論文の内容の要旨

Abstract of Dissertation

Title of Dissertation: A Study on the Use of Microfluidic Platforms for Biomolecular Analysis

(生体分子解析のためのマイクロ流体プラットフォームに関する研究)

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1. Objective

Microfluidics is referred to as a set of techniques for fluid handling in channels with dimensions of 5~500μm and with volumes of famtoliters to microliters. It has favorable features such as efficient heat and mass transport, low thermal mass, and large surface-to-volume ratio. To utilize these features, microfabrication techniques, originated from microelectronics industries, have been introduced into microfluidics for making micrometer-sized structures. Microfluidics is also of intense interests in practical use in biomolecular applications, because of its characteristics to shorten reaction times, reduce sample consumptions, and increase detection sensitivities. Unlike conventional laboratory operation for biomolecular analysis, microfluidic-based analytical systems generally offer fast operation and detection times, and low power consumption.

Despite the rapid progress of technologies in microfluidics, its actual impacts on lifesciences and biotechnology are not fully recognized. One reason seems to be associated with the difficulty in proper transition from simple microfluidic components to highly integrated systems as tools in practical use. Another one can be considered as less controllability of the fluid handling such as sample introduction, transportation, exchange, *etc*. Precise handling by fluid in microfluidics has large potentials to study biomolecules such as DNA and proteins, because the biomolecules are surrounded by liquid solutions with certain concentration of chemical components, pH, and so on.

The aim of the study is to improve the controllability of fluid handling in microscale environments for advanced analysis of biomolecules. A microfluidic platform is proposed to achieve the aim. Different design concepts and measurement methods are proposed depending on the applications.

The platform consists of fluidic chips and a glass chip. The fluidic chips are a micropump chip for actuating liquid samples and a microchannel chip made by PDMS (polydimethylsiloxane) for fluidic pathways of delivering chemicals, reagents, etc. The glass chip works as a window to see through what is occurring in microchannels such as chemical reactions of fluids, dynamic behaviors of biomolecules, and so on. It is also used as functional elements by proper treatments at one plane (called observation plane) facing liquid in microchannel Three chips are assembled into a platform, and this assembly realizes accurate handling of liquids such as delivering reagents or molecules to desired location, exchanging chemicals, and controlling environmental conditions.

Firstly, EIS (Electrical Impedance Spectroscopy) measurement of DNA was conducted on the platform. Secondly, the platform is used for analyzing a molecular rotary motor by controlling environmental conditions such as supply and removal of chemicals. As an extension of the study, an analytical method to estimate flows in close proximity of the surface is developed using analytical

analytical method to estimate flows in close proximity of the surface is developed using analytical method based on experimental results of rotation of F_1-ATPase under flows. And the method was validated by the measurement using micro-PIV (Particle Image Velocimetry) methods.

2. A Microfluidic platform for EIS measurement of DNA

A microfluidic platform is proposed for impedance measurement of DNA under high electric fields at various frequencies. Impedance response is measured in various field (0.1 MV/m ~ 0.3MV/m) and frequencies (1Hz ~ 10^7 Hz). Sample delivery and exchange are handled by embedded micropumps, and DNA measurement carried out while DNA is flowing through between two measurement electrodes to suppress Joule heating heat by applying high electric fields. **Figure 2-1** shows the structure of the platforms. Microelectrodes are fabricated on the platform for generation electric fields in a sample volume and measuring the response. Two-electrode set-up which current control (electric excitation) and potential sensing (measurement) are shared by the single pair of electrodes is used for inducing high electric field in a small volume. The behaviour of DNA is visualized as well, which is movements of DNA due to the polarity of applying electric field low frequencies (1Hz ~ 10Hz) and being stretched in higher frequencies (≥100kHz).

