Fabrication of a Novel Nanofluidic Device Featuring ZnO Nanochannels

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ABSTRACT: We developed a novel fabrication method for nanochannels that are easily scaled up to mass production by selectively growing zinc oxide (ZnO) nanostructures and covering using a flat PDMS surface to make hollow nanochannels. Nanochannels are used in the biotechnological and environmental fields, being employed for DNA analysis and water purification, due to their unique features of capillary-induced negative pressure and an electrical double-layer overlap. However, existing nanochannel fabrication methods are complicated, costly, and not amenable to mass production. Here, we developed a novel nanochannel fabrication method. The pillar-like dense ZnO nanostructures were grown in a solution process, which is easily applicable to mass production. The size of the fabricated ZnO nanostructures has a thickness of 30–300 nm and a diameter on the order of 100 nm, which are easily adjusted by synthesis times. The ZnO nanostructures were covered by the flat polydimethylsiloxane (PDMS) surface, and then the cracks between ZnO nanostructures served as hollow nanochannels. Because the suggested fabrication process has no thermal shrinkage, the process has higher production efficiency than existing nanochannel mass production methods based on the thermal/pressure process. The mechanical strength of the fabricated ZnO nanostructures was tested with repetitive tape peeling tests. Finally, we briefly verified the nanochannel performance by applying the nanochannel to the micro/nanofluidic system, whose performance is easily evaluated and visualized by current–voltage relation.

INTRODUCTION

A nanochannel is a nanometer-sized (1–100 nm) hollow channel exhibiting unique nanofluidic phenomena, including capillary-induced negative pressure caused by the high surface-to-volume ratio and an electrical double-layer overlap. Nanochannels have been used for DNA analysis,† virus detection,‡ water purification,§ protein research,∥ assessing molecule concentration,∥∥ particle separation,∥∥∥ and environmental monitoring.∥∥∥ The current fabrication methods use conventional lithography and include reactive ion etching,∥ high-energy beam processing,∥∥ and interference lithography.∥∥∥ However, nanochannels fabricated using conventional lithography are not amenable to mass production; the high-energy beam process has relatively low production speed, and reactive ion etching and interference lithography have relatively smaller patternable areas. Therefore, conventional lithography-based nanochannel fabrication systems are hard to scale up to mass production.‡∥ To overcome these limitations, hot embossing,∥∥ thin-film deposition,∥∥∥ and nanoimprinting∥∥∥∥ have been studied; one- and two-dimensional nanochannels, as well as array-type nanochannels, can be fabricated easily at a low cost.∥∥∥∥ Although nanochannel fabrication techniques amenable to mass production have been developed, deformation caused by thermal expansion remains problematic; thermal shrinkage compromises nanochannel precision and greatly reduces production efficiency. A highly efficient fabrication technique ensuring nanochannel integrity is required.‡∥ Here, we fabricated nanochannels via the selective growth of a zinc oxide (ZnO) nanostructure, followed by soft lithography employing a mask pattern. Using hydrothermal synthesis, pillar-like dense ZnO nanostructures were fabricated on silicon and glass wafers to any height desired between 30 and 300 nm by adjusting the synthesis time, and the structures were easily patterned on the surface with conventional lithography mask patterns. The fabricated nanostructures on a glass substrate are coated by plasma bonding. The mechanical strength of the ZnO nanostructures was briefly tested with repetitive 3M tape peeling tests. The performance of the nanochannels is briefly evaluated by applying them to a micro/nanofluidic system, whose performance is easily evaluated and visualized by current–voltage relation.
RESULTS AND DISCUSSION

