DEVELOPMENT OF GATING NANOPORE
TOWARDS SINGLE-BIOMOLECULE ELECTRICAL IDENTIFICATION

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ABSTRACT

We fabricated a gating nanopore (GN) nanostructure, which is composed of a silicon-based nanopore and embodied gold gap-electrode inside the nanopore by using newly developed fabrication scheme. We investigated change in electric current flowing not only between Ag/AgCl electrodes paralled to a nanopore, but also between gap-electrodes when single-particles pass through a nanopore. We found that the change in ion-current flowing between gap-electrodes was related not only to the particle size and surface charge. From the ionic current intensity and its time profiles, we demonstrated the discrimination of sample single-particles among the mixed sample solutions. The electrical measurement by using gating nanopore would be a promising methodology for single-biomolecule sensors.

KEYWORDS

nanopore, nanogap-electrode, single-biomolecule, electrical measurement.

INTRODUCTION

The nanopore-based device enabled discrimination of individual molecules/ions based on the fact that a different value of ionic current is observed for each of these molecules passing through a nanopore. The methodology has emerged as high-throughput single-molecule/ion detection and identification methodology [1]-[5]. The synthetic solid-state nanopore device nanopores were one of the hot-topics in the research field of nanopore device because of the robustness and usability in a wide range of pH, temperatures and electrical voltages. In addition, they also provide flexibility in pore and membrane sizes, which is important for sensing various target analysis [6]-[11]. We have proposed a single molecule analysis by using "Gating Nanopore (GN)", which is composed of solid-state nanopores and nanogap electrodes [12]. This nanostructure is expected to detect molecules that pass through a nanopore not only by a change in electric current flowing between nanogap electrodes perpendicular to a nanopore, but also by the electric current between the nanoelectrodes (**FIG.1**). Herein, we developed a fabrication scheme of a gating nanopore with a diameter in the range from 200 um to 40 nm. By using the gating nanopores, we investigated analytical target molecules translocating through the pore by electrical measurements of ionic current parallel to the nanopore, and between gap-electrodes inside nanopore, respectively.
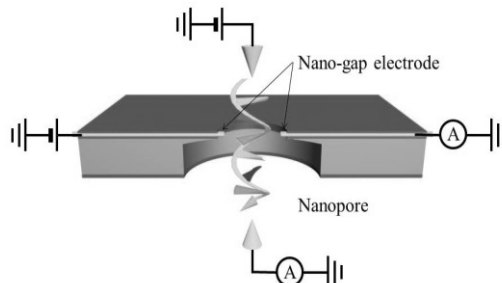

FIG. 1: Schematic of gating nanopore device for identification of single bio-molecules. single-particles / biomolecules that pass through a nanopore only by change in electric current not only flowing paralled to a nanopore, but also flowing between nanogap electrodes perpendicular to the nanopore. By using a gating nanore, we have proposed a single-molecule DNA/RNA sequencer. In the device, it is expected that DNA molecules electrophoretically translocate through the nanopore by applying the dc voltage between the Ag/AgCl electrode, and the DNA sequence is determined by sequentially measuring the tunneling-current of its nucleotide just through the nanogap electrode.

EXPERIMENTAL SECTION

The fabrication scheme of a gating nanopore (GN) is in the following three steps (**FIG.2**). In the first step, a gap-electrode pattern was formed on silicon wafer by a standard electron-lithography. Ti/Au/Ti thin films were sequentially formed by use of a RF sputtering method, and the gap-electrodes were produced by lift-off process. In the second step, the nanopore pattern was made based on alignment marks in order to position the nanopore just in the center of gap-electrode, and the nanopore was produced by RIE dry-etching. In the third step, the deep reactive-ion etching process (BOSCH) was performed on the opposite surface of the nanopore, producing a through-hole. The gap-electrode were coated with insulated silicon layer prepared by chemical vapor deposition (CVD) except the apex of the electrode on the sidewall of the pore. Optical microscopy and scanning electron microscopy were used for the characterization of the pore-size and nanogap-distance of the GN. The measurements of current-time (I-t) and current-voltage (I-V) profiles were performed between the Ag/AgCl electrodes paralled to the nanopore in a 1 M KCl or 1xPBS buffered solution (pH 6.9), and between gap-electrodes perpendicular to the nanopore, respectively. The Ag/AgCl electrodes were prepared by Pt or Ag wire coated with Ag/AgCl ink (BAS, Co., Ltd.). Microfluidics polydimethylsiloxane (PDMS) substrate were put on both sides of the gating nanopore. The KCl

