Dynamic Light-Activated Control of Local Chemical Concentration in a Fluid

Hyundoo Hwang and Je-Kyun Park*

Department of Bio and Brain Engineering, College of Life Science and Bioengineering, KAIST, 335 Gwanhangno, Yuseong-gu, Daejeon 305-701, Republic of Korea

This article reports a method for controlling the chemical concentration in a localized region of a fluid using optoelectrofluidic mechanisms. Optoelectrofluidic fluorescence microscopy (OFM), in which an optoelectrofluidic device is integrated into a conventional fluorescence microscope, allows both modulation and detection of local chemical concentration in an easy and simple way. Here, we present the first experimental investigation of the concentration change of polysaccharides, proteins, and fluorophores, due to the frequency-dependent ac electrokinetics and electrostatic interactions in an optoelectrofluidic device. The dynamic modulation of the local concentration of biomolecules such as dextran and serum albumin was demonstrated in a temporal and spatial manner. This OFM can be a useful tool for controlling the local chemical concentration in several chemical and biological applications.

The local concentration of molecules can affect the activities and assembly of them, modulating whole chemical processes in a biological system. A dynamic control of the chemical concentration in a specifically localized region of a sample fluid with a rapid and noncontact method has been difficult, although such a technology can be valuable for several biological studies, including macromolecular crowding on protein folding kinetics and cellular chemotaxis in a chemical gradient. Recently, an optical heating has been applied for the dynamic modulation of chemical concentration in an aqueous droplet due to its shrinkage and expansion. Optoelectrofluidics, which is also known as optically induced electrokinetics, refers to the motion of particles or molecules and their interactions with an optically induced electric field and surrounding fluid. In 2000, this concept has been demonstrated through the electrophoresis of colloidal particles using an ultraviolet light pattern projected onto an indium tin oxide (ITO) electrode. After that, most of the researchers have started applying a photoconductive material deposited on a metal plate electrode to induce a nonuniform electric field, which results in an electrokinetic motion of particles and fluids, only with a weak white light source or a conventional laser source. Although optically induced dielectrophoresis (DEP) has frequently been employed to manipulate individual microparticles, light-induced ac electroosmosis (ACEO) has recently attracted a great deal of attention because of its capability to rapidly manipulate nanoparticles or molecules, which are too small to manipulate using DEP force proportional to the particle volume. Recently, a new optoelectrofluidic technology that combined both several electrokinetic mechanisms and electrostatic interactions for the rapid and selective concentration of microparticles has been reported.

In the previous studies, conventional display devices such as a digital micromirror device and a liquid crystal display have usually been applied to generate and control the light pattern and electric field gradient. However, the display-based optoelectrofluidic platform has a limitation for detecting signals induced from the light—matter interactions, including fluorescence and Raman scattering, as manipulating them. This limitation is due to the characteristic of the optoelectrofluidic device, in which a photocative material is used to optically induce an electric field. However, the photoconductivity of the optoelectrofluidic device was advantageous for our objective that is dynamic modulation of chemical concentration. We could dynamically control the local chemical concentration using optically induced electrokinetics and electrostatic molecular interactions and detect the concentration change at the same time with a single light source for fluorescence excitation. This concept is almost the same with that described in the previous study about the optically
induced ACEO. However, only the simple concentration of nanoparticles and DNA molecules was performed, and the in-depth investigations into the phenomena were not enough.

In this article, we investigate the behavior of molecules under the optically induced electric field against various factors and demonstrate a simple method for dynamic control of local chemical concentration in a sample fluid using optoelectrofluidic fluorescence microscopy (OFM), in which an optoelectrofluidic device is integrated into a conventional fluorescence microscope. The local concentration change of molecules was determined according to the applied ac signal, the type of molecules, and the initial background concentration of the sample solution. On the basis of these results, the effects of optically induced electrokinetic mechanisms and electrostatic interaction forces at the molecular level on the change of local chemical concentration were investigated. Our experimental results are quite different from the previous notion about the optoelectrofluidic molecular concentration. In addition, a dynamic control of local concentration of macromolecules such as dextran and serum albumin, which have often been applied as a crowding agent, was demonstrated using OFM. The frequency-dependent behavior of molecules were first investigated and applied for dynamic temporal modulation of local molecular concentration.

