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All-in-One Nanowire-Decorated Multifunctional Membrane for Rapid Cell Lysis and Direct DNA Isolation

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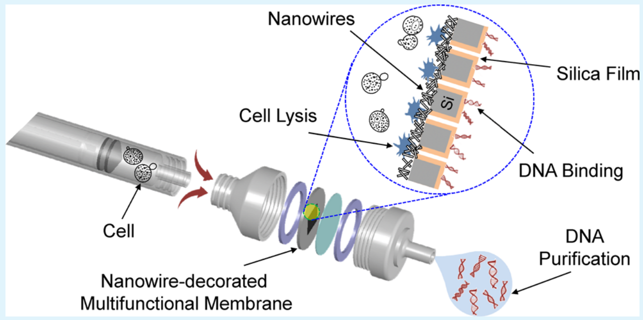
ABSTRACT: This paper describes a handheld device that uses an all-in-one membrane for continuous mechanical cell lysis and rapid DNA isolation without the assistance of power sources, lysis reagents, and routine centrifugation. This nanowire-decorated multifunctional membrane was fabricated to isolate DNA by selective adsorption to silica surface immediately after disruption of nucleus membranes by ultrasharp tips of nanowires for a rapid cell lysis, and it can be directly assembled with commercial syringe filter holders. The membrane was fabricated by photoelectrochemical etching to create microchannel arrays followed by hydrothermal synthesis of nanowires and deposition of silica. The proposed membrane successfully purifies high-quality DNA within 5 min, whereas a commercial purification kit needs more than an hour.



KEYWORDS: *nanowires, porous silicon membrane, cell lysis, DNA purification, point-of-care diagnostics*

I. INTRODUCTION

Effective extraction of nucleic acid from biological samples is an essential technique for a variety of reasons such as genetic testing, objective identification, and analysis of forensic evidence. Since most polymerase chain reaction (PCR) devices have required high-quality DNA to amplify for analysis effectively, various lysis protocols and purification techniques have been extensively investigated during the past decade.[1−3] The emergence of lab-on-a-chip (LOC) has also significantly affected the development of new DNA purification tools. These new tools are compact and compatible with microchips and provide a fast, cost-effective, and high-throughput process with high-quality DNA.[4,5] Among many purification approaches—including selective precipitation[6] and the use of silica-based resins[7] and magnetic beads[8]—silica-based methods have been found to isolate DNA handily without specialized equipment (such as a centrifuge, electrical source, or magnetic controller) and minimize DNA degradation, which might be caused by the shear force resulting from spin centrifugation during non-silica-based processes.[9] As a result, new silica-based DNA purification chips using the LOC platform have recently been developed to fulfil the increasing requirements of market for a faster, easier, and more reliable process.

In all approaches, cell lysis is the first and one of the most important steps to release nucleic acids by disrupting cell and nucleus membranes. In conventional cell lysis methods—including chemical,[10] acoustic,[11] electrical,[12] and mechanical[13,14] methods—mechanical methods, which allow fast cell lysis while maintaining the integrity of extracted components, are the ones most commonly used to obtain nucleic acids.[15] However, since cell lysis must be conducted prior to the DNA purification step, two separate devices or protocols for each step have generally been needed to isolate the DNA, resulting in increased fabrication cost and process time, and loss of extracted DNA for analysis. Clearly, special LOC-based designs are required to rapidly lyse cells and to immediately isolate DNA from the lysate for a simple process, minimum loss of extracted nucleic acid, and direct analysis. Various types of silica-based DNA purification devices using silica pillars,[16,17] sol−gel,[18] or silica-coated beads[19] have been extensively developed during the past decade. However, most of them have concentrated on the integration of structures within a single microfluidic channel and have involved complex fabrication processes in which multiple steps, such as lithography, wet etching, reactive-ion etching, alignment, multilayer deposition, and anodic bonding, have been needed to fabricate a whole device. Such devices also might limit the yield of isolated DNA due to the restricted surface area of silica in a single microchannel. Therefore, the development of a novel DNA purification device combined with an effective cell lysis chip still remains an engineering challenge for simple, rapid, compact, and direct analysis.