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or PBS solutions were poured into the PDMS microfluids, and were flowed through the nanopore by an electric field applied between the Ag/AgCl electrodes. The polystyrene (PS) particles (Thermofisher Co.,Ltd.) were used for the sample particle. The ζ-potentials of the PS particles were found to be around -35 mV from Multi-particle Anaylzer (Kashiwa). All electrical measurements were performed by using a Faraday gauge, and PXIe system and in-house virtual instrumentation written in LabVIEW (National Instrument) at a sampling speed of 10kHz-1MHz.
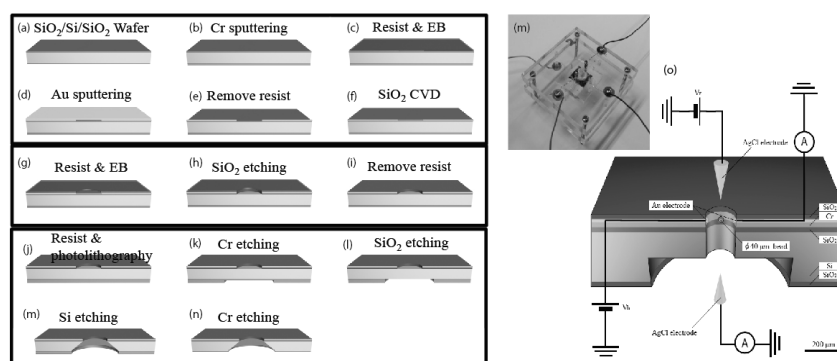

FIG. 2 Fabrication process of Gating nanopore device. Fabrication scheme of a gating nanopore: (Step 1) a gap-electrode formation process. (a) SiO₂/Si/SiO₂ wafer, (b) Cr sputtering. (c) Spin-coating of ZEP resist on the wafer, and gap-electrode pattern is lithographed by electron-beam. (d) Au sputtering. (e) Remove resist. (f) CVD SiO₂ layers. (Step 2: Red) a nanopore formation process. (g) Spin-coating of ZEP resist on the wafer, and nanopore pattern is lithographed by electron-beam. (h) SiO₂ is etching by RIE. (i) Remove resist. (Step3: Green) a through-hole process. (j) Spin-coating of ZEP resist on the wafer, and pore pattern for a through-hole is lithographed by electron-beam. (k) Cr etching for the hole pattern. (l) SiO₂ is etching by RIE. (m) Si etching by the deep reactive-ion etching (BOSCH) process. (n) Cr etching. (m) photo-image of GN device. The device size is 10 x 10 mm. In one fabrication, the obtained number of devices is 32. (o) Schematics of electrical measurements by using a gating nanopore.

RESULT AND DISCUSSION

First, we performed electrical measurements of current-time *(I-t)* between the Ag/AgCl electrodes paralled to the nanopore for standard-sized polystyrene (PS) particles translocating through the pore (**FIG.3a**). Since the surface of the sample PS particles are functionalized with carboxyl group, they are negatively charged. When the negatively charged PS particle was driven by the electrophoretic force and enters the pore, it partially precludes the pathway for ionic conduction and the ionic current through the nanopore decreases. Therefore, the individual PS particle signals were observed as negative-pulses (**FIG.3b**), corresponding to the translocation through the gating nanopore. The magnitude of "resistive-pulse" scaled approximately in proportion to the particle volume, which is similar to coulter counter type measurements in the previously reported study [13]-[15]. In addition, at constant applied voltage, a linear dependence was observed between the particle-translocation event rate and the concentration of particles. These results demonstrated that this change in current would be used to size and determine the concentration of the analyte species recorded as the nanoparticle is driven across the membrane.
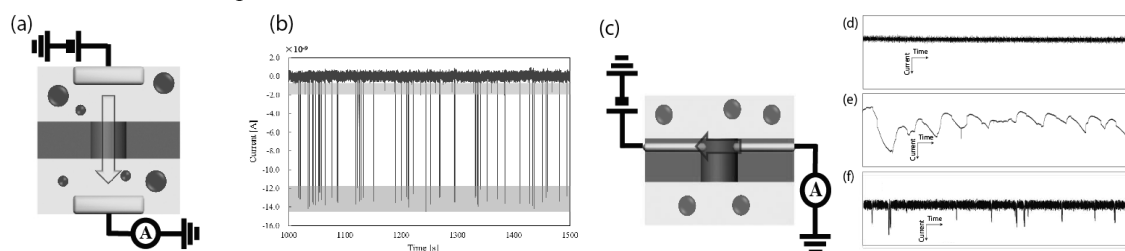

FIG. 3 Electrical Measurements of Gating nanopore for sample particles (a) Schematics of the measurement with nanopore parallel Ag/AgCl electrodes. (b) I-t profiles. The signals is 10, and 4 um PS particle with 20um GN. The signal intensity is found to be approximately in proportion to the particle volume. (c) Schematics of the measurement between gap-electrode perpendicular to a nanopore. (d) Electrical Measurements of 10um Gating nanopore for PS particles with gold gap-electrodes under 1xPBS aqueous solution containing 1mM K₄[Fe(CN)₆] / K₃[Fe(CN)₆]. The negative-current-signals represents the particle translocation of the gap-electrode.

