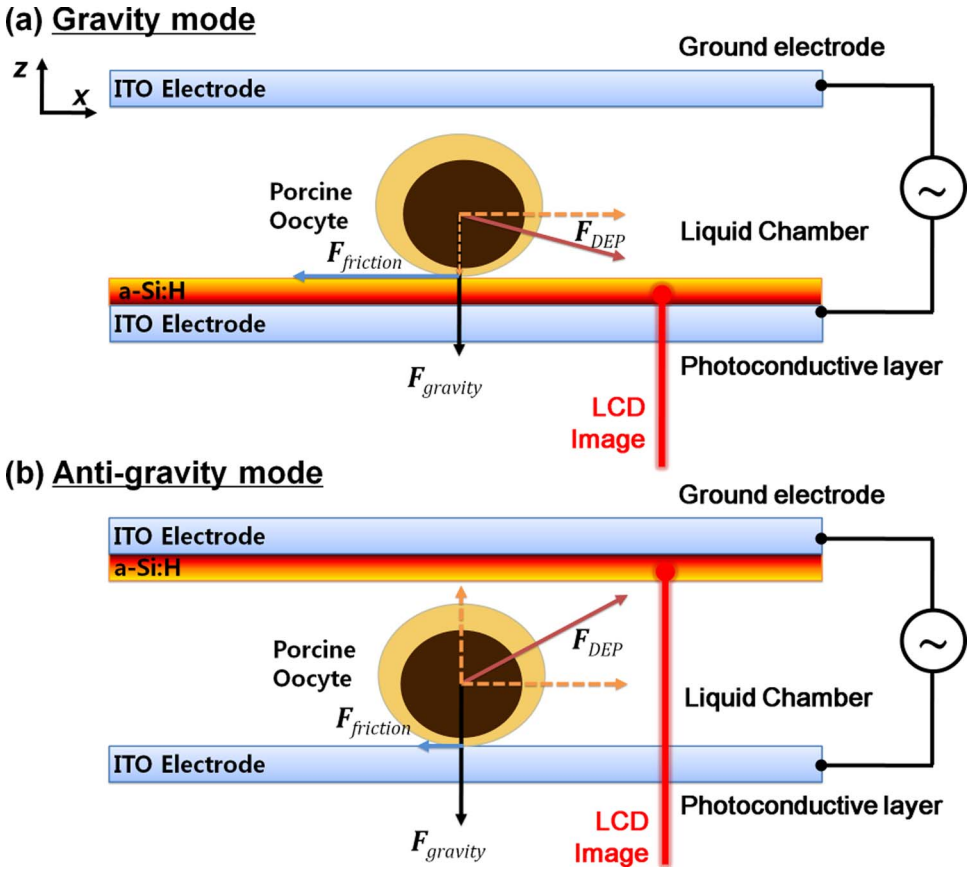

FIG. 2. Cross-section schematic (not to scale) of two distinct manipulation modes: (a) Gravity and (b) antigravity modes based on the gravity effect and optically induced positive DEP in the optoelectrofluidic device. Under the antigravity mode, the oocytes exhibit vertical positive DEP force in the opposite direction of the gravity. Therefore, the friction force becomes weaker due to the reduced net vertical force, resulting in the effective manipulation of normal oocytes.

B. Two manipulation schemes of optoelectrofluidic platform

When we apply the optoelectrofluidic platform for manipulating microparticles including biological cells based on the optically induced DEP force, not only the lateral components but also the vertical ones should be considered. According to our previous study about the particle-surface interactions in the optoelectrofluidic device, the vertical DEP force is not negligible, and it sometimes induces the adsorption of the particles onto the electrode surface and the friction forces that interfere with the effective particle manipulation.[25,26] If the target particles are heavy and sticky cells such as oocytes, the effects of the gravity and the particle-surface interactions are more dominant than other cases applying the typical animal cells. Therefore, the magnitude and the direction of the net force, which is determined by the summation of the forces acting on the particles including not only the DEP force but also the gravity, are important factors to be considered in manipulating those cells.