In this paper, we propose a handheld device using an all-in-one nanowire-decorated multifunctional membrane (NMM) for continuous mechanical cell lysis and rapid DNA purification without the assistance of additional power sources, lysis

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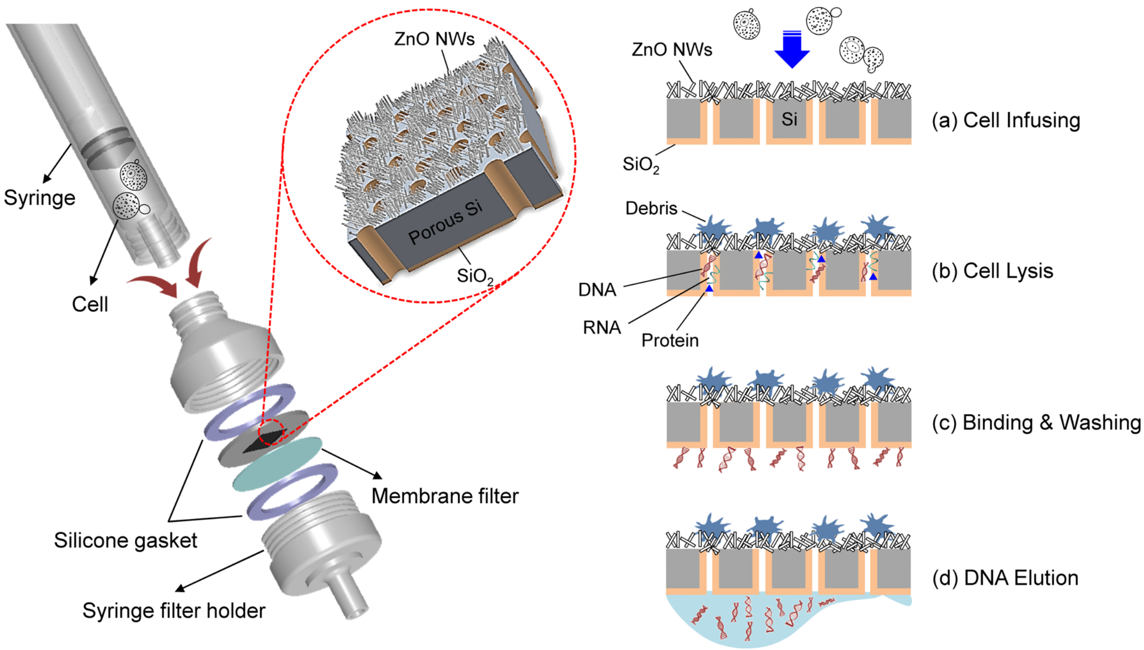

Figure 1. Schematic of the direct assembly of the nanowire-decorated multifunctional membrane with a commercial handheld syringe filter holder for mechanical cell lysis and DNA purification. Overall sequential process: (a)−(d).

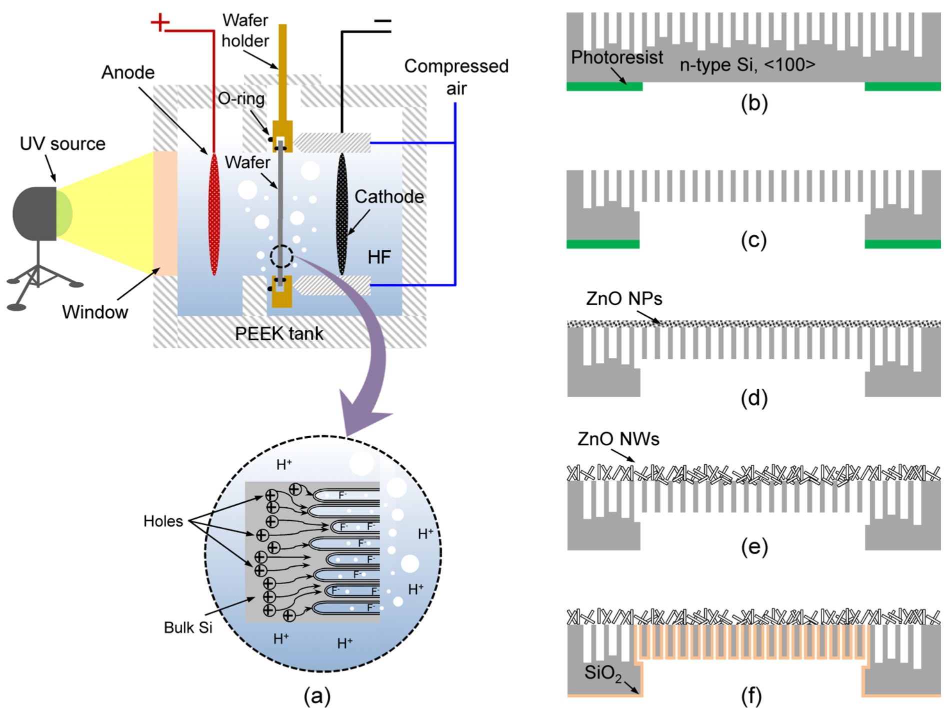

Figure 2. Schematic illustration of the NMM fabrication process: (a) photoelectrochemical etching, (b) pattern of photoresist mask for DRIE, (c) DRIE, (d) deposition of ZnO NPs seed layer, (e) hydrothermal ZnO NWs growth, and (f) PECVD of SiO_2 on the back side.

reagents, and routine centrifugation. As a first step, the efficiency of the cell lysis could be dramatically improved by using the ultrasharp tips of nanowires (NWs) decorated on the periphery of the vertically aligned microchannel, which were fabricated through photoelectrochemical (PEC) etching. The direct assembly of the developed all-in-one membrane with a commercial syringe filter holder also minimizes the overall sample preparation time, as well as the fabrication cost.

