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Low-cost polymer microfluidic device for on-chip extraction of bacterial DNA

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ABSTRACT

A polymer microfluidic device for on-chip extraction of bacterial DNA has been developed for molecular diagnostics. In order to manufacture a low-cost, disposable microchip, micropillar arrays of high surface-to-volume ratio (0.152 μm^{-1}) were constructed on polymethyl methacrylate (PMMA) by hot embossing with an electroformed Ni mold, and their surface was modified with SiO_2 and an organosilane compound in subsequent steps. To seal open microchannels, the organosilane layer on top plane of the micropillars was selectively removed through photocatalytic oxidation via TiO_2/UV treatment at room temperature. As a result, the underlying SiO_2 surface was exposed without deteriorating the organosilane layer coated on lateral surface of the micropillars that could serve as bacterial cell adhesion moiety. Afterwards, a plasma-treated PDMS substrate was bonded to the exposed SiO_2 surface, completing the device fabrication. To optimize manufacturing throughput and process integration, the whole fabrication process was performed at 6 inch wafer-level including polymer imprinting, organosilane coating, and bonding. Preparation of bacterial DNA was carried out with the fabricated PDMS/PMMA chip according to the following procedure: bacterial cell capture, washing, *in situ* lysis, and DNA elution. The polymer-based microchip presented here demonstrated similar performance to Glass/Si chip in terms of bacterial cell capture efficiency and polymerase chain reaction (PCR) compatibility.

1. Introduction

Nucleic acid-based micro total analysis system (μTAS) is a promising approach to fully automate the analytical procedures on a microchip platform. In particular, incorporation of target sample preparation, amplification and detection into a miniaturized lab-on-a-chip format has the potential to revolutionize many life science applications and related areas, including clinical diagnoses, biological and forensic analyses. These sample-in-answer-out devices can offer many advantages, such as low consumption of reagent, rapid analysis, easy operation, and improved reproducibility [1–4].

In contrast to protein analysis, an extraction or purification step to acquire DNA of interest from desired cells is a prerequisite for successful genetic analysis [5–9]. Most conventional bench-top DNA purification techniques are difficult to be used in microchips since these often require centrifugation or other hands-on processing steps. To address these challenges, a large number of solid phase extraction techniques, which can be integrated into microfluidic devices, have been developed [4,10]. In spite of the aforementioned benefits associated with the decreased cost through miniaturization, most of published works on DNA extraction have been predominantly performed with expensive inorganic substrates, such as silicon, glass, quartz because of their superior material properties and easy adaptation to well-established MEMS fabrication techniques [4,7]. However, there are some potential drawbacks with the inorganic-based microdevices. The cost for materials and fabrication processes such as photolithography, etching (dry and wet) and high temperature bonding is high [2,4,10], which has been often ignored in the research stages of microfluidic devices. Therefore, it is potentially beneficial to develop fabrication technologies that allow production of such devices at a cost comparable to that of the conventional bench-top devices. In this regard, polymer microdevices, which can be manufactured through relatively low cost processes such as injection molding or hot embossing, are an attractive solution to alleviate the above-mentioned cost issues [2,10–13]. Nonetheless, transition from inorganic to organic materials is not trivial, for which several challenges need to be suitably addressed including fabrication of large surface area (high surface-to-volume ratio microstructures) for effective

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biomolecule manipulation [4,10,14] and chemical compatibility of polymer during biochemical assays [11,12]. A handful of studies have been reported on DNA extraction with plastic microfluidic devices. In order to generate large surface area, most of the previous studies employed installation of silica-based matrix, such as packed bead, sol–gel and bead-polymer monolith, on a chip scale after fabrication of polymeric microchamber [15–18]. In addition, micropost-anchored carboxyl groups on polycarbonate substrate exhibited DNA purification capability with the aid of polyethylene glycol (PEG) solution and ethanol [19,20].

Until now, chaotrope-based DNA purification method has become widely accepted in miniaturized devices because chaotropes (e.g. guanidinium thiocynate) have an ability to disrupt cell membrane as well as to bind DNA onto a solid surface (i.e. silica), which eliminates additional need for chamber or buffer change [4,9,15–18]. However, it is desirable to avoid the use of such chemicals including chaotropes or organic solvent (e.g. ethanol) within microfluidic environments as they are well-known PCR inhibitors [10]. They have been usually applied to bench-top processes involving centrifugation steps. Therefore, a different DNA extraction scheme that would be more compatible with downstream processes (e.g., electrostatic interaction [21,22] and filtration [23]) is still needed.