EXPERIMENTAL SECTION

Device Fabrication. To fabricate an optoelectrofluidic device, glass substrates coated with an ITO were purchased from Samsung–Corning Precision Glass (Korea). Triple layers of a 50-nm-thick heavily doped hydrogenated amorphous silicon (a-Si:H), a 1-µm-thick intrinsic a-Si:H, and a 20-nm-thick silicon nitride were deposited sequentially on the ITO-coated glass substrate by the plasma enhanced chemical vapor deposition (PECVD) method. After the fabrication of a 30-µm-thick photore sist spacer on a bare ITO-coated glass substrate, a 10 µL droplet of sample solution was sandwiched between the bare ITO—glass substrate and the a-Si:H-deposited substrate and a wrapping wire was connected for applying a voltage. This fabrication process is almost the same with that of the conventional optoelectrofluidic devices which have been reported in previous studies.

Sample Preparation. Several target molecules, including fluorescein isothiocyanate (FITC)-labeled dextran, fluorescein-conjugated bovine serum albumin (BSA), fluorescein, and bisbenzimide, were purchased from Sigma–Aldrich (Milwaukee, WI). FITC-dextran was diluted with deionized water into 1, 5, 10, 30, 50, 75, and 100 µM solutions. Fluorescein-BSA, fluorescein, and bisbenzimide were diluted into 10 µM solutions. The samples were stored in an ice box to keep its temperature until injecting into the optoelectrofluidic device.

System Configuration. A fabricated optoelectrofluidic device was put on the stage of an epi-fluorescence microscope (BA400T; Martin Microscope Company, SC) after injecting a liquid sample containing target molecules. A light for excitation of fluorescence signal was projected from a mercury lamp through an iris diaphragm for controlling the light pattern and an objective lens for focusing the light pattern onto the photoconductive layer of the optoelectrofluidic device. At the same time, an ac voltage was applied across the liquid chamber by a function generator for focusing the light pattern onto the photoconductive layer of the diaphragm for controlling the light pattern and an objective lens signal was projected from a mercury lamp through an iris containing target molecules. A light for excitation of fluorescence from molecules was projected onto the optoelectrofluidic device, the number of electron-hole pairs at the partially illuminated area was significantly increased. As a consequence, a nonuniform electric field was formed in the sample solution, resulting in the optically induced electrokinetic flows and electrostatic interactions around the light pattern which induce convergence or divergence of molecules. This OFM system makes both modulation and detection of local molecular concentration easily possible by applying the light source for both fluorescence excitation and electric field activation.

When we applied a voltage and projected a light pattern to the optoelectrofluidic device, a sudden change of fluorescence signal due to the change of molecular concentration in the illuminated area was detected (Figure 2a). With application of a voltage of 10 Vpp at 1 kHz, the ACEO flow was induced by the movements of closely packed ions at the electric double layer on the optically induced electrode surface due to the tangential electric field ($E_t$) with a slip velocity defined as

$$\langle v_{\text{slip}} \rangle = \frac{1}{2} \frac{\lambda_D}{\eta} \Re(\sigma_q E_t^*)$$

where $\lambda_D$, $\eta$, and $\sigma_q$ are the Debye length, the fluid viscosity, and the charges contained in the electric double layer, respectively. The ACEO flow converging into the illuminated area forms a stagnation point, where the flow velocity approaches zero, near the center of the area, and FITC-dextran molecules in the bulk fluid were concentrated into the point. This local chemical
concentration of molecules in the illuminated area was saturated into a specific constant value within a few seconds. Figure 2b shows the rapid change of local chemical concentration according to the amplitude of the applied voltage. The time for the change of FITC-dextran concentration was almost independent of the amplitude of the applied voltage, while the value of steady-state concentration depended not only on the amplitude of the applied voltage but also on various parameters, including the applied ac frequency, the type of molecules, and the bulk chemical concentration of the solution.