Figure 1 depicts a schematic illustration of the overall device, which comprises three main parts: a commercial syringe, the developed all-in-one membrane, and a syringe filter holder. The NMM is also composed of three main parts: a porous silicon surface decorated with zinc oxide (ZnO) nanowires for mechanical cell lysis, vertically aligned microchannels for the transport of the lysate after lysis, and a silica surface for binding DNA, as seen in the circle showing the zoomed-in view of the membrane in Figure 1. The NMM is placed in the middle of a syringe filter holder, and two silicone gaskets are used to tightly hold the NMM minimizing flow leakage through the edges of the membrane. This assembled device makes cells flow directly

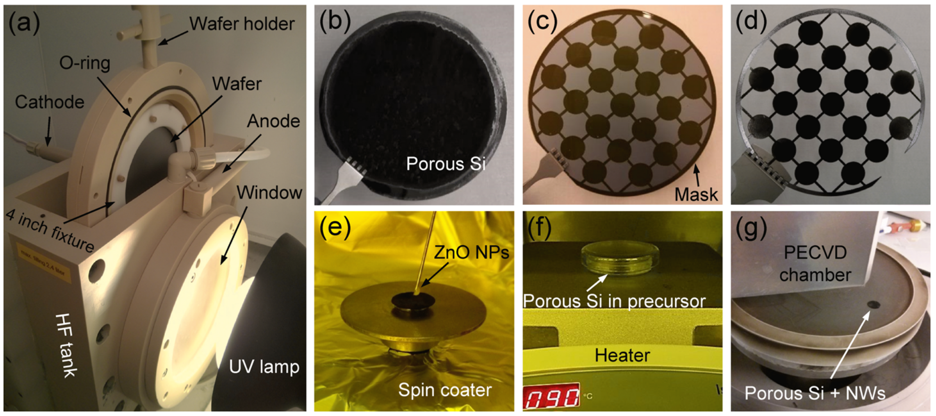

Figure 3. Experimental images of the NMM fabrication process: (a) photoelectrochemical etching, (b) porous silicon after PEC etching, (c) photoresist mask pattern on back side for DRIE, (d) array of through-hole porous silicon membrane after DRIE, (e) deposition of ZnO NPs seed layer, (f) hydrothermal ZnO NWs growth, and (g) PECVD for SiO_2 deposition.

across the NMM, and thus be disrupted by the ultrasharp tips of nanowires for lysing. The illustration on the right side in Figure 1 depicts the sequential and automated process of separating DNA from proteins and other cellular contaminants using the proposed all-in-one membrane. Cells are first mixed with a binding buffer. After the solution is put in a commercial syringe, the syringe filter holder combined with the NMM is directly plugged into the syringe. Although some of the cells can be chemically disrupted by the binding buffer containing chaotropic salts, mechanical cell lysis method by ultrasharp tips of the nanostructures was adopted again based on our previously reported study to significantly improve the cell lysis efficiency.[14] The cells are then released through the NMM by easily pressing a plunger piston (Figure 1a). The intracellular proteins and nucleic acids inside the cells are released and transported through the silica-coated microchannels where the DNA is selectively adsorbed due to the intermolecular electrostatic force and hydrogen bond formation in the DNA-silica contact layer[20] (Figure 1b). By the injection of a wash buffer, all other cellular contaminants and proteins pass through the channels while the DNA remains bonded to the silica surface (Figure 1c). Finally, the captured DNA is released with an elution solution (Figure 1d).

II. EXPERIMENTAL SECTION

Fabrication of the NMM. The overall fabrication process for the all-in-one NMM includes four main processes: PEC etching to create coherent porous silicon, deep reactive-ion etching (DRIE) to form a thin membrane, hydrothermal synthesis to decorate the nanowires over the porous silicon membrane, and plasma-enhanced chemical vapor deposition (PECVD) to coat the inside of the pores and the back surface with 1 μm thickness of silicon dioxide (SiO_2). For an anisotropic wet etching to form a porous silicon, n-type ⟨100⟩ silicon wafer with 10−20 Ω-cm resistivity was anodized by the PEC etcher (MPSB 150, AMMT GmbH, Frankenthal, Germany) in 5.5 wt % hydrofluoric acid for 120 min. During the etching, the back side of the wafer was illuminated by a near-ultraviolet (UV, 365 nm wavelength) source (Figure 2a). The applied voltage of 3.6 V and current density of 8.6 mA/cm^2 were maintained constant during the PEC etching. The bulk silicon on the back side of the sample was then patterned using standard lithography (Figure 2b) and removed by DRIE to create a through-hole thin membrane with 13 mm diameter (Figure 2c), which fits perfectly into a 13 mm commercial syringe filter holder (Swinnex 13 Filter Holders, Millipore, Bedford, MA).