Here, we define the optoelectrofluidic platform, wherein the direction of the optically induced DEP force is identical with that of the gravity as shown in Fig. 2(a), as the gravity manipulation mode. Under this mode, if the target particles follow the negative DEP, the particles move toward the ground electrode, while they move toward the photoconductive layer if they follow the positive DEP. Accordingly, if the photoconductive layer is at the bottom of the device, the oocytes, which follow the positive DEP force in general, move in the same direction of the gravity when an LCD image was projected onto the surface of the photoconductive layer. The net forces make the oocytes settle down and stay on the bottom photoconductive layer of the device; thus the friction

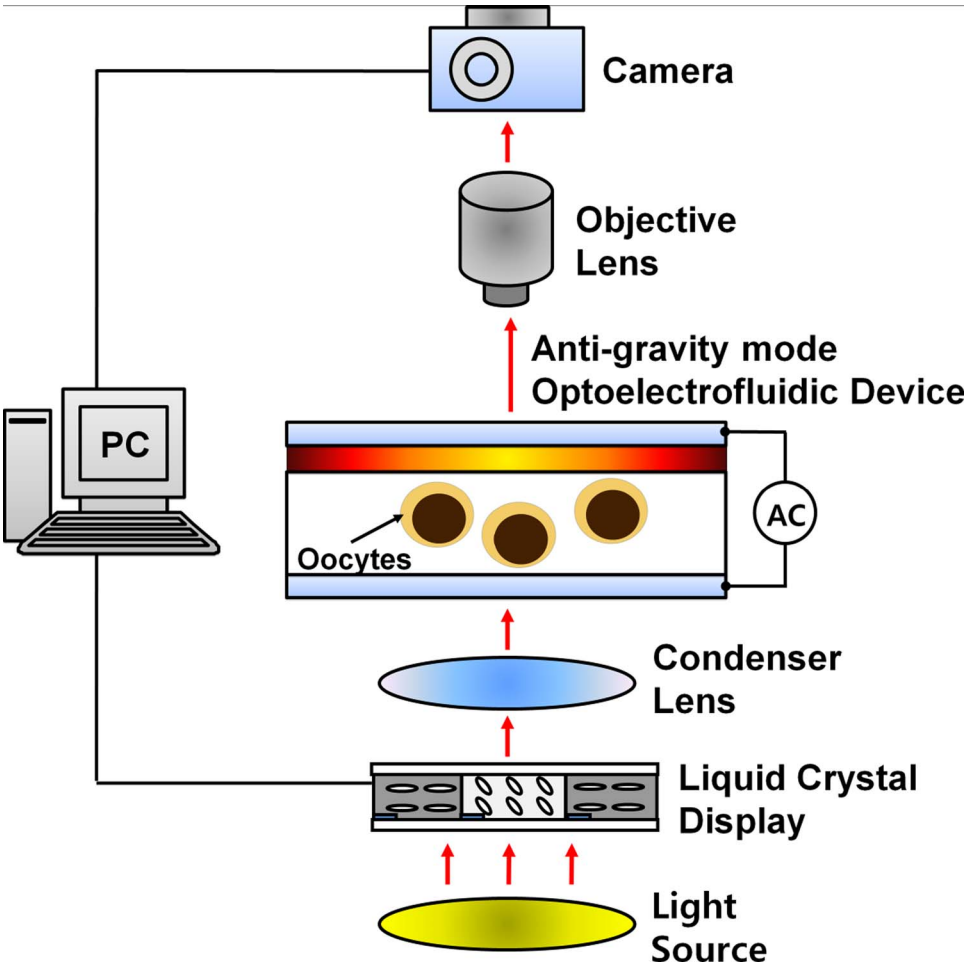

FIG. 3. Experimental setup of the LCD-based optoelectrofluidic platform for oocyte discrimination in the antigravity manipulation mode. At the gravity mode, the optoelectrofluidic device is turned upside down without any modification of other components.

force increases by the combination of these forces as well as the sticky cell surface. Consequently, these undesired attachment problems hinder the efficient manipulation and reduce the discrimination performance of the oocytes. Also, the increased selection time has adverse effects on the cell viability due to the prolongation of exposure time to the electric field.

