Solid-state nanopores: a new tool for biomedical diagnostics

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ABSTRACT

In this work, we present a novel electrode/nanopore architecture that can be used to characterize ion and DNA transport under the influence of a local alternating electric field. The characteristic of the ion passing through the micrometer-sized pore was investigated at the different KCl concentrations by measuring the pore conductance.

Keywords: solid-state nanopore, single molecular detection, ionic translocation, DNA sequencing, electrodes

1 INTRODUCTION

In recent years, solid-state nanopores have been proposed as a novel tool for single-molecule detection of DNA, RNA and other biomolecules in solution. In particular, prospects of ultra-fast DNA fragment sizing or sequencing have fueled considerable commercial and academic interest, the idea being that the composition of a translocating biopolymer could be detected in real-time and extremely rapidly as the (linear) polymer chain travels through the nanopore. [1] For DNA sequencing applications, single-base resolution would be crucial. Although significant progress in this direction has been made, the full implementation of this approach has not been achieved yet. One of the major obstacles is related to controlling the speed of the translocation process and the interaction between the DNA with the surface of the nanopore. Therefore, the physical mechanisms underlying the translocation process and the factors controlling the reproducibility and specificity of the translocation event require further study. [2, 3]

Sigalov G. *et al.* suggested that the application of an AC field at or close to the nanopore can induce back-and-forth motion of the DNA inside the nanopore. According to their simulations, this process exhibits specific-sequence hysteresis, potentially resulting in detectable changes in the translocation characteristics.[4] This could improve the detection and sensing capabilities of solid-state nanopores considerably.

In this work, we present initial results from the experimental implementation of such an electrode/nanopore architecture with the aim of characterizing ion and DNA transport under the influence of a local alternating electric field. This includes the fabrication of nanopore/electrode devices, their incorporation into microfluidic structures, and initial characterization of these devices at different modulation frequency and amplitude of the applied AC field.

2 METHODOLOGY

2.1 Fabrication of the pore / electrode devices

Nanopore/electrode devices are fabricated by conventional semiconductor processing technology on a 4" wafer substrate. 100nm low-stress Si_3N_4 film is deposited via LPCVD on Si <100> wafers. Electrodes are lithographically patterned and metalized by thermal evaporation of 30 nm thick Au film with 5 nm Cr adhesion layer on one face of the wafer. The other face of the wafer is subsequently processed by optical lithography, reactive ion etching, and wet etching to fabricate 100 nm thin Si_3N_4 membrane windows aligned to the Au electrodes. The fabrication is carried out on a wafer allowing for the production of approximately 250 devices with a size of 5 mm x 5 mm. A typical device is shown in Figure 1A.

Focused ion beam (FIB) is then used to mill simultaneously a nanopore through the Si_3N_4 membrane and the Au electrode. Under typical experimental conditions Ga^+ ions are accelerated by potential of 30 kV, at beam current of 1 pA. Beam exposures between 1 s – 10 s results in nanopores with diameters in the order of 20 nm – 100 nm (Figure 1B).

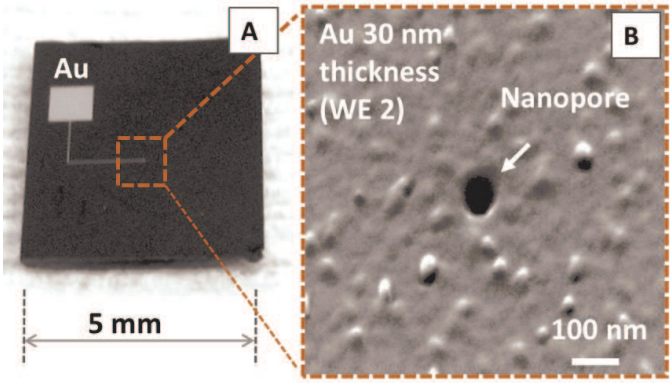

Figure 1: [A] A typical 5 mm x 5 mm chip with an Au electrode. [B] A blow-up of the Au electrode surface, showing a nanopore fabricated by FIB

2.2 Electrochemical Cell and Bipotentiostatic Setup

The nanopore/electrode devices are mounted in a flow cell consisting of two compartments made from poly(dimethylsiloxane)/glass. These two compartments are filled with an electrolyte solution (potassium chloride) and connected only via the nanopore.

Two Ag/AgCl electrodes were placed in the top and bottom reservoir respectively, Figure 2. The Ag/AgCl electrodes were used as working electrode one (WE1) and combined Counter/Reference electrode (CE/RE). A bias voltage was applied between them to drive the transport of ions through the nanopore. The gold micrometer-size electrode on the membrane was used as working electrode 2 (WE2); its potential is defined relative to CE/RE. The Au electrode is always placed in the same compartment with the CE/RE.

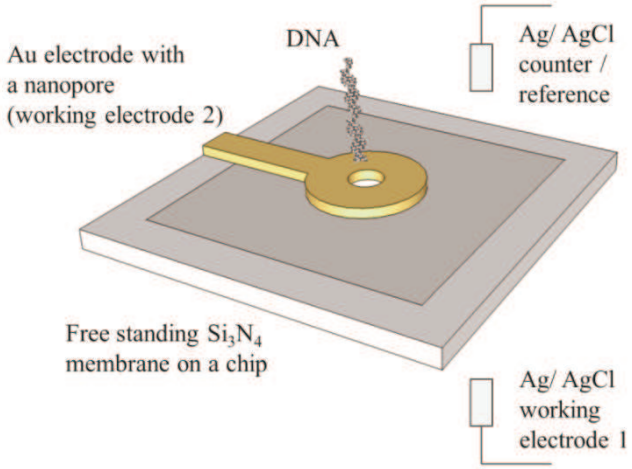

Figure 2: Schematic of the device structure. Microfluidic system, solvent, and ions are not shown for clarity. The Si_3N_4 membrane separates two reservoirs, DNA translocates through a nanometer-sized pore in the center of the Au electrode (WE2).