Figure 1 shows a schematic of ZnO nanostructure fabrication using a mask pattern, PDMS surface covering, for the formation of ZnO nanochannels and integration to micro/nanofluidic devices. The experimental setups of nanofluidic visualization and particle separation experiments are described in Figure 4. As shown in Figure 1, nanostructured patterns were fabricated by coating of a seed layer, UV pattern lithography, and hydrothermal synthesis on a bare glass substrate. In Figure 1, the patterned ZnO nanostructure was bonded to a prepatterened PDMS microchannel replica for the fabrication of nanochannels and integration to the micro/nanofluidic system at once. For evaluating the fabricated nanochannel performance, we applied an electric field to the micro/nanofluidic system to induce a common nanofluidic, electrohydrodynamic phenomenon called “ion concentration polarization (ICP)” (Figure 4A). To explore future application to biotechnology, the micro/nanofluidic device was used to continuously separate microparticles with the ICP phenomenon (Figure 4B). As shown in Figure 1, the device features two microchannels (main and buffer channels) connected by ZnO nanochannels. As shown in Figures 3B and 4A, the fabricated nanochannels are marked with red dashed rectangles between the two microchannels. Unmarked nanostructures exist only for the convenience of fabrication and have little impact on the experiments. The main channel has two inlet reservoirs (two cylinder-type reservoirs at the left side of the main channel) that are connected to a syringe pump system for applying a pressure-driven flow to the micro/nanofluidic device. The other three reservoirs (three cylinder-type reservoirs at the right side of the main channel) are exposed to air to act as outlets. The buffer channel also has two reservoirs that are connected to syringes filled with an electrolyte solution, but these syringes are locked at certain reservoir levels to prevent solution flow in the buffer channel. In the nanofluidic visualization experiment setup (Figure 4A), there is also no solution flow in the main channel, so only two reservoirs are connected to locked syringes and the other reservoirs (gray-colored reservoirs) are blocked by a commercial epoxy before injection of the electrolyte solution. The anodes are inserted at each side of the main channel reservoirs, and the cathodes are inserted at each side of the buffer channel reservoirs, as shown in Figure 4A. In the continuous particle separation experiment, all the reservoirs are used as inlets or outlets. In the main channel, two types of microparticles are continuously injected from the inlet reservoir located above. At the same time, a sheath flow is injected from the other inlet reservoir that merges in front of the nanochannel and flows to the outlet. The sheath flow pushes the particle flow, keeping the particles located at the side wall before they are spatially separated by the ICP phenomenon. Details about particle separation are explained in Figure 6.
Figure 2 shows scanning electron microscopy (SEM) images of the ZnO nanostructure pattern. As shown in Figure 2A, a linear ZnO nanostructure pattern (width, ~200 μm) was fabricated on a glass substrate, consistent with the UV lithography pattern. Figure 2B shows an enlargement of the nanostructure pattern; a flower-shaped ZnO structure was densely synthesized on the surface. As shown in Figure 2C, the height differences of the nanostructures were ~100 nm, and the cracks created between the sharp tips of the ZnO nanopillars act as nanochannels after PDMS bonding. A previous study found that the density and height of ZnO nanostructures could be controlled by specifying the distribution of the seed layer and the duration of hydrothermal synthesis.

Figure 3 shows the mechanical strength test of the fabricated ZnO nanostructures. Figure 3A shows an SEM image of the zinc oxide nanostructure synthesized on a glass substrate without tape peeling test. After attaching and detaching 3M Scotch tape 10 times on the synthesized zinc oxide nanostructures, the surface was observed by SEM (Figure 3B). Comparing SEM images before and after the experiment, the morphology of the zinc oxide nanostructures did not change. Based on the experiment, it was confirmed that the adhesion between the zinc oxide nanostructure and the substrate is very strong and that the mechanical rigidity of the nanostructure surface is excellent.

Although it is generally meaningful to evaluate the performance according to the cross-sectional size of hollow nanochannels fabricated through PDMS bonding, the size of many nanochannels fabricated through the bonding process is not constant and it is not practical to measure it directly. Also, from the standpoint of making a system connecting nanochannels and microchannels, the unique phenomenon of nanochannels such as the ICP phenomenon is almost impossible to use when nanochannels exist alone. To access fluids or apply electric fields to nanochannels, fusion with microchannels is absolutely necessary. Therefore, we conducted a characterization experiment using a micro/nanofluidic device, deciding that the evaluation of the characteristics of the micro/nanofluidic device was as meaningful as that of the nanochannel alone. We fabricated the nanochannel between the two microchannels to check whether the nanochannels are connected to the desired position without disconnection and indirectly confirmed the connection whether or not the ICP phenomenon occurs. In addition, it was confirmed that the performance of the fabricated nanochannels can be controlled by varying the total number of nanochannels by adjusting the width of the ZnO nanostructure pattern.