To overcome this physical hurdle in the optoelectrofluidic device, the minimization of lateral friction force acting on the heavy and sticky cells is required. Hence, we introduce a novel disposition method named as the antigravity manipulation mode, in which the direction of the optically induced DEP force is opposite to that of the gravity [Fig. 2(b)]. When we applied the optoelectrofluidic device for the oocyte discrimination, the photoconductive layer is positioned at the top of the device. Under this mode, when an ac bias voltage is applied and the optical image is formed on the surface of the top photoconductive layer, the electric field gradient in the device is generated in the opposite direction from that of the gravity mode. As a result, the heavy cells are forced toward the upper photoconductive layer by positive DEP force, which pulls them up. Consequently, the vertical positive DEP force counterbalances the gravity; thus the net force along the z axis can be canceled out, resulting in weaker friction force. Therefore, we can achieve effective manipulation of heavy and sticky cells such as oocytes using the antigravity manipulation mode of optoelectrofluidic platform.

III. EXPERIMENTAL

A. Preparation of porcine oocytes

The oocyte samples were obtained from porcine ovaries and then cultured in a 500 μl drop of maturation medium[13] covered with paraffin oil and incubated for 22 h at 39 °C. After maturation, normal oocytes were manually selected by microscopic observation and gentle pipetting, where abnormal oocytes were defined as the oocytes maintained without medium for three days and would be regarded as unhealthy for lack of nutrition. Samples were immersed in Zimmermann cell fusion medium (0.13 mS/cm). This cell fusion medium was comprised of 0.3 *M* mannitol, 100 μM $CaCl_2$, 100 μM $MgSO_4$, 0.1 mg/ml bovine serum albumin (fraction V, Sigma), and 1.192 mg/ml *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer. All the prepared oocytes have a uniform diameter of 119.82 ± 3.96 μm, which is a fully developed size after maturation *in vitro* under the same conditions. Overall research including oocyte preparation was performed according to the ethical guideline from the Institutional Review Board (IRB) at KAIST.

B. Fabrication of optoelectrofluidic device

The optoelectrofluidic device consists of a photoconductive layer and a transparent indium-tin-oxide (ITO) electrode. The photoconductive layer was comprised of four layers: (1) A 180-nm-thick ITO layer, (2) a 50-nm-thick n^+ doped hydrogenated amorphous silicon (n^+ *a*-Si:H) layer, (3) a 1-μm-thick intrinsic hydrogenated amorphous silicon layer (intrinsic *a*-Si:H), and (4) a 20-nm-thick silicon nitride (SiN_x) layer. The ITO-coated glass substrates (Samsung-Corning Precision Glass, Asan, Korea) were prepared and a triple layer of n^+ *a*-Si:H, intrinsic *a*-Si:H, and SiN_x was consecutively deposited by plasma-enhanced chemical vapor deposition onto the substrate. Afterward, some regions were etched by reactive-ion etch for electric connections. A wire was connected after dicing the fabricated device into 37.5 mm $\times$ 25.0 mm sections. Finally, the ITO ground electrode was placed on the photoconductive layer facing each other with 240-μm-thick double-stick tapes as spacers for the liquid chamber, of which the height is high enough to manipulate the prepared oocytes without jam.