In preparation for NWs synthesis, the front side of the porous silicon membrane was spin-coated by a droplet of ZnO nanoparticles (NPs) (40 wt % in ethanol, Sigma-Aldrich, St. Louis, MO) as shown in Figure 2d. The sample was then rinsed by ethanol after 30 s, and this seeding process was repeated two times to deposit a uniform seed layer for ZnO NWs growth. NWs were synthesized by immersing the seeded sample in a solution containing 25 mM zinc nitrate hydrate ($Zn(NO_3)_2\cdot 6H_2O$, Sigma-Aldrich), 5 mM polyethylenimine (PEI, C_2H_5N, branched, low molecular weight, Sigma-Aldrich) and 25 mM hexamethylenetetramine ($C_6H_{12}N_4$, Sigma-Aldrich) at 90 °C for 2 h[21−23] as seen in Figure 2e. For binding DNA, SiO_2 was finally deposited using PECVD (PlasmaLab 80plus, Oxford Instruments) with 25 W at 350 °C, which allows conformal coverage through the pore walls and back side of the porous silicon membrane (Figure 2f). In the process, nitrous oxide (N_2O, 800 sccm) and 10% silane (SiH_4, 100 sccm) in argon (Ar, inert carrier gas, 100 sccm) were used as the source of oxygen and silicon, respectively. The approximated deposition rate of SiO_2 film was 50 nm/min at 0.9 Torr pressure. The deposited amorphous SiO_2 has a compressive stress of 255.7 MPa (FLX-2320, KLA-Tencor, Milpitas, CA) and refractive index of 1.442 (Stokes LSE, Gaertner, Skokie, IL). Figure 3 shows the experimental images of the NMM fabrication process for more detailed experimental description. Figure 4 shows SEM images of the porous silicon membrane before and after deposition of ZnO NPs. As seen in Figure 4c,d, ZnO NPs were uniformly deposited on top of the porous silicon membrane with an average diameter of 75 nm.

Cell Preparation. In this study, hepatocellular carcinoma cell line (HepG2) and immortalized human keratinocyte (HaCaT) and HeLa cell line, gifts from Berkeley Tissue Culture Facility, were cultured in a 5% (v/v) CO_2 incubator at 37 °C and kept in Dulbecco's modified eagle medium (Gibco, Grand Island, NY), which was mixed with 1% (v/v) penicillin-streptomycin (10 000 units/mL, Gibco) and 10% (v/v) fetal bovine serum (Gibco).[14] The cells were cultured for 5 days prior to the experiment and separated from the culture dish using 0.05% trypsin-ethylenediaminetetraacetic acid (Life Technologies, Grand Island, NY) treatment and then prepared in culture media just before the experiment. The cells

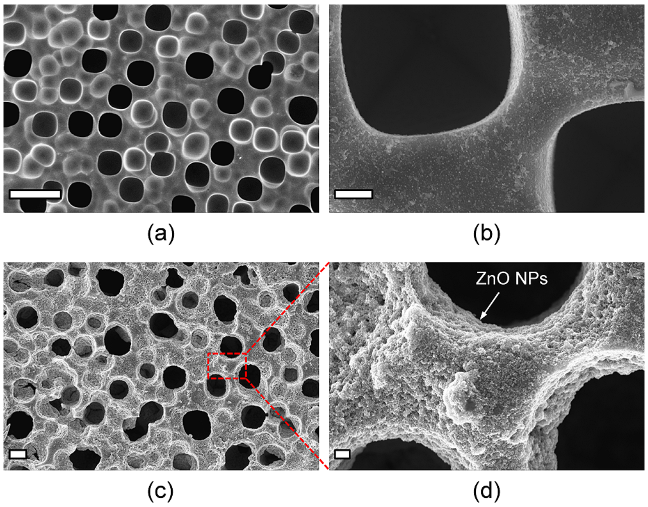

Figure 4. SEM images of the porous silicon membrane before deposition of NPs: (a) top surface view and (b) zoomed-in view of macropores and the porous silicon membrane after deposition of NPs: (c) top surface view and (d) zoomed-in view of uniformly deposited NPs on the porous membrane. Scale bars: (a) 10 μm, (b) 1 μm, (c) 3 μm, and (d) 400 nm.