Frequency-Dependent Concentration Changes. Figure 3 shows the change of fluorescence signal of FITC-dextran for different ac frequencies such as 100, 10, and 1 kHz according to the time. The fluorescence intensity was maintained in a certain value with application of a voltage. The fluorescence intensity profiles of FITC-dextran at 1 and 10 kHz had always triangular and square shapes, respectively (Figure S1 in the Supporting Information), although the steady-state magnitude of the fluorescence signal was changed proportionally to the amplitude of the applied ac signal. When we turned off the applied voltage, the concentration was rapidly changed into a specific value and then slowly recovered due to the diffusion transport of target molecules. From these results, we concluded that the electrostatic interactions among the molecules due to their polarization in the optically induced electric field must also affect the behavior of molecules, since the electrohydrodynamic flow by ACEO cannot raise these phenomena for itself. For example, the first rapid decrement of molecular concentration as soon as the voltage is turned off, before the second slow recovery due to the diffusion, might be due to the sudden disappearance of the electric field-induced attractions between the FITC-dextran molecules. The positive DEP forces can also affect the molecular concentration, but the effect of DEP will be described later.

Effect of Molecular Type. Figure 4a shows the microscopic pictures of the FITC-dextran solution in the OFM for different ac frequencies from 100 Hz to 100 kHz. The fluorescence intensity was normalized to the average intensity value at the no voltage condition as a control. Figure 4b shows the microscopic pictures of the bisbenzimide solution for different ac frequencies. The fluorescence intensity was normalized to the average intensity value at the no voltage condition as a control. The fluorescence intensity of FITC-dextran was significantly decreased when the ac signal of 1 kHz frequency was applied, while the fluorescence intensity of bisbenzimide was almost unchanged. This result suggests that the electrostatic interactions among the molecules due to their polarization in the optically induced electric field must also affect the behavior of molecules, since the electrohydrodynamic flow by ACEO cannot raise these phenomena for itself. For example, the first rapid decrement of molecular concentration as soon as the voltage is turned off, before

**Figure 2.** (a) Microscopic pictures showing the change of the chemical concentration of FITC-dextran in the illuminated region of a sample fluid after application of a voltage of 10 Vpp at 1 kHz. (b) Temporal change of the chemical concentration according to the amplitude of the applied voltage at 1 kHz.

**Figure 3.** Change of chemical concentration of FITC-dextran in the illuminated area against the applied ac frequency with application of a voltage of 10 Vpp (black/white triangle: on/off).

**Figure 4.** Microscopic pictures of (a) FITC-dextran and (b) bisbenzimide solutions against the ac frequencies from 100 Hz to 100 kHz. (c) Fluorescence intensity according to the type of molecules. The fluorescence intensity was normalized to the average intensity value at the no voltage condition as a control.
This was due to the weaker ACEO flow around the illuminated area at the high-frequency range. The weak focusing flow, which is slightly stronger than the Brownian motion of molecules at 10 kHz, decreased the amount of concentrated molecules in the illuminated area. As a consequence, the weak dipole attraction among the molecules due to the low-volume fraction of them makes the resulting chemical concentration in the illuminated area become lower.