C. Experimental setup of optoelectrofluidic platform

A liquid sample containing oocytes was sandwiched between the photoconductive layer and the ground electrode, and an ac voltage bias was applied across the liquid chamber. This electric bias was generated by a function generator (MXG-9802A, Seowon Family, Korea or AGF3022, Tektronix, USA). Optical patterns were generated by a 1.3 in. monochromatic LCD module (800×600 pixel array with 33 μm pixel pitch) and projected onto the surface of the photoconductive layer. A condenser lens, which is located between the optoelectrofluidic device and the LCD module, was integrated for focusing the LCD image patterns. The motion of oocytes was observed and recorded using an upright microscope (Zeiss Axioskop 40, Carl Zeiss, Germany) with a charge coupled device camera (DS-U1, Nikon Instruments Inc., NY). To measure and analyze the velocity data of oocytes, we used an analysis program that we developed using MATLAB. The maximum moving velocity of the oocytes, which chase the scanning virtual electrodes, was measured. The experimental setup of optoelectrofluidic platform for discrimination of normal oocytes based on the antigravity mode is shown in Fig. 3. At the gravity mode, only the optoelectrofluidic device is turned upside down without any modification of other components.

IV. RESULTS AND DISCUSSION

For predicting the dielectrophoretic oocyte behavior, we characterized the electric field distribution using a commercial CFD solver (CFD-ACE+, ESI, Huntsville, AL) (Fig. 4). We assumed the antigravity manipulation mode, wherein the ac voltage of 10 V at 1 MHz was applied across the illuminated area of the photoconductive layer and the ground electrode. As shown in the simulation results, the strongest electric field was obtained at the edges of illuminated area and the overall field strength declined along the *z* axis. We could predict that the oocytes following the

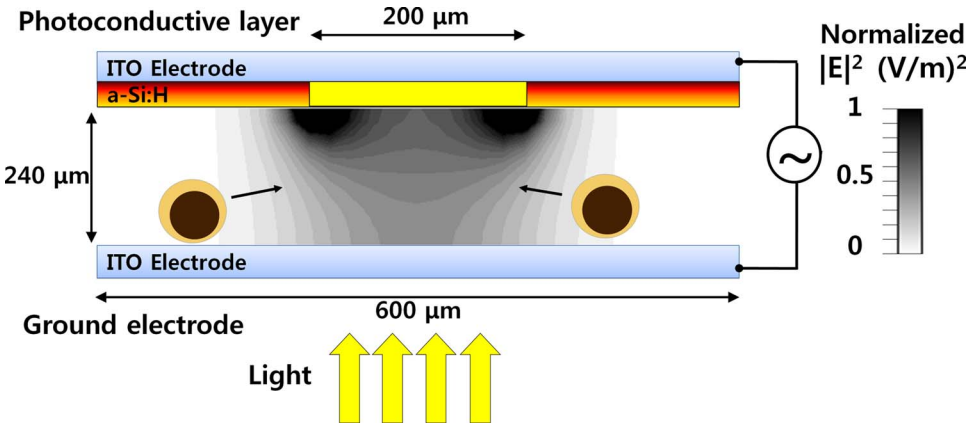

FIG. 4. The simulated electric field distribution formed by an optically induced virtual electrode (yellow region) under the antigravity mode. An ac signal of 10 V at 1 MHz is assumed to be applied. The estimated moving direction of the oocytes by the optically induced positive DEP is also represented.

positive DEP would move to the edge region of the virtual electrodes on the photoconductive layer.

