Nanochannel system fabricated by MEMS microfabrication and atomic force microscopy

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Abstract: A silicon nanochannel system with integrated transverse electrodes was designed and fabricated by combining micro-electro-mechanical systems (MEMS) micromachining and atomic force microscopy (AFM)-based nanolithography. The fabrication process began with the patterning of microscale reservoirs and electrodes on an oxidised silicon chip using conventional MEMS techniques. A nanochannel, approximately 30 µm long with a small semi-circular cross-sectional area of 20 nm × 200 nm, was then mechanically machined on the oxide surface between the micro reservoirs by applying AFM nanolithography with an all-diamond probe. Anodic bonding was used to seal off the nanochannel with a matching Pyrex cover. Continuous flow in the nanochannel was verified by pressurising a solution of fluorescein isothiocyanate in ethanol through the nanochannel in a vacuum chamber. It was further demonstrated by translocating negatively charged nanobeads (diameter ~20 nm) through the nanochannel by using an external DC electric field. The passage of the nanobeads caused a sharp increase in the transverse electrical conductivity of the nanochannel.

1 Introduction

Nanopores have shown tremendous promises in significantly lowering the cost of genome sequencing [1]. Among the current nanopore designs, the solid-state nanopores in particular can achieve a pore size similar to that of a single nucleotide, which is critical in reaching single-base accuracy [2]. A major obstacle in the present nanopore technique is that the speed with which the DNA chain is translocated through the nanopore is too high for single-base readout. A potential solution for reducing the translocation speed and increasing the readout resolution is by extending the length of the nanopores and thus converting them into nanochannels so that a large drag force is imposed on the translocating DNA [3].

In the field of nanofluidics, the design and fabrication of nanochannels is not a standardised technique [4]. Current methods for realising nanochannels include bulk nanomachining [5, 6], surface nanomachining [7], the buried channel technique [8] and nanoimprint lithography [9, 10]. Nanochannels with cross-sectional dimensions in the range of 50 nm × 5 µm (depth and width) [5, 6], 20–100 nm × 0.5–20 µm [7] and 10 nm × 50 nm [9] have all been previously demonstrated. Among the fabrication techniques used, photolithography-based methods such as bulk and surface nanomachining are capable of generating one-dimensional (1D) nanochannels (only channel depth in the nm scale). Direct-write methods (e-beam and focused ion beam) and nanoimprint, by comparison, can realise 2D nanochannels (both depth and width in the nm scale), which were successfully applied to DNA translocation and analysis [10, 11].

Atomic force microscopy (AFM) nanolithography is a direct-write nanomachining technique capable of fabricating 2D nanochannels. Originally, designed as a high-precision surface profiler, AFM has been widely used to study material properties in material science, biomedicine and more recently nanofabrication [12]. In AFM nanolithography, a sharp probe tip with a large spring constant mechanically machines a surface by removing the substrate material with high precision. This technique has been previously demonstrated on a wide range of substrate materials including polymers [13–16], metals [17] and semiconductors [18, 19]. However, not all substrate materials are suitable for nanochannel fabrication through AFM. For example, ductile materials such as polymers will not be ideal since significant material pileups can occur along the nanochannel during the machining process, which can lead to channel collapse during the subsequent channel capping process.

Semiconducting materials such as Si and SiO₂ exhibit good mechanical properties for AFM nanolithography. Nanopatterning on SiO₂/Si surfaces can be achieved by transferring AFM-machined photoresist through wet chemical etching [20]. However, channel depth control presents a significant challenge in this approach. In the present paper, nanochannels are realised on SiO₂ surface through direct AFM nanomachining. Prior works on AFM
nаномачинане и SiO₂ have explored the relationship between the dimensions of the machined channels and the control parameters of the AFM for both single and multiple machining passes [21–23]. The present paper describes the design and fabrication of a silicon nanochannel system with integrated transverse electrodes by combining MEMS micromachining and AFM nanolithography. In this approach, conventional photolithography techniques were first used to fabricate a silicon chip consisting of micro inlet/outlet reservoirs and parallel Pt/Ti microelectrodes. AFM nanolithography was then applied to machine a 30-μm-long nanochannel between the reservoirs. During this process, the parallel electrodes that lay along the machining path are dissected into electrode pairs along the length of the nanochannel, which can be used to probe the electrical property of the nanochannel in the transverse direction. A transparent Pyrex cover caps off the nanochannel through anodic bonding. Fluid and particle flow in the nanochannel system is characterised optically through the transparent cover and electrically through the integrated electrodes.

