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Solid-State Nanopore System for Label-free DNA Sequencing

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Abstract

This paper summarizes the latest developments regarding solid-state nanopores fabricated in our laboratory. For direct DNA sequencing with solid-state nanopores, a simple method based on a voltage pulse injection has been developed to precisely fabricate nanopores with diameters of 1 to 3 nm. By measuring DNA translocation events through the fabricated nanopores, it was confirmed that single-stranded DNA (ssDNA) can pass through the nanopores with diameters as small as 1.2 nm. In addition, it was discovered that the translocation speed of ssDNA is higher than that for double-stranded DNA (dsDNA) by three of four orders of magnitude.

1. Introduction

A nanopore-based DNA sequencer is promising technology to achieve high-throughput, long-read DNA sequencing at comparatively low cost thanks to its direct, reagent-less, amplification-less sequencing capability[1]-[4]. A nanopore is a nanometer-scale hole formed with either biological molecules[1][2] or solid-state materials[1][3]. It is utilized as an ultra high-sensitivity sensor for biological applications[1]. Advantages of a solid-state nanopore (i.e. nanopore formed with the solid-state material) over biological one are its robustness and capability of large-scale integration thanks to semiconductor fabrication technology[3].

Fig.1 shows an overview of a solid-state nanopore DNA sequencer. A several-nm (typically 5 nm to 10 nm) thin silicon-nitride membrane separates a chamber into upper (*trans*) and lower (*cis*) chambers. Both chambers are filled with electrolyte like a KCl solution. Ag/AgCl electrodes in both chambers apply electric fields near the membrane, and a single-stranded DNA sample translocates through the nanopore from *trans* to *cis* chamber. Here, the ionic current through the nanopore ("blockade current"; shown as I_{BKD}) varies depending on the type of nucleotide located in the nanopore, as shown in Fig.1 (b). We can determine the sequence of the DNA according to the variation of the blockade current. To realize this concept with solid-state nanopores, we believe that there are four key issues to be resolved: (1) fabricating a small nanopore with a diameter of the same order as that of DNA, (2) fabricating an ultrathin membrane, (3) decreasing the DNA translocation speed through the nanopore, and (4) reducing electrical noise during the measurement. In this paper, we describe a method for fabricating small nanopores and measurement results of ionic currents obtained with the nanopores.

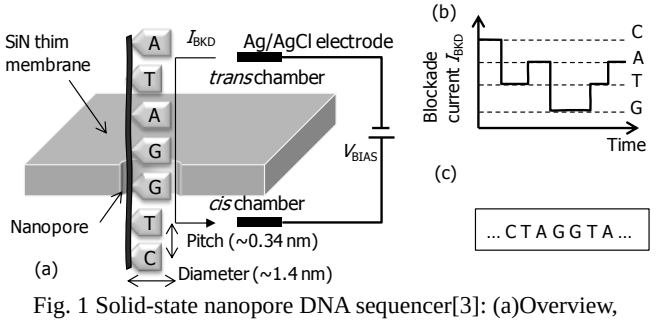

Fig. 1 Solid-state nanopore DNA sequencer[3]: (a)Overview, (b)blockade current and (c) decoded sequence of target DNA.

2. Multilevel-Pulse-Voltage Injection method

To achieve DNA sequencing with nanopores, it is necessary to fabricate nanopores with diameters of approximately 2 nm in a membrane. This requirement cannot be satisfied by current lithography technology. Therefore, we have developed a technique called Multilevel Pulse-Voltage Injection (MPVI) to precisely fabricate small nanopores [3][4]. The nanopore-fabrication mechanism utilized in MPVI is based on the dielectric breakdown induced by a high electrical-field stress. Fig. 2 shows a brief introduction of MPVI scheme. It consists of two phases: pore generation and pore enlargement. During the pore generation phase, the V_{BIAS} is periodically driven to high voltage V_{P1} to generate pore by an electrical breakdown. Then, V_{BIAS} is driven to lower voltage $V_{R,}$ and ionic current is measured and compared to predefined threshold level (I_{TH1}) to check if a pore is generated or not. Note that each pulse width can be fixed or dynamically increased from 1 ms to around 1 s for faster pore generation. Once a pore is generated, V_{P2} is applied to V_{BIAS} to enlarge the diameter of pore. Similar to the pore generation process, pulse is applied periodically until the diam-

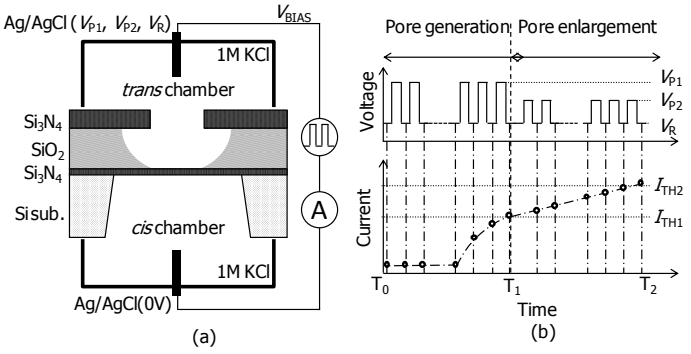

Fig. 2 Schematic diagram of MPVI scheme: (a) setup and (b) pulse chart

eter of the pore reached to desired level.

To demonstrate nanopore generation, we applied MPVI to 10-nm-thick SiN membranes. As shown in Fig. 3(a), the pore generation could be detected very clearly according to the sudden increase in the current. The ionic current increases as the cumulative time (t_{sum}) of the applied-pulse (V_{PI} and V_{P2}) increases. Here, V_{P1}, V_{P2} were set to 7 V and 2.5 V, respectively, and current at each point is measured at V_R = 0.1 V. As shown in Fig.3 (a), the diameters of the generated nanopores (φ_{NP}) were less than 1 nm, and its probability is 90% (Fig.3 (b)). The time required for nanopore generation is within 0.1-10 s, which demonstrates that MPVI enables rapid nanopore formation. After the nanopore was generated, its diameter was enlarged to the intended size with sum-nanometer precision.