In order to examine the effect of two manipulation modes—gravity and antigravity—the velocities of normal and abnormal porcine oocytes were measured under each mode (Fig. 5). These experiments were conducted in the same condition of the simulation as 10 V bias at 1 MHz. We measured the velocity of moving oocytes when they are only around the edge of the virtual electrode where they move with the maximum velocity. For both modes, the normal oocytes show

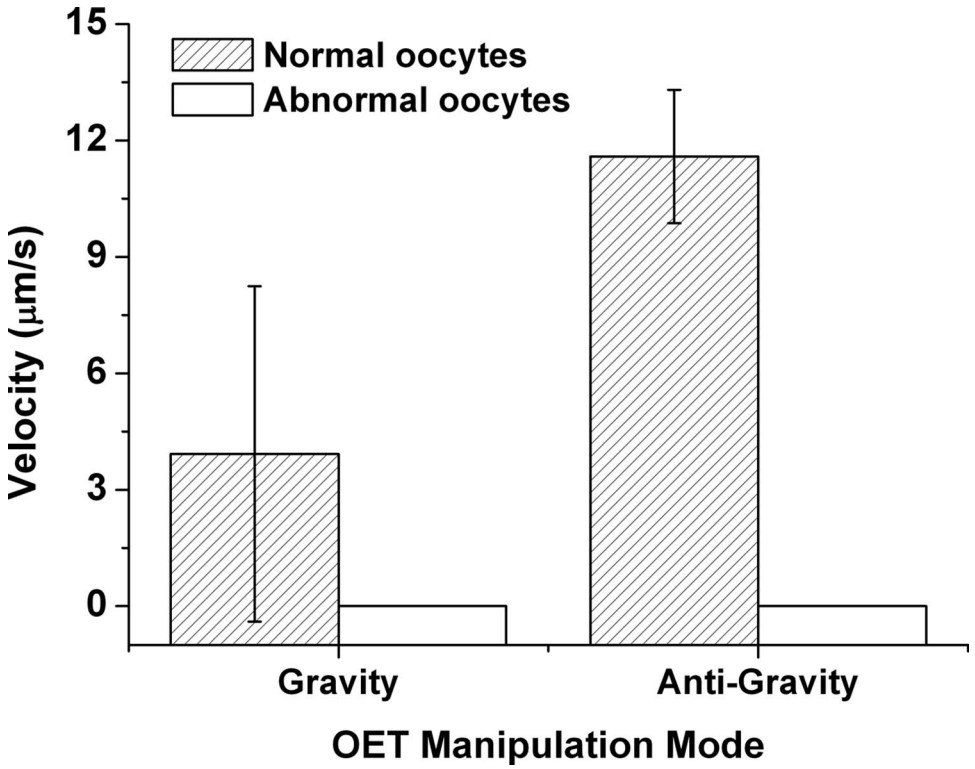

FIG. 5. Measured velocity of normal and abnormal oocytes under two manipulation modes—the gravity and the antigravity modes. The operating voltage was 10 V bias at 1 MHz.

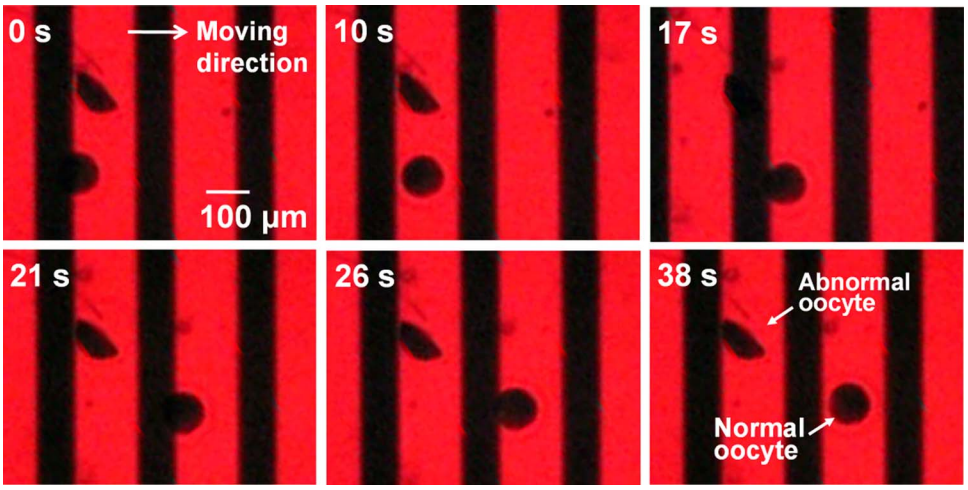

FIG. 6. Captured images of the discrimination of normal and abnormal oocytes using an optical line scanning from left to right under the antigravity manipulation mode. Samples were manipulated under the voltage of 10 V at 1 MHz. When the voltage was applied and the LCD image was moved, only the normal oocyte were manipulated in the moving direction of image pattern, while the abnormal oocytes remained at the initial position.