2 Fabrication of nanochannel system

2.1 Design and fabrication of silicon chip

Fig. 1 shows an enlarged picture of the silicon chip fabricated by MEMS process. Both the micro reservoirs and channels are 20 μm deep, which is designed to prevent the Pyrex cover from adhering to the reservoir bottom during the anodic bonding process. The reservoir diameter is 4 mm. The Pt/Ti electrodes are 40 nm thick, which is also designed to accommodate anodic bonding. Previous experience has indicated this electrode thickness to be ideal in terms of guaranteeing a close bond around the electrodes. The electrodes reside on top of a 500-nm-thick silicon oxide layer. High-temperature Pt/Ti is selected as the electrode material owing to processing difficulties arising from the behaviour of low-melting-point metals. In an early chip design, Au electrodes were adopted and subsequent testing indicated that the resistance of the electrodes tends to rise significantly because of the high temperature used in the bonding process. The resistance of the Pt/Ti electrodes was shown to be significantly more stable. The length of the microchannels in the silicon chip is designed to be asymmetrical; whereas the upstream microchannel is 20 mm long, the downstream microchannel is only a few mm long. Such design has two advantages: (i) it allows the microscope objective to reach the chip surface for fluorescent microscopy, and (ii) it permits easy backing filling of the downstream microchannel during flow testing.

Fig. 2 is a close-up picture of the location on the silicon chip where the nanochannel is machined. The dashed line in this figure indicates the path of the nanochannel. Five 1-μm-wide parallel electrodes cross the path of the nanochannel. As the nanochannel is machined, each electrode is dissected into two matching electrodes bordering the outline of the channel. With these dissected electrodes, it becomes possible to measure the transverse electrical conductivity of the nanochannel at five distinct locations in the longitudinal direction. When needed, external bridge-type circuits can be added to the system to monitor the instantaneous conductivity of the nanochannel as a way to track the movement of a nanoscale particle inside the channel.

2.2 AFM machining of nanochannel

The nanochannel on the silicon chip was mechanically machined in a dimension 3100 AFM (Veeco Inc., CA, USA) under the control of a Nanoscope IIIa controller. The AFM probe selected for the process was an all-diamond nanoindenting tip (P DNISP from Veeco) with a calibrated spring constant of 215 N/m and a nominal tip radius of 40 nm. Fig. 3 demonstrates the basic layout of the AFM machining method. In the machining process, the AFM tip is pressed against the silicon chip surface with a constant force achieved by keeping the vertical deflection of the position sensitivity detector constant. The nanochannel is realised by translating the tip along a pre-planned path at a fixed tip speed.

Prior to system fabrication, a calibration study was carried out to relate the control parameters of the AFM to the resulting dimensions of the nanochannel. Fig. 4 demonstrates typical nanochannels machined on a flat SiO₂ substrate at different normal force ranging from 25.26 to 151.57 μN and different cutting speed from 0.1 to 1.0 μm/s. Data analysis indicates that the nanochannel dimensions are not strongly correlated with the tip speed, as previously described in our earlier study [24].