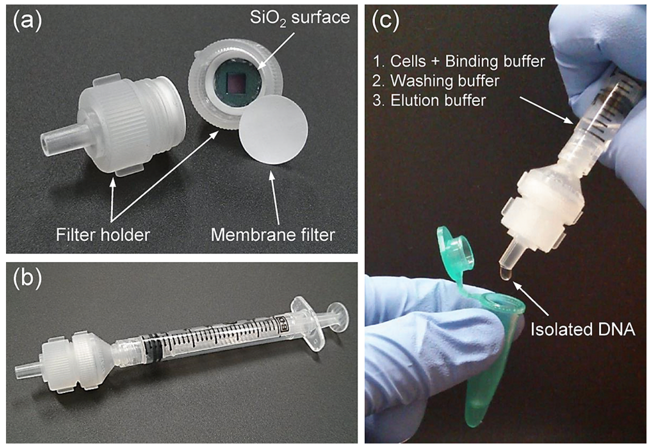

Figure 5. (a) Assembly of the proposed all-in-one membrane with a commercial syringe filter holder and membrane filter; (b) direct connection of the filter holder to a handheld syringe; (c) rapid DNA purification after flowing three different buffers through the all-in-one membrane.

populations at a concentration of 5×10^5 mL^{-1} in phosphate buffered saline (PBS, pH 7.4, Gibco) were counted by a hemocytometer and used for the proposed method and commercial purification kit method.

Protocol for DNA Purification. Cells in PBS are first mixed with 100 μL of binding buffer (High-Cutoff, Invitrogen, Carlsbad, CA). After putting the solution in a commercial syringe, the syringe filter holder combined with the NMM was connected to the syringe. The solution was then infused toward the NMM by simply pushing a syringe piston. As a second step, a wash buffer of 100 μL (Invitrogen) was injected to remove all other cellular contaminants and proteins through the pores while DNA remains bound to the silica surface. Captured DNA was finally eluted with 5−20 μL of elution buffer (10 mM Tris-HCl, pH 8.5) and collected into a microcentrifuge tube.

Gel Electrophoresis. To obtain as DNA band for qualitative analysis, eluted DNA from each method was analyzed with gel electrophoresis. 1% agarose gel was used for electrophoresis at 140 V for 30 min. Gel staining was conducted with a Sybr Safe DNA gel stain (Life Technologies) and the gel image was taken with a ChemiDoc XRS (Bio-Rad Laboratories, Hercules, CA).

PCR Amplification of Human Papillomavirus Gene. Primers were designed to amplify human papillomavirus (HPV) protein E6 and E7. DNAs isolated from the developed method and conventional method were PCR-amplified using Phusion High-Fidelity DNA polymerase (New England Biolabs, Ipswich, MA). PCR cycles were set up with the manufacturer's manual and the C1000 Touch Thermal Cycler (Bio-Rad Laboratories) was used. After PCR amplification, samples were analyzed by 1% agarose gel electrophoresis, followed by staining and imaging as described above.

III. CHARACTERIZATION

To characterize the purification performance of the NMM, HepG2 and HaCaT were used in this study. The detailed cell preparation for the experiment is described in the Experimental Section. Figure 5 shows the proposed all-in-one device and experimental protocol for rapid DNA purification using the handheld syringe assembled with the NMM and a syringe filter holder. The NMM is fixed in the middle of a syringe filter holder, and the NWs surface of the membrane faces the syringe tip, which allows rapid disruption of cells by the ultrasharp tips of the NWs (Figure 5a). After a solution containing cells and binding buffer was added to a commercial syringe, the syringe filter holder combined with the NMM was simply connected to the syringe (Figure 5b). Finally, the cells were infused toward the NMM by easily pushing a syringe piston, followed by the injection of a wash buffer to remove other cellular contaminants with proteins and an elution buffer to release the captured DNA from the NMM surface (Figure 5c). To evaluate the purification efficiency of the NMM method including total process time, concentration, and quality of extracted DNA, the DNA purification protocol using a commercially available DNA purification kit (PureLink Genomic DNA Mini Kit, Invitrogen) was performed and compared to one obtained using the developed all-in-one device. The concentrations of purified DNA were measured using a spectrophotometer (NanoDrop 2000, Thermo Scientific, Waltham, MA) with a wavelength of 260 nm, the appropriate wavelength to measure the optical absorbance of nucleic acids in soluble cell lysates.

To investigate the mechanical limitation of the NMM, the membrane was assembled with a syringe filter holder and the breakage of the membrane was monitored by increasing the internal pressure in the syringe. The experimental setup is shown in Figure 6a. The pressure in the syringe was controlled by changing the volumetric flow rate from 1 to 10 mL/min using a syringe pump (KDS210, KD Scientific, Holliston, MA) and was monitored using a pressure sensor (40PC500G2A, Honeywell, Morristown, NJ). As a result, it was observed that the NMM was not fractured at all even at the internal pressure of 275 kPa as seen in Figure 6b. Considering an experimental volumetric flow rate between 4 and 5 mL/min (corresponding average internal pressure: 30.6 and 54.7 kPa, respectively) generated by the finger force used in this study, it was confirmed that the NMM was sturdy enough to endure the applied pressure during mechanical cell lysis and DNA isolation process.