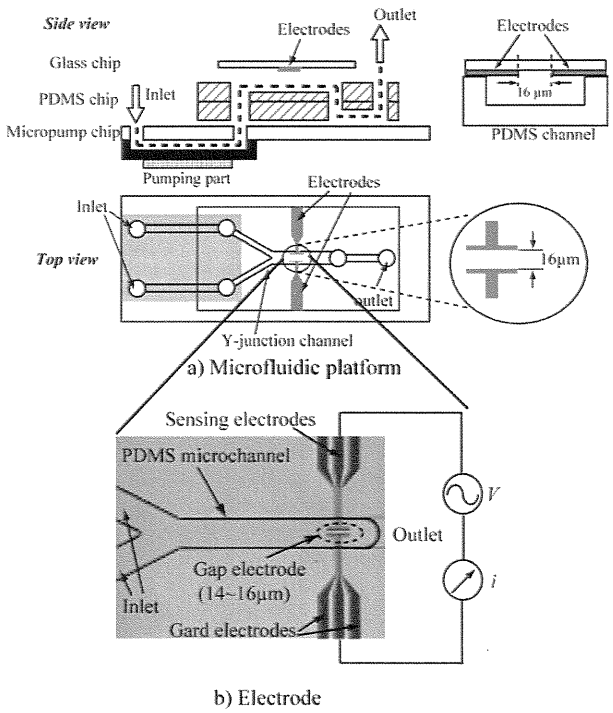


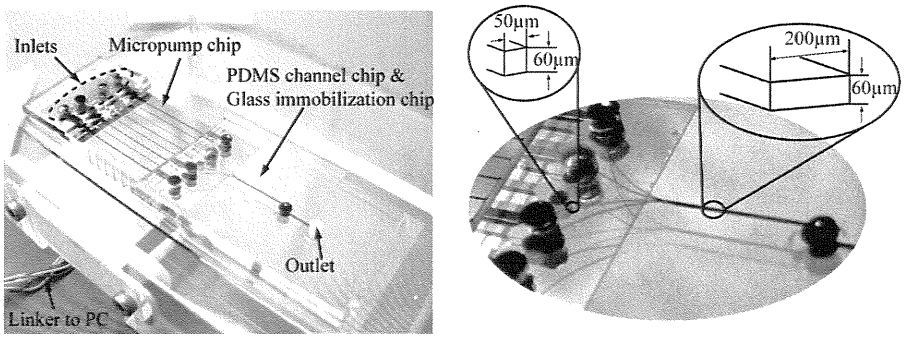
Figure 2-1. Schematic pictures of the microfluidic platform for EIS measurement of DNA

As a result, two different behaviors of DNA were observed. In low frequencies (< 10 Hz), DNA maintains random-coil conformation and moves between the electrodes following the polarity of the applying electric field. The absolute values of impedance signal were lower than that of buffer solution (solutions without DNA). On the contrary, DNA is stretched in high frequency (> 10^6 Hz) and high field strength (> 0.8 MV/m) and the absolute value of impedance were higher values than that of buffer. The applied electric field may play a role in governing the static and dynamic properties of polymeric biological materials. It will exteriorize the general behavior of conformational change of biopolymer in frequency domain using DNA as a model through its visualization, and utilize the phenomena to DNA detection enhanced by the conformational change using microfabricated electrode system.

3. A microfluidic platform for Single Molecular Analysis

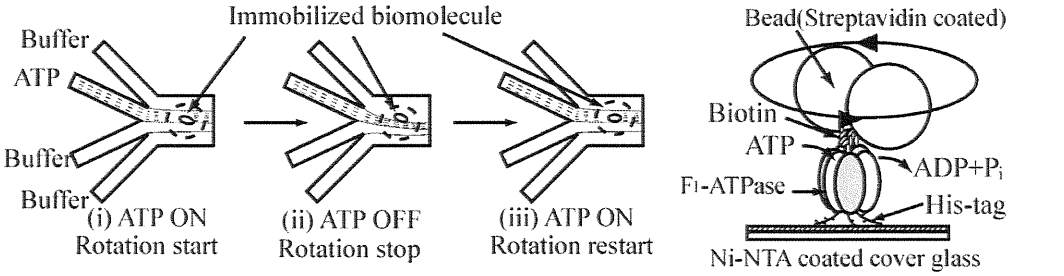
The fluidic operations, such as chemical delivery, hydrodynamic force control, solutions exchange, etc., to a desired location in a microfluidic channel are one of the most important technical aspects to progress single-molecule studies in microfluidic devices. For single molecular analysis, a microfluidic platform is designed to generate multilaminar flow by fluidic operations. The platform has three components, which Ni-NTA coated glass chip for binding proteins with His-tagged on the surface, PDMS (polydimethylsiloxane) chip having microchannels inside, and the micropump chip in which 4 micropumps are embedded. The whole structure of the platform and size of the microchannel is shown in **Figure 3-1**.

The concept of the chemical delivery system and an example of single-molecular analysis using this chemical delivery system are schematically shown in **Figure 3-2**. In the Figure, an enzyme of F_1-ATPase is immobilized in the center of a microchannel under 4 numbers of laminar streams are formed in the microchannel. By controlling the flow rate of each laminar stream, one can position the laminar stream containing ATP to an arbitrary location, so that ATP can be delivered or undelivered to the immobilized F_1-ATPase.