The relationship between the average nanochannel depth and the normal force is depicted in Fig. 5. The average
depth is determined based on the results of 20 evenly spaced locations along each fabricated nanochannel shown in Fig. 4. When the nanochannel depth is much smaller than the radius of the AFM probe tip, the relationship between the normal force and depth is mostly linear, as demonstrated in Fig. 5. In repeated machining, each normal force corresponds to a unique horizontal projected area of the interface. In Fig. 6, the interface between the AFM tip and the initial nanochannel in repeated machining is shown. By computing the area of the interface, the relationship between the final nanochannel depth, the initial nanochannel depth and the normal force can be determined. If \( D \) is the first nanochannel depth \( D_0 \) is the initial nanochannel depth and \( D_1 \) is the final nanochannel depth, the relationship between \( D, D_0 \) and \( D_1 \) can be defined as

\[
D_1 = kD_0
\]

(1)

\[
D_0 = k_0D
\]

(2)

where \( k \) is proportionality constant between the final nanochannel depth and the initial nanochannel depth, and \( k_0 \) proportionality constant between the initial nanochannel depth and the first nanochannel depth.

Fig. 7 shows the variation of \( k \) with respect to \( 1/k_0 \), which was previously described in [23]. The exact equation can be written as

\[
k = -0.24 \left( \frac{1}{k_0} \right)^3 + 0.74 \left( \frac{1}{k_0} \right)^2 + 0.012 \frac{1}{k_0} + 1
\]

(3)
Based on this equation and the relationship between the initial nanochannel depth and the corresponding normal force [22], to achieve a total nanochannel depth of 20 nm on SiO₂ surface using repeated AFM machining, the normal force applied is about 200 µN and the number of machining runs required is 2.

Based on the result of the calibration study, the nanochannel on the silicon chip was machined by using a normal force of 200 µN and a tip speed of 1 µm/s. The process was repeated twice along the same machining path. The resultant nanochannel is demonstrated in Fig. 8. Using the surface scanning function of the AFM, the nanochannel is determined to be about 20 nm deep and 200 nm wide. It is 30-µm long, which corresponds to the distance between the inlet and outlet microchannels. As previously described, the nanochannel cuts across the parallel electrodes and dissect them into matching electrode pairs along the length of the channel.

2.3 Anodic bonding

Following AFM nanomachining, the silicon chip was sealed off by a matching Pyrex cover through anodic bonding. In this process, the 500-µm-thick silicon chip was placed on a hot plate and linked to the anode of a voltage-adjustable direct current supply. The Pyrex cover (0.5-mm thick) with pre-drilled through holes was placed on top of the silicon chip and linked to the cathode of the current supply. The hot plate was maintained at a temperature of 550°C. At this temperature, the surface temperature of the silicon chip was measured as 420°C by an infrared radiation thermometer. The anodic bonding process was performed at a voltage of 600 V. The current supply showed a current level between 0.2 and 0.4 mA at the beginning of the process. After about 20 min, the current dropped to about 0.01 mA at which point the bonding process was terminated. A separate study indicated that a satisfactory bonding could be achieved at 420°C and 600 V for a silicon chip with a 500-nm thick silicon oxide layer. The capped-off silicon chip was fitted with a microfluidic connector to complete the nanochannel system fabrication process (Fig. 9).

3 Flow tests

Prior to flow testing, the wettability characteristics of the nanochannel were enhanced by soaking the nanochannel sequentially in acetone, methanol and isopropanol alcohol (IPA) in a vacuum chamber [25]. Each soaking step lasted about 40 min. These soaking steps are necessary since deionised water has a larger viscosity and a lower volatility than acetone and methanol. Once the nanochannel was treated, two different tests were conducted to demonstrate through flows in the channel. In the first test, a solution of fluorescein isothiocyanate (FITC) in ethanol was pumped through the channel by using a combination of syringe pump and vacuum chamber. The test began with the injection of 20 µl FITC ethanol solution into the inlet reservoir. Next, the inlet reservoir was sealed off and the nanochannel system was placed in a vacuum chamber for a duration of about 12 h. Afterwards, the nanochannel system was removed from the vacuum chamber and inspected by a fluorescent microscope.