Next, we measured the current-time profiles between the gold gap-electrodes of the gating nanopore (**FIG.3c**). In the absence of the sample particle inside the gap electrodes, a steady-stable ionic current was observed between the gap-electrodes because some kinds of ions in the solution were reacted at the exposed gap-electrodes. The steady-flow current is called as a background current. In aqueous solutions containing sample PS particles, when a PS particle translocate between the gap-electrodes and/or approaches the electrodes, it is expected to be partially reduced the ionic conduction, resulting in temporally decreasing and then recovering the background current. **FIG.3d** shows a typical *I-t* profile for the 1xPBS containing 6um PS particles when the applied potential is +0.4V. The *I-t* profile shows the current change for the signal was too small to be detected in this condition. This would be mainly due to the low background current. The I-V profiles in the same solution shows the current was increased in the

range of over ± 1.5 V, but the current was almost zero in the range of under ± 1.0V. These results suggest that the low background current would be not due to the regulation of ion conduction between the gap-electrodes but due to the low chemical reaction on the electrodes surface. Next, in order to increase the background, we applied the voltage of over ± 1.5 V applied between the gap-electrode. **FIG.3e** shows a typical *I-t* profile for the 1xPBS containing 6um PS particles when the applied potential is -1.2V. The background current was increased and the negative signals were observed, but the background-current is not stable. This is due to gas-evolving reactions, i.e., oxygen, and/or hydrogen gas, under the buffered aqueous solutions.

In order to stabilize the background ionic current even under high applied voltage conditions, the $[Ru(NH_3)_6]Cl_3$ or $K_3[Fe(CN)_6]$ were used as the redox-reaction mediator at the gap-electrode surface. This is because the redox potential of $K_4[Fe(CN)_6]$ and $[Ru(NH_3)_6] Cl_3$ were found to be about +0.16 V, and −0.23 V (vs Ag/AgCl) in the aqueous solution, respectively so that the redox reaction would be easily occurred, compared to those without the redox-reaction mediators. Moreover, the redox products of these mediators were soluble so that the diffusion of the chemical reaction was not inhibited by the reaction products, while the gas-evolving reactions inhibited the redox-reaction on the surface without these mediators. **FIG.3f** shows a typical current-time profiles for the 6um PS particle in 1xPBS buffer solution containing 1mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ for redox-mediators. We found that the background current should be a steady-stable flow, and some negative-signals were observed in the I-t profiles. When the negatively charged PS particle approached the electrode, the concentration of the redox anion mediateor of $[Fe(CN)_6]$ was significantly reduced on the electrode surface, so that the negative-current signals was observed in the I-t profiles. We also found that the negative-signal intensity was related to the sample size. For example, the average of the signal-change for 8um PS is larger than the 6um PS particle signals. This result suggested that, when the size of particle is increased, the distance between particle and the electrodes decreased so that the mediator redox reaction became inhibited. In addition to this size effect on the signals, we also found that the pH of the sample solution also have effect on the current-intensity. For example, the averaged signal-change for 6um PS in pH 6.9 was found to be larger than that in pH 1.2 in 1xPBS buffer solution containing 1mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ for redox-mediators. The ζ-potentials measurements for the 6um PS particles in pH 6.9, and pH 1.2 were found to be -38 mV, and -12.5 mV, respectively. Since the negatively charged sample particles were naturally repulsive to the anion redox mediator, the difference in the surface charge of the sample particle might induced the difference in the concentration of the redox mediator around the gap-electrode, resulting in the difference in the signal intensities between the 6um PS particles in pH 6.9 and pH 1.2.

CONCLUSION

We fabricated a gating nanopore nanostructure, which is composed of a solid-state nanopore and embodied nanogap-electrode inside the nanopore by using a simple fabrication method developed here. By using the gating nanopore, we detected the translocation phenomena of single-particles/biomolecules through a nanopore by the change in electric current flowing between Ag/AgCl electrodes paralled to a nanopore and between gap-electrodes. We found that the change in the current between the Ag/AgCl electrodes is in proportion to the volume of the sample. We also found that the change in ion-current flowing between gap-electrodes was related not only to the particle size and surface charge. The gating nanopore-based electrical measurement potentially serves to obtain several kinds of the characteristic properties for analytical targets so that it would be promising for high-throughput single-biomolecules single-molecule sensors.

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