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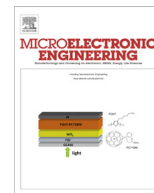

Controlled sealing of nanopores using an easily fabricated silicon platform combining *in situ* optical and electrical monitoring

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ABSTRACT

Nanopores are of great interest for biosensing applications. However, using such sensing platforms requires long and costly fabrication processes and the implementation of simultaneous electrical recording and optical imaging is not straightforward. Here, we present a new approach for silicon nanopore fabrication and integration avoiding backside etching of the substrate and allowing direct microfluidics integration for electrical recording and optical imaging. We demonstrate an electrical and optical live *in situ* detection of the electrophoretic trapping of polystyrene microspheres on silicon nanopores. This trapping procedure could also be used to partially close already made nanopores, in order to increase their sensitivity to small analytes.

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1. Introduction

Solid-state nanopores are promising tools for molecular biosensing. Recent advances in nanoprocessing enabled the fabrication of nanopores with diameters of a few nanometers, which are comparable in size to biological macromolecules and allow direct detection by measuring the variation of an applied ionic current through the pore [1–4]. Indeed, when filled with an aqueous electrolyte, nanopores behave as a variable resistance that increases when a macromolecule comes in the vicinity or inside the pore. In the literature, many studies have been dedicated to DNA sequencing and single molecule detection, with devices made of two electrolyte-filled chambers separated by a membrane in which one or several nanopores have been fabricated [3].

In order to discriminate different DNA nucleic acid molecules or to achieve a high sensitivity in the detection of macromolecules, it is necessary to control and generally reduce the dimensions of the pore down to few nanometers and very promising solutions have been developed in the last few years [5,6]. However, to date most of these methods are not integrated and directly upscalable.

Among the different accessible technologies to produce nanopores, silicon based platforms have three main advantages: the maturity of silicon industry for upscaling, the ability to precisely control nanopore's shape and position with silicon nanopatterning technologies and finally the wide variety of available silicon oxide chemical functionalizations. However, today's silicon nanopore sensing platforms lack simplicity in their fabrication and implementation. First, nanopores with diameters of less than 10 nm involve critical fabrication step as, for example, focused ion beam. Moreover, a backside etching of the wafer is usually performed, with a necessary alignment of the back and front sides patterns.

Then, since nanopores are fabricated across the substrate, performing simultaneous electrical and optical monitoring requires the development of an appropriate setup. Integration of this setup with polydimethylsiloxane (PDMS) has previously been described for thin polymer nanopore membranes [7,8], but these methods are not relevant to thick substrates such as silicon. In another study, simultaneous electrical and optical recordings have been performed on silicon platform using a mechanically engineered cell made of chlorotrifluoroethylene (CTFE) [9] but, to our knowledge, there is no straightforward integrated system allowing simultaneous optical and electrical monitoring with minimum handling for the user.

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Here, we describe a new approach in silicon nanopore technology, which consists of a line of nanopores positioned above a buried channel and that is easier to implement. The fabrication of the nanoholed substrate does not require a backside etching, advantageously limiting the process fabrication to four steps. The front side of the silicon substrate using an integrated PDMS microfluidic chip enables electrical, chemical and optical access to both sides of the pores. This system (Fig. 1) will allow easy handling of the silicon nanopore platform for biomedical applications requiring simultaneous optical and electrical characterization.

To demonstrate ability to perform electrical recording as well as optical imaging, we used our platform to trap 500 nm carboxylate-modified polystyrene fluorescent microparticles on the nanopore line by applying an electrical field through the pores. The trapping was electrically and optically monitored. This trapping could also be used as a method to reduce the diameter of already made nanopores, in order to increase the detection sensitivity without using critical nanopatterning process such as focused ion beam.

2. Material and methods

2.1. Sample fabrication

The silicon chips were fabricated using a method derived from previous works [10,11]: a line of circular nanopores (200 nm diameter, 1 μm pitch, 8 mm length) was patterned on the top silicon layer of an SOI substrate (Si: 205 nm, SiO_2: 200 nm, Si: 650 μm) (Soitec, France) by electrical lithography (Raith 150, Germany) using conventional ebeam lithography and reactive ion etching. The buried oxide (BOX) below the nanopore line was then etched through the nanopores by a 50% vol/vol hydrofluoric acid solution during 1 min and 30 s. Consequently, a buried channel, linking all the nanopores together under the top silicon membrane was created. In order to make an insulating substrate, an 80 nm thermal oxide was then thermally grown to prevent any leakage current (we measured a leakage current of less than 1 nA) through the silicon membrane. The size of the pores was reduced to 130 nm by the oxidation process and the 200 nm-high buried channel was also reduced to 100 nm (see Supplementary data).