As demonstrated in Fig. 10, both the inlet and the outlet microchannels of the silicon chip are filled with the injected FITC ethanol solution. Although the fluorescent image in the figure fails to illuminate the nanochannel, this result indirectly indicates that the solution has successfully passed through the AFM machined nanochannel. The fact that the boundary of the microchannels is well defined in the fluorescent image further demonstrates that there is no leak around the micro- and nanochannels.

In the second test, 20-nm carboxyl-modified nanobeads (FluoSpheres® from Invitrogen) were translocated through the nanochannel by using an externally applied DC electric field. Fig. 11 demonstrates the experimental setup. Since the FluoSpheres® are negatively charged, a positive voltage at the outlet reservoir, if high enough, tends to pull the
nanobeads from the negatively biased inlet to the outlet through the nanochannel. Once initiated, this nanobead flow can be monitored by the transverse electrical current across the electrode pairs positioned along the nanochannel. The electrical connections in Fig. 11 show the current measurement of one electrode pair by a System SourceMeter.

As in the first test, the nanochannel was pre-soaked sequentially in acetone, methanol and IPA prior to the nanobead experiment. For the experiment, 20 μl of the FluoSpheres® suspension was first injected into the inlet reservoir of the nanochannel system. Then the inlet reservoir was sealed off and the nanochannel system was placed in a vacuum chamber for 12 h. Fig. 12a demonstrates the filling of the upstream microchannel by the FluoSpheres® suspension at the end of the vacuum chamber step. After the nanochannel system was retrieved from the chamber, the outlet reservoir was filled with a 0.01-M phosphate-buffered saline. The nanobead translocation experiment then began with the application of a 10-VDC bias between the upstream and downstream reservoirs. Fig. 12b demonstrates the result after a 3 min delay. The fluorescent image clearly indicates that the upstream FluoSpheres® have been successfully translocated through the nanochannel into the downstream reservoir by the voltage bias. Together with the result from the first test, it can be concluded that the nanochannel in the silicon chip is a through channel, capable of transporting both fluids and nanobeads from the upstream to the downstream reservoirs.

Fig. 13 demonstrates the transverse electrical current measured by one electrode pair on the nanochannel during the nanobead translocation experiment. A voltage of 5 VDC was supplied by the System SourceMeter to the electrodes for the measurement. As indicated by Zone I in this figure, without a positive voltage at the outlet reservoir to initiate the flow of FluoSpheres®, the transverse current across the nanochannel is extremely low, indicating a high electrical resistance. When a positive voltage is applied (Zones II and IV), a large transverse current appears between the electrodes, indicating the flow of the conductive nanobeads significantly enhances the electrical conductivity across the nanochannel. When the voltage is removed, the electrical current reverses back to its original level, as demonstrated by the result in Zones III and V.

4 Conclusions

The present paper demonstrates a new fabrication method, which combines MEMS microfabrication and AFM nanolithography for a nanofluidic channel system with integrated transverse electrodes. Using this method, a nanochannel with a length of 30 μm, a depth of 20 nm and a width of 200 nm, was machined on an MEMS silicon chip equipped with micro reservoirs and electrodes using an all-diamond AFM tip. Anodic bonding was used to cap off the silicon chip with a matching Pyrex cover. Two different experiments were carried out to demonstrate through flows
in the nanochannel. In the first experiment, a solution of FITC in ethanol was successfully pumped through the nanochannel using a combination of syringe pump and vacuum chamber. In the second test, negatively charged nanobeads with an average diameter of 20 nm were driven through the nanochannel by applying a positive voltage to the outlet reservoir. Transverse electrical current measurements by the electrodes on the nanochannel indicate that the flow of the charged nanobeads leads to an increase in the electrical conductivity in the nanochannel. Together, the fluorescent images and the electrical conductivity experiment point to the possibility of using the nanochannel with its embedded transverse electrodes as a chip-based system to detect and quantify charged nanoparticles and perhaps biomolecules such as DNA and proteins.

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6 References