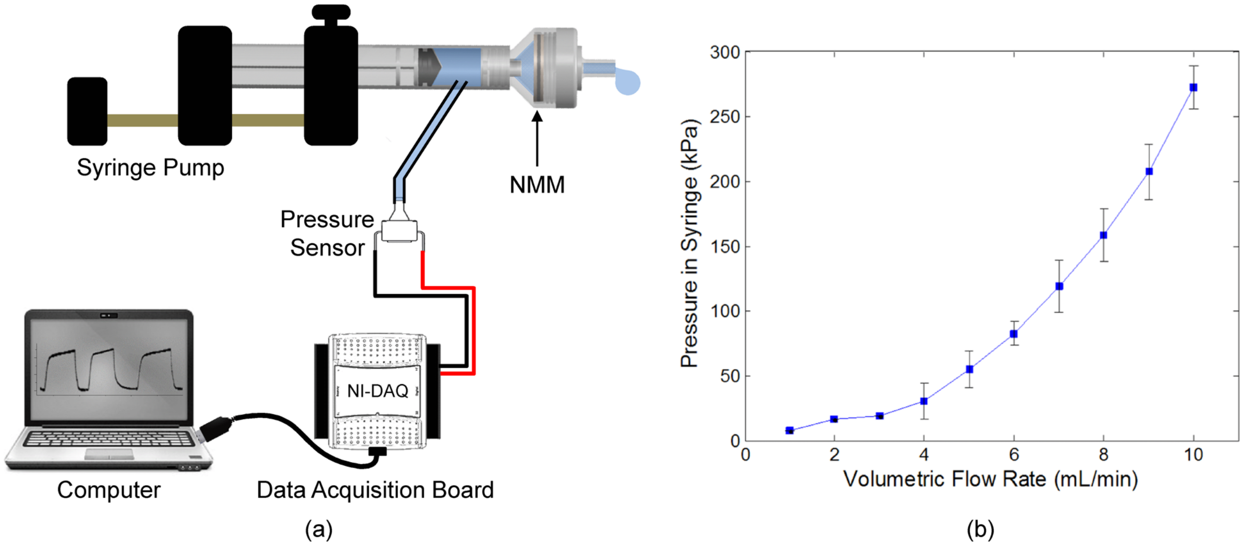

Figure 6. (a) Schematic illustration of experimental setup to monitor the pressure in the syringe while pushing a plunger piston and (b) measured internal pressure in the syringe with respect to the volumetric flow rate.

IV. RESULTS AND DISCUSSION

Figure 7a,b shows the actual size of the all-in-one membrane and a tilted surface view of the fabricated NMM with an average pore radius of 2.95 μm and a porosity of 32.8% (12 000 pores/mm^2) after PEC etching. Figure 7b shows the high-aspect-ratio and vertically aligned microchannel arrays with an average length of 130 μm used for the transport of lysate after lysis. Close-up views of the ultrasharp tips of the NWs and macropores decorated with NWs after hydrothermal synthesis are shown in parts (c) and (d), respectively, of Figure 7. The ultrasharp tips of NWs on the periphery of the straight microchannels are able to lyse cells rapidly by disrupting the membranes of both the cell and the nucleus immediately after infusing the cells into the NMM with finger pressure.

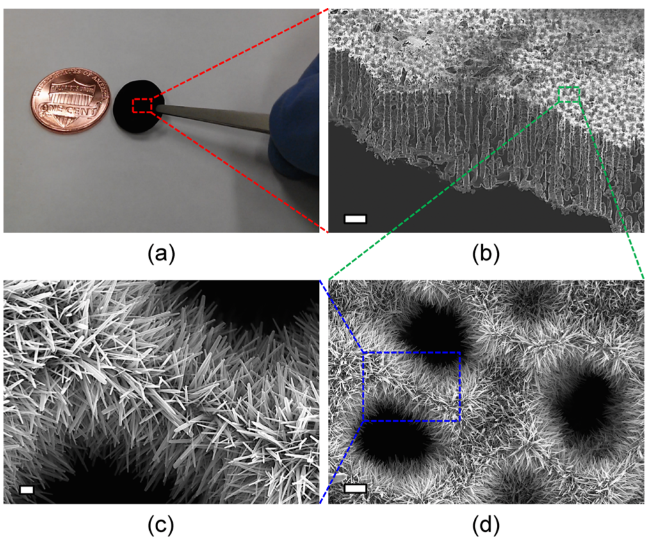

Figure 7. SEM images of fabricated all-in-one nanowire-decorated multifunctional membrane: (a) size comparison between the fabricated 13 mm diameter all-in-one membrane and a U.S. one-cent coin, (b) 45° tilted view, (c) close-up view of ultrasharp tips of nanowires, and (d) close-up top view of macropores decorated with nanowires. Scale bars: (b) 20 μm, (c) 200 nm, and (d) 1 μm.