ICP is a fundamental electrochemical phenomenon developing around ion-selective membranes when an electric field is applied across the ion-selective membranes. When a microchannel is filled with an electrolyte solution, the surfaces of the microchannels have certain net charges based on their material property. To compensate the net charges, counterions near the surface adhere to the surfaces, forming an “electric double layer”.

When the channel distances are reduced to a nanometer scale, the effect of the electric double layer is increased, resulting in an unbalanced ion concentration around the surface. Therefore, the nanosized channel passes only cations or anions, thus generating ion mismatches on both sides when an electric field is applied to the electrolyte solution. Thus, an ion depletion region develops on one side of the membrane and an ion enrichment region on the other side. Cells and charged particles become depleted or enriched around the ion depletion region; concentration and desalination can thus be achieved.

As the behavior of charged particles changes in the ion depletion region, nanochannel performance can be easily visualized using fluorescent dyes. We found that the operating voltage at which ICP developed varied by the width of the ZnO nanostructure pattern. The details are shown in Figure 5.

Figure 5 shows an experimental result where ICP can be induced with the fabricated ZnO nanochannel pattern. These figures are the magnified view of Figure 4A, where the nanochannel is located. As shown in Figure 5A, the two microchannels (main channel on the top and buffer channel at the bottom) were filled with green fluorescent dyes in 1 mM DSP solution. It was hard to observe ZnO nanostructures and bonded nanochannels with a fluorescence microscope because the ZnO patterns were thin and had no fluorescence. Therefore, we performed the visualization experiment using the fluorescence filter after confirming the position of the nanochannel through the white light mode. When no electric field is applied, the concentrations of ions and fluorescent particles are identical over the entire microchannel (Figure 5A). When an electric field above a certain level is applied, ion depletion commences near the nanochannel at the anodal side. Because the fluorescent dyes have net charges in the electrolyte solution, the green fluorescent particles are also depleted rapidly around the nanochannel (Figure 5B). Because the ICP phenomenon is a kind of dynamic equilibrium, the depletion region cannot be distributed evenly in all directions. The width of the nanochannel pattern in the figure is 200 μm; ICP is observed when a voltage of 140 V is applied. The relationship between the ZnO nanopattern width and the ICP operation voltage is shown in Figure 5C. The nanochannel pattern width was varied over 100, 200, and 400 μm; both limiting and overlimiting current regions are observed at different voltage sections, which means that the electrical property of a ZnO nanochannel is controllable by adjusting the pattern width. In general, the ICP phenomenon shows three phases of current response depending on the voltage increase. When the voltage is lower than the certain voltage required for the ICP phenomenon to occur, the current–voltage curve follows Ohm’s law. If the electric field around the nanochannel is sufficiently strong to trigger ICP, the limiting period commences; the low ion concentration of the ion depletion...
region limits ion transport through the nanochannels, so there was almost no current rise accompanying the voltage rise and this situation persists until the overlimiting period commences. When the voltage becomes higher than that of the overlimiting region, the depletion region is disturbed by a vortex caused by the excessive electric field, and the current rises as ions pass through the depletion region.26,27 As shown in Figure 5C, the results show that the smaller the width of the pattern, the higher the voltage when the limiting period commences. This is because when the pattern is small, the number of effective nanochannels that can pass ions is fewer than the number of wide nanochannels so that ion movement at the same voltage is reduced. Therefore, a higher voltage is required to move ions sufficiently rapidly to generate a depletion region.

Figure 6 shows that microparticles are continuously separated in the fabricated nanochannels. As the ion depletion region has a higher electrical resistance than the surrounding region, the electric field becomes concentrated in the former region. A previous study showed that charged particles were more efficiently separated under such circumstances.28 Particles in the electric field are subject to electrophoretic forces applied in the direction of that field, imparting
of particles of greater electrophoretic mobility are shifted more. The two particles are now separated spatially; the trajectories are spatially separated and directed in a constant path. Additional experiments under the same conditions show that however, most of the particles move in a similar path, and so on will be adjusted in various ways to check the performance according to the dimension difference of individual nanochannels and to confirm the possibility of large-area nanochannel fabrication. We expect that our novel nanochannel fabrication method will contribute to nanotechnological applications in biotechnology and environmental science.