Fig. 4 (a) shows an entire image of the membrane after MPVI. It confirms that only one nanopore was fabricated in the membrane. Fig. 4 (b) is TEM images of nanopores with diameters from 1 nm to 3 nm obtained by MPVI.

3. DNA translocation through nanopore

After the nanopore fabrication, ionic current through the nanopore was measured with DNA sample (1 nM 5.3-kb ss-poly(dA)). As shown in Fig. 5, each histogram of ionic current blockades (ΔI) contains a discriminative peak (i.e., ΔI_P, calculated from Gaussian fits to each histogram), indicating ssDNA translocations through the nanopore. The peak values reflect the size of the ssDNA.

Fig. 6 shows dwell time/nucleotide for 5.3-kb ssDNA and 1-kbp dsDNA in several nanopores with different diameters at V_R = 0.1 or 0.3 V. MD simulation results are also shown. As shown in Fig. 6, dwell time/nucleotide of ssDNA is shorter than that of dsDNA by three or four orders of magnitude. The result implies difficulty in sequencing ssDNA by solid-state nanopores and translocation control of DNA is desirable[5].

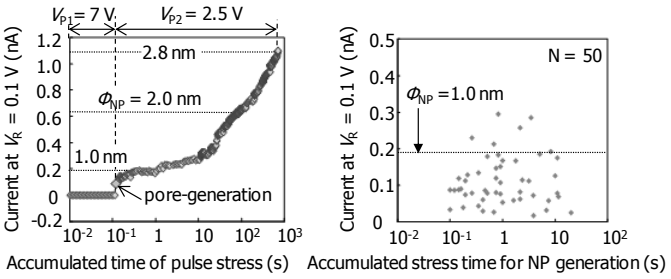

Fig. 3 Time-dependent characteristics of current I during MPVI: (a) I vs cumulative time of applied-pulse durations (t_{sum}), (b) scatter plot of I at V_R = 0.1 V at the nanopore-generation point.

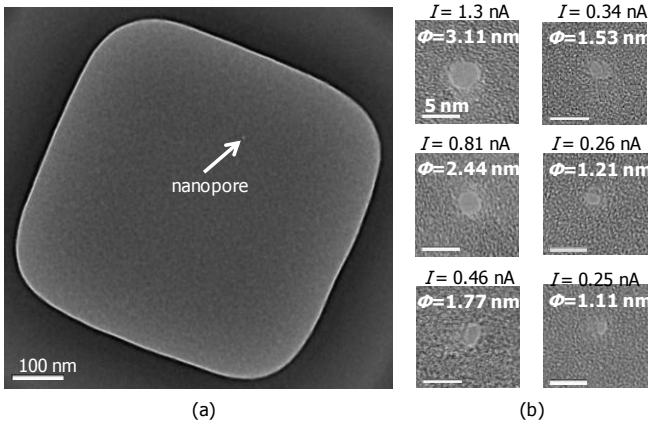

Fig. 4 (a)A single nanopore fabricated on membrane and (b) nanopores with various diameters and currents measured at 100 mV.

4. Conclusions

We have developed the MPVI technique to precisely fabricate small nanopores. Experimental results show that MPVI can be utilized to fabricate nanopores with diameters of 1 nm to 3 nm at sub-nanometer precision.

References

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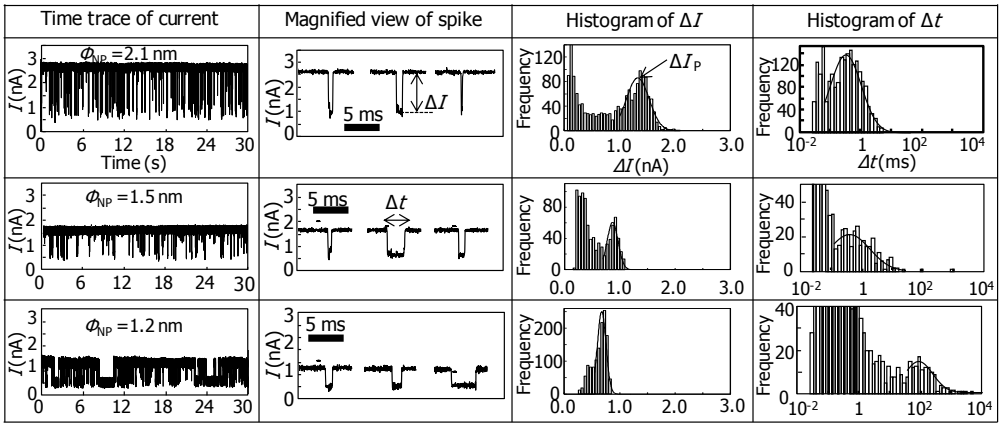

Fig. 5 ssDNA translocations thorough nanopores with different diameters: time traces of ionic currents, magnified views of typical current blockades, histograms of height(ΔI) and dwell time(Δt) of each blockade.

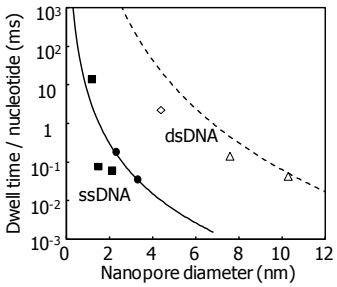

Fig. 6 Measured and simulated dwell time vs nanopore diameter. Data points represent experimental results. Solid and dashed lines are obtained by MD simulation.