(a) Microfluidic chip (b) PDMS channel chip and its sizes

Figure 3-1 Photograph of integrated microfluidic platform and PDMS channel



a) Switching of laminar stream of ATP for chemical stimulation

b) Rotational motion of F_1-ATPase by catalytic reaction

Figure 3-2 Schematic image of multi-laminar controlled analysis

As a result, it was applied to single molecular analysis of F_1-ATPase with dynamic control of environment ,i.e, switching ATP on and off. A multilaminar flow was formed in the platforms and those can be manipulated in spatially resolved way by fluidic operation. Finally, F_1-ATPase was successfully immobilized on the platform and its dynamic behavior, which rotation is on and off following switching operation of ATP, was also monitored in spatially and temporally resolved way.

4.Flow measurements based on Single molecular Analysis

Hydrodynamic effects on single-biomolecule will have drawback and advantage depending on analysis. The drawbacks would be that a strong hydrodynamic force will peel an immobilized single-biomolecule from a measurement area on a surface or if the biomolecule stay at the surface, the biomolecules might deform its molecular structure to be lost the activity. The advantage is that a hydrodynamic force would be used as a probe to evaluate molecular motor proteins, such as linear motor of actin-myosin, tubulin-myosin, etc., and rotary motor of ATPase, bacterial flagellar motors, etc.

In any case, quantitative evaluation of hydrodynamic effects on single-biomolecule must be required at the beginning. In this work, I investigated the laminar flow induced hydrodynamic force acting on a single-biomolecule using a molecular motor of F_1 subunit of ATPase (F_1-ATPase) and its rotational motion by varying the laminar flow velocity. A physical model to estimate flow velocity in the close proximity of the surface was proposed as a probing method of flow.

For precise control of flows acting on the single molecules, the same microfluidic platform of previous chapter was used. The rotational observation of beads attaching to F_1-ATPase is done under 5 different flow conditions. One is rotation of F_1-ATPase when the channels were filled with ATP but no fluid flows, second was done under ATP flows when the micropump actuated 3V power, third and fourth were observed at 4V and 5V, respectively. Much higher power (8V pumping power) is applied to the pump to stall the rotation. The physical model based on the observation of rotational motion of the beads attaching to F_1-ATPase under various flow conditions was proposed (See **Figure 4-1).** Conventional method to observe flow such as micro-PIV method was also carried out in order to validate the method.

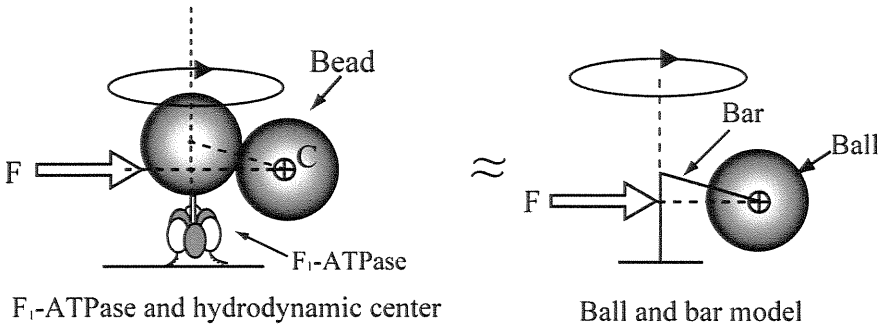


Figure 3-2 Simple

As a result, the rotations were decreasing in proportional to the increasing of flow velocities near the surface and it finally stopped. The flow velocity calculated by the model showed fairly good agreement with the value measured by micro-PIV even though some discrepancy appeared. It makes it possible to offer new way to measure flow in submicrometer scale using single molecules of F_1-ATPase as a probe.

5. Conclusions

The microfluidic platform is very efficient to operate fluid flexibly: 1) deliver reagents to a desired location at which the target single-molecule is immobilized; 2) rapid switch from a reagent to others for time-resolved analysis by chemical stimulus, 3) control hydrodynamic force to the target molecule during fluidic operations to prevent damages or to enforcedly apply an external force. It is also useful to control flow in very slow speed without any pulsation. Those kinds of fluid handling make it possible to study various biomolecular analyses with dynamic control of environments. Therefore, the microfluidic platform is very efficient to study biomolecular analysis with its controllability of fluid environment.