**CONCLUSIONS**

We developed a new and simple method of nanochannel fabrication, which is easily scaled up to mass production through a solution-based fabrication process. The pillar-like dense ZnO nanostructures were hydrothermally synthesized on a glass substrate and covered by a PDMS surface to make hollow nanochannels. The nanochannels and the PDMS surface became the ceiling of the nanochannels. The suggested fabrication does not require expensive equipment or a complicated process such as conventional lithography; moreover, our method affords a much more stable pattern than conventional mass production via electrochemical etching. The fabricated nanochannels were evaluated by a fundamental nanofluidic phenomenon called ion concentration polarization, which is easily evaluated by the current–voltage response and visualized by fluorescent dyes. We proved that the performance of the fabricated nanochannel could be controlled by adjusting the ZnO nanochannel pattern width. The pattern widths of ZnO nanostructures were fabricated differently in the same type of micro/nanofluidic device as 200–400 μm, thereby varying the approximate number of nanochannels. Then, the current–voltage response confirmed that the ICP phenomenon easily occurs as the number of nanochannels increases.

Finally, we confirmed the possibility of the nanochannels for biotechnological applications by performing continuous separation of two kinds of microparticles using the fabricated micro/nanofluidic device. The experiments resulted in the successive separation of two types of particles with different electrical properties, and in the future, it is expected to be useful for the treatment of proteins, DNAs, and cellular materials with different electrical properties in solution. The ZnO nanochannels could be fabricated on any surface on which a seed layer can be grown, including flexible films or polymer membranes. In the future, the synthesis times of ZnO nanostructures, seed layer formation method, substrate, and so on will be adjusted in various ways to check the performance according to the dimension difference of individual nanochannels and to confirm the possibility of large-area nanochannel fabrication. We expect that our novel nanochannel fabrication method will contribute to nanotechnological applications in biotechnology and environmental science.

**EXPERIMENTAL SECTION**

**Materials.** Polyvinylpyrrolidone (PVP, analytical reagent (AR), MW: 1,300,000) powder, ammonia solution [NH₄OH, AR, 28.0–30.0% (m/m)], and HCl (AR) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Zinc chloride (ZnCl₂, AR) and zinc nitrate hexahydrate [Zn(NO₃)₂·6H₂O, AR] were purchased from Junsei Chemical Co. Ltd. (Tokyo, Japan). The PDMS (AR) was purchased from Dow Corning (Midland, MI, USA). All reagents were used as received without further purification.

**ZnO Nanostructure Fabrication.** We spin-coated a uniform seed layer onto a glass substrate. A polymer solution containing Zn ions was prepared by adding 500 mM Zn(NO₃)₂ and 0.1 g/mL PVP (final concentrations) to deionized water, followed by stirring at 600 rpm with a magnetic stirrer for 2 h. The polymer solution was spin-coated at 5000 rpm to deposit a seed layer onto a glass substrate; the polymer film was then decomposed and calcinated in air at 500 °C to form a ZnO seed layer. Hydrothermal growth was used to fabricate nanostructures on this seed layer. The growth solution was prepared by adding 10 mM ZnCl₂ and 5 μL/mL NH₄OH (final concentrations) to deionized water at 40–80 °C to initiate the reaction. NH₄OH was used to raise the pH via generation of OH⁻ species. ZnO nanostructure formation is influenced by pH, the concentration of Zn⁺⁺ ions, and the solution temperature; it is important to keep these conditions constant. In particular, maintenance of a constant temperature (here, 60 °C) is very important; even a small increase in temperature changes the response rate.

**Patterning of ZnO Nanostructures.** We used a lithographic method to pattern bulk ZnO nanostructures on the substrate when fabricating nanochannels at a desired position. Masking tape or a standard microelectromechanical system (MEMS) lithographic technique was used. Masking tape was cut using a paper cutter (CAMEO; Silhouette America, Inc., Orem, UT, USA) to obtain the desired patterns. We also used UV lithography. AZ 5214 photoresist was spin-coated onto glass at 3000 rpm for 30 s and then soft-baked in an oven at 100 °C for 1 min; the surface was exposed to 150 W of UV light for 7.5 s and then developed with a 2.38% (w/v) tetramethylammonium hydroxide solution. The seed layer and the ZnO nanostructures can be selectively modified/removed using dilute HCl (0.5 mM). We fabricated ZnO nanostructural patterns with widths of 100, 200, and 400 μm.