Considering the extremely rough surface of the NMM, the developed membrane can also filter out cell debris by catching it between the nanowires, which significantly reduces overall sample preparation time for the DNA purification protocol by skipping routine centrifugation to remove debris after cell lysis.

To demonstrate the rapid and direct cell lysis by the nanowires as a first step for DNA isolation, the HepG2 cells in PBS with different concentrations were infused toward the NMM by pushing a syringe piston. Parts (a) and (b) of Figure 8 show the scanning electron microscope (SEM) images of the NMM after cell lysis, including the top view and the close-up view of the membrane lipids captured between the NWs, respectively. The HepG2 cells with a diameter of approximately 10 μm were successfully disrupted by the ultrasharp tips of the NWs, and the NWs were unbroken. To demonstrate the filtering performance of the NMM, the lysate was directly collected into a microcentrifuge tube and centrifuged at 15 000 rpm for 15 min. As a result, the debris (lipids of cells membrane) were not detected in the lysate that had filtered through the NMM, allowing fast cell lysis and direct analysis due to the absence of routine centrifugation. For quantitative analysis, concentrations of extracted proteins and nucleic acids were evaluated using a spectrophotometer where 280 and 260 nm wavelengths were used to measure the optical absorbance

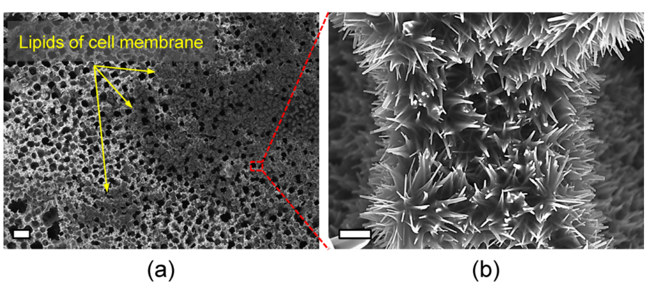

Figure 8. SEM images of the nanowire-decorated multifunctional membrane after cell lysis: (a) ruptured and filtrated lipids of cell membrane and (b) close-up view of membrane lipids between NWs, ruptured by ultrasharp tips of NWs. Scale bars: (a) 20 μm and (b) 600 nm.

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of proteins and nucleic acids in lysates, respectively. Figure 9 shows the total concentrations of extracted protein and nucleic acid in each lysate with different cell populations. It was found that both intracellular protein and nucleic acid concentrations were proportionally increased as the cell concentration was increased as shown in the figure. Therefore, the NMM could provide the lysate within 1 min by ultrasharp tips of nanowires allowing rapid mechanical cell lysis, and thus significantly reducing the total lysis time.

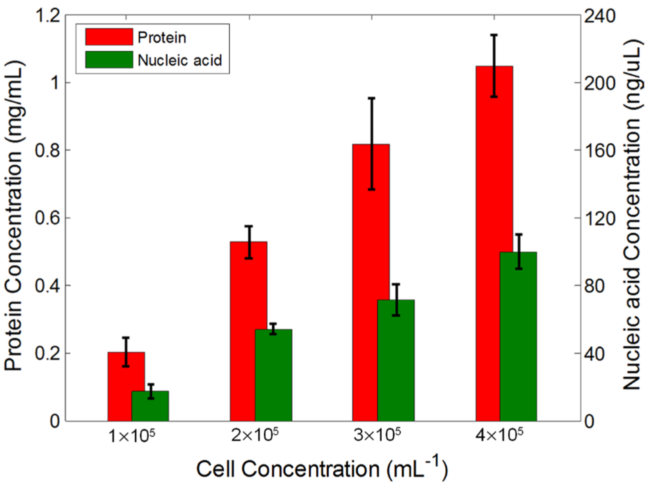

Figure 9. Concentrations of the intracellular protein and nucleic acid with different cell concentrations after HepG2 cell lysing using all-in-one membrane.