2.2. Microfluidic chip preparation

The molds for polydimethylsiloxane (PDMS) reticulation were fabricated using conventional SU-8 resist on silicon process. The thickness of the SU-8 coating was 50 μm. An anti-adhesive monolayer of perfluorodecyltrichlorosilane (FDTS) was then deposited by plasma enhanced chemical vapor deposition (PECVD) to allow the demolding of PDMS.

PDMS solution (Sylgard 184, Dow Corning), which is a mixture of PDMS oligomers and curing agent (10:1 mass ratio), was degased under vacuum during 30 min, poured on the SU-8 master and finally cured at 100 °C for 1 h. Reticulated microfluidic chip was then removed from the master and through-holes chambers were created at the end of each microchannel with a punch. Finally PDMS microfluidic chip was activated (2 min, 60 W O_2 plasma) and bond to previously activated SOI chips (5 min, 400 W O_2 plasma). The bonding was reinforced by heating the chips at 120 °C for 1 h (Fig. 1).

2.3. Electrodes, electrolyte and particles

Phosphate buffered saline (PBS, 1×, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, 2 mM KH_2PO_4) was filtered through a 0.2 μm filter. Silver/silver chloride (Ag/AgCl) electrodes (0.8 mm diameter, Phymep, France) were used. Polystyrene yellow-green fluorescent FluoSpheres® beads (500 nm, carboxylate-modified, Molecular Probes, life technologies) were suspended at a concentration of 3. 10^9 beads/mL in PBS 1×.

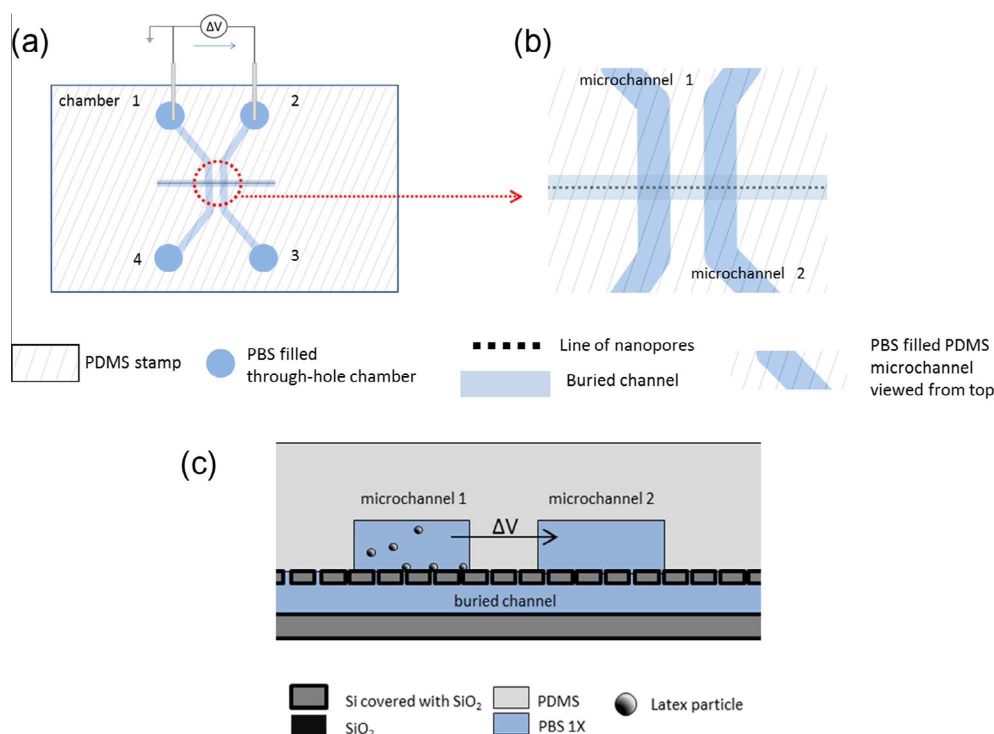

Fig. 1. Schematic illustrations of the platform for simultaneous optical and electrical monitoring of trapping of microparticles. (a) Schematic view of the resulting structure after microfluidic chip PDMS bonding on the SOI chip. (b) Zoom on the schematic view. (c) Schematic cross-section of the device showing the two PDMS microchannels, the line of nanopores and the buried channel.

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