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**Figure 6.** Continuous particle separation using a fabricated nanofluidic device. Particles that have similar sizes but different electrophoretic mobilities are selected for electrical force-based continuous separation (1.0 μm-diameter particle with a relatively large electrophoretic mobility (μ_{ep,1.0 μm} = \(-8.6 \times 10^{-4} \) cm² V⁻¹ s⁻¹) and 1.1 μm-diameter particle with a relatively low electrophoretic mobility (μ_{ep,1.1 μm} = \(-6.5 \times 10^{-4} \) cm² V⁻¹ s⁻¹)).
3M Scotch Tape Peeling Test for Confirming Mechanical Strength of the ZnO Nanostructures. ASTM (American Society for Testing and Materials) certified tests were performed to verify the mechanical stability of the zinc oxide nanostructures. ASTM D3359-02 is a test that verifies mechanical stability by attaching a commercial 3M Scotch tape to a target device and then removing it repeatedly. We performed repetitive 3M tape attachment and peeling to ZnO nanostructures that are fabricated on a glass substrate without lithographic patterns. A total of six ZnO nanostructure samples were prepared: three samples without the taping test and three other samples with 10 tape peeling tests. SEM images were used to confirm the results before and after the taping test.

Fabrication of the Micro/Nanofluidic Device. Nanostructured ZnO was used to create a nanofluidic device via a PDMS-to-glass plasma bonding method.30 As can be seen in Figure 1, the ZnO nanostructures are patterned in strips on a glass substrate to connect two parallel microchannels. The two PDMS microchannel patterns are fabricated by soft lithography, with SU-8 inverse pattern molds and a PDMS replica (main and buffer channels; length, 2 cm; width, 100 μm; height, 35 μm; between-channel gap, 200 μm). When the two microchannels are bonded on the top of the glass substrate, the ZnO nanostructures at the gaps between the two microchannels are bonded to the flat PDMS surface. Because the ZnO nanostructures are rough (Figure 2C), the PDMS surface does not completely fill the gaps, and this does not completely fill the cracks between the nanostructures and these cracks eventually act as a hollow nanochannel connecting two microchannels.

In this paper, the fabricated micro/nanofluidic device was used for two types of experiments: the visualization experiment of nanochannel phenomena to evaluate the nanochannel performance (Figure 5) and continuous particle separation experiment for future biotechnological application (Figure 6). In the nanofluidic visualization experiment, microchannel outlets that are not used for electrolyte injection were blocked with commercial epoxy before electrolyte injection (Figure 1).

Microfluidic Device Measurements. In the two experiments, 1 mM dibasic sodium phosphate (DSP) dissolved in distilled water was used as the electrolyte solution. In the visualization experiment, fluorescein sodium salt was diluted in the buffer to allow observation of the nanofluidic phenomenon near the fabricated nanochannel. As can be seen in Figure 4A-B, anodes and cathodes consisting of a platinum rod were connected to the reservoirs of both microchannels and used to apply electric fields by a source measurement unit (B2902A; Keysight Technologies, Inc., Santa Rosa, CA, USA). An inverted fluorescence microscope (IX71; Olympus Co., Tokyo, Japan) and a charge-coupled device camera (DP72; Olympus) were used to observe the behavior of fluorescent dyes and to collect images. In the particle separation experiment, two types of fluorescent polystyrene particles (Thermo Scientific Corp., Waltham, MA, USA) differing in terms of diameter (1.0 and 1.1 μm) and electrophoretic mobility (μ_{ep}) (μ_{ep,1.0μm} = -8.6 × 10^{-4} cm^2 V^{-1} s^{-1} and μ_{ep,1.1μm} = -6.5 × 10^{-4} cm^2 V^{-1} s^{-1}) were separated. Particle mobility was measured using a zeta potential analyzer (Zetasizer Nano Z; Malvern Instruments, Malvern, U.K.); particles were diluted in a 1 mM DSP buffer.

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Notes
The authors declare no competing financial interest.

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