larger DEP velocity than abnormal oocytes. This result shows that the CM factor, which can be affected by the dielectric properties of the cytoplasm and the membrane, of the abnormal oocytes is smaller than that of the normal ones because all other parameters affecting their DEP mobility, such as the oocyte radius, the electric field gradient, and the surrounding media, are equal to the normal ones. According to our simulation and the experimental observation, the decreased conductivity of the cytoplasm may be one of the most dominant factors for the decreased DEP mobility of the abnormal oocytes. Their decreased conductivity may be promoted from the difference in the cytoplasmic contents of abnormal oocytes, which might be affected by the artificial starvation. We could also estimate the change of their cytoplasmic contents through the microscopic pictures (Fig. 6), in which the abnormal oocytes have different-sized nucleus. The normal oocytes under the antigravity mode moved faster than those under the gravity mode due to the reduced friction forces: The moving velocities were 3.92 ± 4.32 and 11.59 ± 1.72 μm/s under the gravity and the antigravity modes, respectively. The larger standard deviation of the normal oocyte velocity under the gravity mode also verifies that the larger friction force acts on the target oocytes.

Based on the experimental results of the different DEP responses between normal and abnormal oocytes, the experiments for the selection of normal oocytes were demonstrated. The discrimination of normal oocytes by scanning LCD image is shown in Fig. 6. These optical scanning lines are constructed and interactively controlled by a computer-controlled software that we developed. Those virtual electrode lines were about 170 μm in width, with 95-μm-width gaps among them. As the optical lines move from left to right, the normal oocytes were transported to the leading direction while the abnormal oocytes stayed in the initial position. Finally, we could spatially separate two different oocytes in 38 s after applying the voltage.

To evaluate the physical mechanisms involved in the oocyte discrimination, we organized a model for movements of oocytes under the optically induced electric field. When an oocyte is directly above the electrode edges, it experiences the lateral DEP and the friction force due to the electrode surfaces along the x axis. The magnitude of the friction force is proportional to the forces oriented along the z axis. Due to the resultant force, we can describe the movement of oocyte using Stoke's formula, $V_{\mathrm{DEP}}=(F_{\mathrm{DEP}x}+F_{\mathrm{friction}})/6\pi\eta R$, where η is the fluid viscosity, R is the radius of oocyte, and F_{friction} is the friction force acting on the oocyte, which can be defined as the multiplication of the net vertical force, F_z, which is composed of the gravity force (F_{gravity}) and the vertical DEP force ($F_{\mathrm{DEP}z}$), and the friction coefficient, k. The gravitational force is represented as

