論 文 の 内 容 の 要 旨

論文題目 Microfluidics-based analytical platform for nanoparticle characterization
（ナノ粒子の特性評価のための流体デバイスに基づく解析プラットフォーム）

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The developed methods for characterizing nanoparticles (NPs) are an important part in materials engineering. Nanoparticles can be spherical, tubular, or randomly shaped with nanometer sizes. They may display distinctive physical, optical, and electrical properties. The application of nanoparticles includes nanobiotechnology such as targeted drug delivery, molecular biology, and novel composite of nanoparticles and polymers. Typically, electron microscopy and atomic force microscopy are used to estimate the structure, composition, and properties of nanoparticles. However, the evaluation of nanoparticle using the abovementioned techniques has considerable disadvantages such as high cost of operation and time consumption. In addition, the small sizes of nanoparticles (i.e., several tens to one hundred nanometers) complicates their quantification. Hence, there is a need to develop a new characterization technique.

The technological application of lab-on-a-chip systems and the development of an entire bio-chemical laboratory on a micron-sized polymer-based chip is increasing. Chip-based microcapillary electrophoresis (μCE) is one of the characterization techniques of nanoparticles. In recent years, outstanding studies on the characterization of biological nanoparticles using the chip-based μCE by laser dark-field imaging have been published. Specifically, particle-by-particle measurement and visualization were achieved by measuring the light scattered by nanoparticles under the influence of electric field. The μCE

chip, which is fabricated from polydimethylsiloxane (PDMS) and glass substrate, consists of a microfluidic channel (length: 10 mm, width: 200 μm, height: 200 μm) and reservoirs (diameter: 5 mm) with open ends.

The microscale dimension of the chip results in undesirable issues, which complicate the measurement of electrophoretic mobility (EPM) of nanoparticles. Thus, it is necessary to solve these issues in a μCE chip by augmenting the analytical method for characterizing nanoparticles. The issues presented below.

1. Hydrodynamic issue arises due to the low fluid conductance of the microchannel, electroosmotic flow (EOF) in the microchannel producing an imbalance of the fluid height between the two reservoirs during the measurement, and meniscus instability in the reservoirs originating from the complexity of the interface dynamics.

2. Electrochemical or Joule heating issue arises from the increase in the mobility of ions and generation of convection flow, pH change of fluid, and bubble formation at the electrodes by electrolysis.

3. PDMS-based microchannels of the μCE chip exhibit a negative surface potential, which causes nanoparticle adsorption due to hydrophobic and/or electrostatic interaction, and EOF in the microchannel.

To solve the above-mentioned issues, we proposed a new chip design by adding a wider bypass channel (length: 10 mm, width: 2 mm, height: 2 mm) to balance the fluid level of the two open-end reservoirs to readily determine the EPM. This approach facilitates the technological advancement of μCE chips.

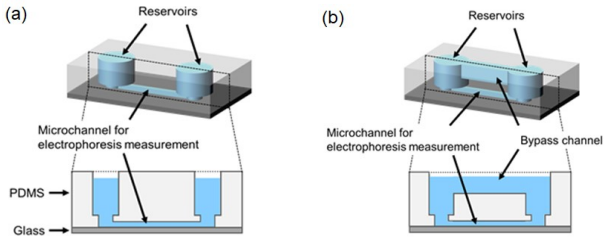

Fig. 1. Microcapillary electrophoresis (μCE) chip: (a) without and (b) with the bypass channel

To test the new chip design, the fluid level adjustment of air-water interface with laminar fluid and phase field interface was simulated using the computational fluid dynamics and microfluidic module. During the simulation, appropriate model materials and boundary conditions were applied. Moreover, the following equations were used in the simulation: the Navier-Stokes equation was used to model the flow in the channel; electroosmotic velocity and electric field strength were governed by the Helmholtz-Smoluchowski equation; air-water interface motion was simulated by the Cahn-Hilliard equation; and phase field and contact angle were modeled by the wetted wall approach. The simulated results, which show the volume fraction of fluid and fluid velocity distribution in the μCE chip with the bypass channel at the applied electric potential of 5 V, demonstrate the presence of hydrostatic counter flow in the bypass channel, which compensates the unbalanced hydrostatic pressure. These results confirm the assumption that the addition of a wider bypass channel compensates the hydrostatic pressure flow in the microchannel.

After successfully simulating the μCE chip with the bypass channel, which is used to compensate the hydrostatic pressure, experimental evaluation was carried out to compare the simulated and experimental EPM. Thus, μCE chips with and without the bypass channel were fabricated, and individual NPs were measured by laser dark-field imaging. To confirm the compensation in the chips, the images of polystyrene NPs in the microchannel were captured for the time periods between 0 and 30 min; at 1 min, a 1-μL droplet of the NP suspension was added. Surprisingly, in the μCE chip without the bypass channel, we observed fluctuation in the fluid velocity during the balancing time, which resulted from the meniscus instability at the fluid-wall interface. This phenomenon is referred to as the Saffman-Taylor-like meniscus instability. However, in the μCE chip with the bypass channel, only the Brownian motion of NPs was observed, which shows the complete elimination of unstable hydrostatic pressure in the microchannel.

The current-voltage (I-V) characteristics of the μCE chips were compared because the chips have different cross-sectional areas and use solutions with different conductivities. The measured current was 0.8-1.0 $\times$ 10^2 times higher in the μCE chip with the bypass channel. In addition, high electrical conductivity and ionic strength result in the increase in the measured current in phosphate buffered saline with a deviation from linearity over 10 V due to Joule heating. Finally, the EOF of non-charged NPs from the experimental result was compared with

the simulated result. It was determined that the addition of the bypass channel did not affect the EOF value.

The adsorption behavior of charged nanoliposomes in the presence of cationic polyethylenimine (PEI) and neutral polyethylene-block-PEG polymer-coated PDMS microchannel of the μCE chip with the bypass channel was studied. Neutral 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC), cationic 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), and anionic L-α-phosphatidylserine (PS) nanoliposomes were prepared using the thin film hydration method with 4:1, 1:1, and 5.55:0.45:4 molar ratios, respectively, and extruded to a 100-nm size. By observing the stable Brownian motion and particle number of nanoliposomes in the region of interest (ROI) with the polymer-coated microchannel, it was determined that the adsorption phenomenon of nanoliposomes on the microchannel surface originated from hydrophobic and electrostatic interactions. The adsorption phenomenon can be attenuated based on the electrostatic repulsion and excluded volume effect, which was evident by the approximately constant particle number throughout the experiment.

In the last part of this thesis, the temperature-dependent adsorption behavior of 50-nm, 100-nm, and 150-nm DLPC nanoliposomes on the polyethylene-block-PEG-coated microchannel of the μCE chip with the bypass channel was observed. The concentrations of nanoliposomes were adjusted, and their adsorptions were measured at room temperature and as a function of a gradual increase in temperature. As usual, nanoliposomes in the chip at room temperature showed the excluded volume effect with a relatively constant particle number in ROI. However, in the chip with a gradual increase in temperature, nanoliposomes showed the gradual increase in the particle number in ROI due to high adsorption coefficient and inefficiency of the excluded volume effect of PEG at low temperature.

We have successfully developed an analysis platform for nanoparticles by extending the potential applicability of the microcapillary electrophoresis chip with new design and improved accuracy of the electrophoretic measurement. This chip is a fundamental tool for the characterization of extracellular vesicles (EVs), including exosomes, and application in the field of nanobiotechnology.