When we increased the applied frequency to 100 kHz, there was no significant change of the chemical concentration compared to that of the no voltage condition. This was due to the frequency characteristics of ACEO, which is generally activated at the low-frequency range below 10 kHz. On the other hand, the positive DEP can be activated and move the molecules at the high-frequency around 100 kHz. Nevertheless, the molecular concentration in the illuminated area at 100 kHz was not much different from that at the no voltage condition in this experiment. It might be due to the weak strength of the positive DEP acting on the FITC-dextran molecules comparable to the Brownian motion of the molecules. We could determine the effect of the weak DEP force in the experiments for dynamic temporal modulation of the local molecular concentration using a consecutive frequency change later.

In the previous studies using the optically induced ACEO, the electrohydrodynamic flow always concentrated the molecules into the illuminated area. According to our experimental results, however, the local chemical concentration of FITC-dextran could be decreased at a few hundred Hertz. It has been shown to be driven by the electric field-induced flow due to the lateral electric field component within the electric double layer at the low-frequency range where the Faradaic reaction becomes considerable. In addition, the weaken dipole interaction forces among the molecules could also help the depletion of molecules at the illuminated area.

In the case of bisbenzimide, the molecular concentration in the illuminated area was decreased into a specific steady-state value even in the high-frequency range above 1 kHz as shown in Figure 4b. These results are quite different from the previous notion that the molecules are always concentrated and trapped by the optically induced ACEO vortices into the illuminated area.

To investigate these phenomena more deeply, we observed the optoelectrofluidic change of local chemical concentration with various types of molecular solutions. The frequency-dependent concentration change showed a different tendency according to the type of molecules. In the case of bisbenzimide and fluorescein, which are small fluorophores, their local concentration decreased as the ac frequency decreased from 100 to 1 kHz, while the concentration of FITC-dextran and fluorescein-BSA, which are macromolecules, had a maximum value at 1 kHz and decreased as the frequency increased to 100 kHz (Figure 4c). On the basis of the experimental results for four different types of molecules, we inferred that the size of molecules is one of the important parameters to determine the tendency of the chemical concentration change in the illuminated area at the low-frequency range above 1 kHz. The smaller molecules such as fluorophores, which the size is approximately 1 nm, have a larger diffusion coefficient and a smaller dipole interaction energy than relatively larger molecules such as dextran and BSA, of which the size is about 22 nm. In the case of small molecules, therefore, the dispersion forces due to the Brownian motion would be more dominant than the concentration forces due to the electrostatic dipole interactions and DEP, resulting in the interference with the molecular aggregation in the illuminated area. When we applied a frequency of 100 Hz, all the molecules rapidly disappeared from the illuminated area. The mechanisms regarding the significant decrement of molecular concentration, which was independent of the molecular size, in the low-frequency range below 1 kHz is considered to have different physical origin from that in the high-frequency range above 1 kHz. The causes which induced the difference between the absolute fluorescence intensity of each molecule could not be completely determined. However, it might be caused by the difference of physical and chemical properties of each molecule.

Electrostatic Aggregation of Molecules. We also determined the electrostatic aggregation of FITC-dextran molecules, which was mentioned above as a dominant factor for molecular concentration changes. When we projected a light pattern of larger area, we could clearly observe the aggregation of molecules due to the dipole–dipole interactions with application of a voltage of 10 Vpp at 1 kHz (Figure 5). The aggregated FITC-dextran molecules were dispersed as soon as the applied voltage turned off after about 14 s. This electrostatic aggregation of molecules under an electric field have also been observed using DNA molecules in a capillary tube for electrophoresis. The physical mechanism of our experimental observation might be much the same with that of the previous study using DNA molecules. The electrostatic interactions among the molecules became dominant near the stagnation point, where the ACEO flow can be negligible. Therefore, the molecules concentrated by ACEO flow into the stagnation point must be aggregated by the electrostatic attraction forces with application of a voltage of 1 kHz.