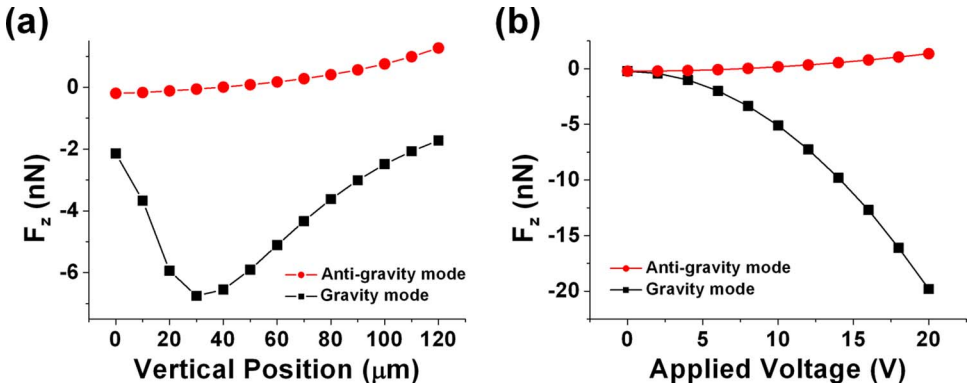

FIG. 7. Calculated net vertical force, which is the summation of the gravity and the vertical positive DEP force, acting on the oocyte, which is assumed to be positioned directly above the electrode edges according to (a) the vertical position of the oocyte and (b) the applied voltage. The gap height of liquid chamber was 240 μm and the ac frequency was 1 MHz.

$F_{\text{gravity}}=4\pi r^3(\rho_c-\rho_m)g/3$, where ρ_c is the density of oocyte, ρ_m is the density of medium, and g is the gravitational acceleration. On the basis of those equations, we could calculate the net vertical force according to the function of the vertical position of oocyte, which is the distance between the oocyte and the bottom surface of the device [Fig. 7(a)]. The vertical component of the DEP force was obtained using the gradient of the electric field in the z direction, $\nabla|E_z|^2$, which was calculated by the numerical simulation. The gap height of the liquid chamber and the radius of oocyte were 240 and 60 μm, respectively. At the ac frequency condition of 1 MHz and the medium conductivity of 0.13 mS/cm, the calculated value of $\text{Re}(f_{\text{CM}})$ was 0.9229. In the case of the antigravity manipulation mode, the magnitude of net vertical force becomes smaller than that in the gravity mode by balancing the gravity and the vertical DEP force; thus the friction force becomes weaker. Especially, when the oocyte is settled down on the bottom surface, at which the vertical position of the oocyte is assumed to be the same with the cell radius, 60 μm, the oocyte exhibits a net vertical force of -5.11 nN for the gravity mode and 1.72 nN for the antigravity mode. The negative sign $(-)$ indicates that the force has orientation in the $-z$ axis, which is identical with the gravity. Accordingly, the vertical DEP force can overcome the gravity force and cancel its effects at the antigravity manipulation mode. Consequently, the velocities of moving oocytes, V_{DEP}, under the antigravity mode would be increased due to the lower friction force, and this result corresponds to the experimental values. If the settled oocyte is slightly larger than the normal value, the vertical position where the point of the forces acts on the oocyte becomes higher, resulting in the smaller friction force under both manipulation modes. Figure 7(b) shows the calculated net vertical force according to the applied voltage. In this calculation, the oocyte was assumed to be settled down. As a result, the difference in vertical forces between two modes was dramatically increased as the applied voltage increased. This result is caused by the vertical component of the positive DEP force induced by the optical patterns, which is proportional to the square of the applied voltage while the gravity has a regular value. Consequently, we can predict that the higher voltage condition is more advantageous for the manipulation of heavy and sticky cells such as oocytes using the antigravity manipulation mode of the optoelectrofluidic device.

V. CONCLUSIONS

In this paper, a new approach for discrimination of normal oocytes based on optically induced positive DEP has been described. The discrimination performance could be improved by balancing the gravity effect and the pulling-up DEP force and reducing the friction force acting on the heavy and sticky oocytes. The normal oocytes could be separated with a scanning LCD image pattern and shows significantly different moving velocities under each manipulation mode: 3.92 ± 4.32 and 11.59 ± 1.72 μm/s under the gravity and the antigravity modes, respectively. The enhanced discrimination performance based on the antigravity manipulation mode would be more useful to

select oocytes with a good developmental potential; thus the efficiency of IVF would be increased. This optoelectrofluidic platform for dielectrophoretic separation can be utilized for automatic and interactive selection of fertilizable oocytes.

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