Figure 10a shows the concentration of the eluted DNA obtained from the proposed all-in-one device and commercially available purification kit. Because the volume of elution buffer used for the NMM method was smaller than the volume used for the commercial kit, it is obvious that the DNA concentrations purified using the all-in-one membrane (80.37 ± 7.62 ng μL^{-1} for HepG2 and 69.13 ± 3.5 ng μL^{-1} for HaCaT) were higher than the concentrations purified by the commercial purification kit (52.5 ± 13 ng μL^{-1} for HepG2 and 37.3 ± 4.9 ng μL^{-1} for HaCaT); the total average amounts of eluted HepG2 DNA using the commercial kit (5250 ng; 3735 ng for HaCaT) were about 13 times (11 times for HaCaT) higher than the sample purified by the NMM method (401.8 ng; 345.6 ng for HaCaT). This is mainly because the spin column in the commercial kit has a much larger surface area of silica structure than that of the NMM. However, since the maximum amount of capturable DNA is proportional to the surface area of silica medium, the dimensions of the NMM including pore size, porosity, membrane thickness, and diameter can be scaled up to capture more DNA. In terms of cost and efficiency, it is also noticeable that the NMM method can be usefully utilized for fast and point-of-care diagnostics on DNA because the NMM method can extract PCR-amplifiable DNA in 5 min with a relatively small volume of elution buffer while at least 50 μL of elution buffer—enough to flow across the thick silica medium of the spin column—is necessary for the commercial kit to produce a minimum yield of isolated DNA. The total processing time from cell lysis to DNA purification using the all-in-one membrane was also less than 5 min compared to at least 1 h for purifying the DNA using the commercial purification kit. This is mainly because the purification method using all-in-one membrane does not require the incubation and centrifugation steps, which are essential for chemical cell lysis and DNA purification using a spin column in a commercial kit process.

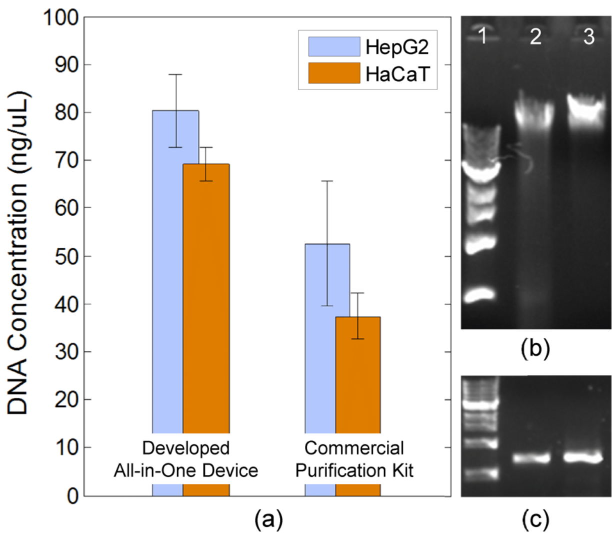

Figure 10. (a) Concentration of DNA eluted from the developed method using all-in-one membrane and the commercial purification kit (cells: HepG2 and HaCaT), (b) comparison of quality of extracted DNA using commercial kit and all-in-one device by gel electrophoresis (cell: HeLa), and (c) PCR amplification product of human papillomavirus gene from the extracted HeLa DNA. Lane 1, ladder; lane 2, commercial kit; and lane 3, developed all-in-one device.

To demonstrate qualitatively the reliability of rapid DNA purification using the fabricated all-in-one membrane, DNA purification was conducted on a HeLa cell line (a gift from the Berkeley Tissue Culture Facility, an immortal cell line derived from HPV-infected cervical cancer cells) and analyzed with agarose gel electrophoresis, followed by Sybr nucleic acid staining to visualize the purified nucleic acids. Figure 10b shows that genomic DNA was successfully purified by the developed method using the NMM, whereas DNA purified by the commercial kit for 1 h still had some remnant RNAs that showed up as smeared broad bands. This result shows that pure genomic DNA of high quality could be isolated using the NMM method in about 10 times less time than the conventional method. Considering that a major application of DNA purification is in the diagnosis of disease, the HPV genome, which causes cancer by integrating itself into a human genome, was detected by amplifying the HPV sequence in HeLa DNA. Figure 10c demonstrates that the PCR detection of amplified HPV sequence from HeLa DNA purified by the NMM was as clear as one from HeLa DNA purified by the commercial kit. Therefore, the results proved that the NMM purification method can be applied for fast and simple diagnostics by isolating high-quality DNA within a shorter period of time.

V. CONCLUSIONS

In this study, an all-in-one device using nanowire-decorated multifunctional membrane was developed for rapid cell lysis and DNA purification to extract nucleic acids from cells without the assistance of power sources and isolate genomic DNA using selective adsorption to a silica surface. This membrane was created by a combined fabrication process by the PEC etching for forming bulk porous silicon, DRIE for forming a thin membrane, hydrothermal synthesis for decorating nanowires over the porous silicon membrane, and PECVD for coating a thick silica layer to capture the DNA in the lysate. The fabricated all-in-one membrane was handily assembled with a commercially available syringe filter holder and a syringe. As a first step for DNA purification, rapid mechanical cell lysis was successfully achieved by ultrasharp tips of nanowires, significantly reducing the total lysis time. After injecting sequential buffers, DNA could be rapidly isolated and released from the back side of the NMM, providing fast and highly efficient DNA isolation for diagnostics based on DNA sequence. This study shows the feasibility of rapid and facile DNA purification for point-of-care diagnostics of disease within a short period of time by reducing many complicated process steps and the use of specialized equipment.

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Notes

The authors declare no competing financial interest.

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