Effect of Bulk Chemical Concentration. We measured the concentration of FITC-dextran which could be increased by the optoelectrofluidics according to its initial concentration, which

---

means the bulk concentration of the sample droplet (Figure 6). The maximum molecular concentration with application of a voltage of 10 Vpp at 1 kHz frequency was proportional to the initial concentration. The local concentration of molecules converged on a specific amount with a very fast response time. The time that the molecular concentration reaches the steady state was not affected by both the magnitude and frequency of the operating voltage signal, while the steady-state concentration of the molecules and the fluorescence intensity profiles were affected.

According to these results, we concluded that the molecules must be in a dynamic state and not be accumulated into the illuminated area. If the molecules concentrated by the optically induced ACEO flows are accumulated, the maximum concentration must have a constant value independent of the applied voltage and the initial concentration of the sample solution. However, the concentration was saturated rapidly into a specific value, which is proportional to the initial concentration and the applied ac signal and depends on the physical and chemical properties of the molecules.

The electrostatic interaction of the molecules could also account for this concentration-dependent phenomenon. If the amount of FITC-dextran molecules in the fluid is too small, the dipole attraction among the molecules might not be strong enough to keep them in the illuminated area as well as the amount of the molecules, which exist around the light pattern and can be concentrated into it, is also insufficient.

**Dynamic Control of FITC-Dextran Concentration.** On the basis of the frequency-dependent change of the local chemical concentration, dynamic control of the FITC-dextran concentration was performed as shown in Figure 7a. As switching the frequency of the applied ac signal to 1, 10, and 100 kHz with an interval time of 10 s, the maximum fluorescence intensity was measured according to the time. In this temporal modulation, the differences of the molecular concentration against the applied ac frequency increased as the amplitude of the ac signal increased from 6 Vpp to 10 Vpp. Under the 6 Vpp, the change of the fluorescence signal due to the molecular movements was too small to detect over the signal noise, since the ACEO flow and the dipole attraction among the molecules were induced enough to concentrate the molecules. The response time at the frequency for increasing molecular concentration, i.e., from 100 to 10 kHz and from 10 to 1 kHz, was shorter than for decreasing it, i.e., from 1 to 10 kHz and from 10 to 100 kHz. This was due to the diffusion of the concentrated molecules, which is a much slower transport mechanism than the optically induced ACEO flows.

The chemical concentration at the frequency of 100 kHz was also dependent on the amplitude of the applied ac signal, although the change of local chemical concentration due to the optically induced ACEO did not appear at 100 kHz according to Figure 4a. This phenomenon was due to the effect of a weak positive DEP force, which could be induced by an optically induced nonuniform electric field, as mentioned above. Under the consecutive concentration and release processes, the molecules, which were already concentrated into the illuminated area at 1 and 10 kHz, would also be affected by the weak DEP force trapping them before they were diffused out from the area. Therefore, the positive DEP as well as the ACEO flows and the electrostatic interactions must affect the molecular behavior in the OFM.

Figure 7b shows the spatial control of the local chemical concentration of FITC-dextran using OFM. With application of a voltage of 10 Vpp at 1 kHz, FITC-dextran molecules were rapidly concentrated into a specific area by being exposed to the light source for fluorescence excitation. When we observed the

**Figure 6.** Plot of the maximum concentration of FITC-dextran modulated by the optoelectrofluidics according to the initial concentration of the sample droplet with application of a voltage of 10 Vpp at 1 kHz. A linear regression line was also represented.

**Figure 7.** (a) Temporal control of the local concentration of FITC-dextran with changing the frequency and amplitude of the applied ac signal with an interval of 10 s (red/blue/green triangle: 100/10/1 kHz frequency). (b) Spatial control of the local concentration of FITC-dextran was also possible as the light pattern was controlled with application of a voltage of 10 Vpp at 1 kHz (dotted circle: light pattern).

---

molecular weight is 65 kDa.24


(24) Krouglova, T.; Vercammen, J.; Engelborghs, Y.