2.3 Cyclic Voltammetry

An electrochemical cell was filled with KCl solution. The ionic current through the nanopore was detected with a CHI 760c bipotentiostat (CH Instruments, Austin, USA) at different salt concentrations of 1 mM, 10 mM and 100 mM. During these experiments, a constant bias voltage between -0.5 V and 0.5 V (vs. CE/RE) was applied to WE2. The Figure 3 shows the voltammetry responses of KCl solution at 100 mV/s scan rate.

2.4 Chronoamperometry

The chronoamperometry experiments were performed to investigate the ionic current of KCl while a fixed potential was applied to both working electrodes. These experiments were conducted to compare the current with the values provided by the cyclic voltammetry. In this technique, the potential applied to WE1 was altered in the range of ±0.01 V, ±0.02 V, ±0.05 V, ±0.1 and ±0.2 V while the potential of WE2 was kept constant at 0 V. Then, the experiment was reproduced by changing the WE2 potential to ±0.10 V and ±0.20 V respectively.

3 RESULT AND DISCUSSION

Initial experiments in the current project were carried out on micrometer-sized pore. The ionic current passing through the micropore was measured by cyclic voltammetry for four different KCl concentrations, Figure 3. It showed reproducible ohmic (linear) behavior at any potential applied to the WE2, Figure 3 for E(WE2) = +0.1 V.

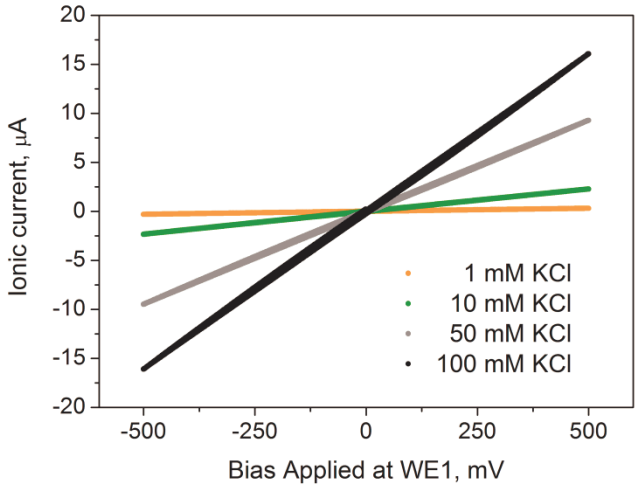

Figure 3: Cyclic voltammetry of ionic current passing through the pore at different bias voltage applied to WE2.

The pore conductance was determined from the derivative of I-V curves and displayed in Figure 4. The conductance varies from 0.6 µS to 32 µS for a variation in KCl concentration between 1 and 100 mM reflecting approximately linear relationship for all the potentials applied to WE2. For every KCl concentration, there is an increase in conductance passing from zero bias to either positive or negative potentials and this variation is steeper at low bias and tends to reach a plateau for potentials of hundreds of mV.

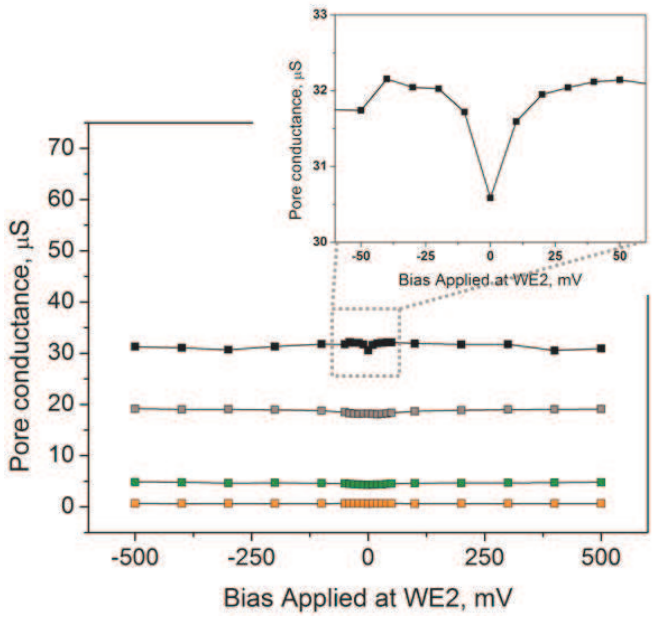

Figure 4: Pore conductance versus bias voltage applied to the WE2

4 CONCLUSIONS AND FUTURE WORK

We characterized the electrical behavior of a micrometer pore/electrode structure measuring the conductance of the pore by cyclic voltammetry and chronoamperometry (data not shown for the second experiment). We found conductance dependence from the potential applied to the electrode at the pore (WE2). This dependence has still to be quantified in terms of pore size and potential modulation at WE2. For this reason in future work we will investigate nanometer pores and AC modulation for WE2. The final experiment will involve DNA translocation both in the presence and absence of AC modulation.

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