The diffusion coefficient of BSA (5.1

diffusivity. However, the time for diffusing out of BSA was

the ac frequency showed very similar tendency to

Figure 8. (a) Temporal control of the local concentration of fluorescein-BSA as the frequency of the applied ac signal is changed (red/blue/green triangle: 100/10/1 kHz frequency). (b) Spatial control of the local concentration of fluorescein-BSA was also possible as the light pattern was controlled with application of a voltage of 10 Vpp at 1 kHz (dotted circle: light pattern).

sample after moving the light pattern and opening the iris diaphragm, the molecular concentration at the localized area along the trajectory of moved light was significantly increased. Finally, the concentrated molecules were diffused out according to the time.

Dynamic Control of Fluorescein-BSA Concentration. Fluorescein-conjugated BSA, which is one of the most widely used crowding agents for protein folding and aggregation research,1,3 was also applied as a target molecule to control its local concentration in a temporal and spatial manner as shown in parts a and b of Figure 8, respectively. The fluorescence intensity profiles of BSA against the ac frequency showed very similar tendency to that of dextran. However, the time for diffusing out of BSA was longer than that of 10 kDa dextran. It was due to the smaller diffusion coefficient of BSA ($5.1 \times 10^{-11}$ m² s⁻¹), of which the molecular weight is 65 kDa.24

We could also trap an aggregate, which is shown in Figure 8b, using the optoelectrofluidic vortices. However, in this experiment, the moving velocity of the light pattern was too fast to transport the aggregate. The trapped aggregate could be manipulated, only when we moved the light pattern with much slower velocity (data not shown). This phenomenon is in contradiction to the results from the previous study, which emphasized that the trapping and transport of nanosized particles without losing them is possible using the light-induced ACEO.17 According to these results, however, it is too difficult to trap and transport molecules without any losses because of their dynamic behavior and electrostatic interactions. We concluded that the trapping and transport of molecules using the optically induced ACEO flow is possible, but the dynamic control of local chemical concentration is the more correct function of this OFM technique. As in the case of colloidal particles, some molecules may be permanently trapped and assembled in a very thin layer on the surface of the optically induced virtual electrode7 but the further studies for its verification are necessary.

CONCLUSIONS

Dynamic control of the local chemical concentration was demonstrated using a simple optoelectrofluidic platform, where a conventional fluorescence microscope was combined with an optoelectrofluidic device. In this OFM system, the light source for fluorescence excitation was applied for both detection and modulation of molecular concentration in a sample fluid. We first investigated the frequency-dependent change of the local chemical concentration using the OFM. The optoelectrofluidic change of the local chemical concentration was dependent on the type of molecules and the bulk concentration as well as the applied ac signal. The investigation of dipole–dipole attraction among the molecules in the optoelectrofluidic device was also performed. Temporal control of the local molecular concentration has been demonstrated by applying the frequency-dependency of several electrokinetic mechanisms and electrostatic interactions of biomolecules such as FITC-dextran and fluorescein-BSA. Spatial control of their concentration at the localized area was also performed by controlling the light pattern.

This OFM system will be utilized as a useful tool in several applications such as molecular patterning and self-assembly as well as studies about protein folding kinetics, cellular chemotaxis, molecular aggregation, and diffusion kinetics. Moreover, it can be applied for the analysis of micro/nanoparticles and molecules based on their size, structure, and permittivity, which affect their mass transfer and electrokinetic characteristics.

ACKNOWLEDGMENT

Support for this work from the Nano/Bio Science and Technology Program (Grant 2005-01291) and the National Research Laboratory (NRL) Program (Grant R0A-2008-000-20109-0) funded by the Korea government (MEST) is gratefully acknowledged. The authors also thank the Chung Moon Soul Center for BioInformation and BioElectronics, KAIST, and the TFT-LCD Research Center, Kyung Hee University, Korea.

SUPPORTING INFORMATION AVAILABLE

Fluorescence intensity profiles of FITC-dextran according to the applied ac frequency. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review May 14, 2009. Accepted May 18, 2009.

AC901047V