Our interest here lies in fabricating a low-cost polymeric microchip that has bacterial DNA extraction capability comparable to inorganic-based microchips and that can perform full pre-PCR processes from bacterial cell capture to DNA isolation. It was previously reported that bacterial DNA preparation can be done on microchips with surface-modified Si micropillars [24,25]. Bacterial cells can be selectively captured on them from buffer suspension or whole blood by optimizing surface tension of solid substrate and media pH based on bacterial adhesion thermodynamics [26]. Furthermore, it does not require the PCR-inhibitory purification chemicals such as chaotropes, PEG, and organic solvent. According to the thermodynamic adhesion model, decreasing surface tension of the solid substrate (i.e. increasing hydrophobicity) will lead to higher cell adhesion when surface tension of buffer solution (*ca.* 73 erg/cm^2) is higher than that of bacterial cell (*ca.* 66–69 erg/cm^2) [26]. Organosilane containing functional groups reactive to SiO_2 surface was chosen for controlling surface tension of the solid substrate in our previous report [24]. While performance of this approach was found to be equivalent to that of a commercial DNA extraction kit, the microchip was produced from expensive glass and silicon substrates using conventional photolithography processes including patterning, deep reactive-ion-etching and high temperature anodic bonding.

In this work, we have developed a fabrication method to replace inorganic substrates with much cheaper polymer materials. Micropillar arrays on polymethylmethacrylate (PMMA) substrate were generated by hot embossing technique with an electroformed Ni mold, and organosilane was coated following room temperature deposition of SiO_2. Finally, the processed PMMA substrate was bonded to polydimethylsiloxane (PDMS) sheet to enclose the microchannels. PDMS is a well-known polymeric material that has been widely used in various microfluidic devices. In particular, bonding characteristic of PDMS to a variety of hydrophilic inorganic oxide materials such as SiO_2 are attractive in that only surface activation is required without the use of adhesives or solvents. To facilitate PDMS-interface bonding to organosilane-modified SiO_2 surface, photocatalyst was employed to selectively remove a thin organosilane film on top plane of the micropillars without any adverse effects on their lateral surface. This approach allowed us to fabricate a low-cost plastic microchip without loss of bioanalytical activities such as bacterial cell capture and PCR compatibility.

2. Materials and methods

2.1. Organosilane-coated Glass/Si microchip fabrication

The Glass/Si microchip was fabricated using standard MEMS techniques. First, a 6-inch silicon wafer was spin-coated with positive photoresist (AZ GXR 601, Clariant, Switzerland) and patterned using an EV620 mask aligner (EV Group, Austria). The patterned photoresist was developed, and the wafer was dry-etched to 50 μm with inductively coupled plasma (ICP) in a Deep Reactive Ion Etching (DRIE) etcher (STS, UK). The microchip (23 mm × 10 mm) was designed with an internal volume of 5 μL and a micropillar interspacing of 12 μm, and size of each micropillar was 23 μm × 23 μm × 50 μm. Then, 5000 Å of silicon dioxide was grown on the Si wafer and anodically bonded to a glass wafer with inlet and outlet holes formed through a sandblast technique. In order to modify the micropillar surfaces, an organosilane solution containing 200 mM of tridecafluoro-1,1,2,2-tetrahydrooctyl trimethoxysilane (Gelest, USA) in toluene was injected into fluidic ports with a syringe. After allowing the reaction for 1 h, the micropillar surfaces were washed with fresh ethanol three times, and then dried at 110 °C for 50 min.

2.2. Ni mold fabrication and hot embossing

In order to fabricate PMMA pillar arrays, additional steps, such as Ni electroplating for mold, hot embossing, and SiO_2 evaporation, were processed over the Si pillar arrays. For efficient embossing process, nanoscallops on the lateral surfaces of the Si micropillars formed during the Bosch process were removed through wet oxidation (8000-Å-thick SiO_2 layer) and etching with hydrofluoric acid solution. Chromium (1000 Å) and copper (1000 Å) were sequentially deposited onto the etched silicon surface with a sputtering system (Atech, Korea). Cr/Cu served as a conducting seed layer for electroforming in the next step. The Cr/Cu layer was connected to a negative electrode and electroplated in a nickel solution-basedsulfamic acid with current density of 20 mA/cm^2 at 55 °C for 20 h. Finally, silicon was etched for 8 h at 85 °C in 25 wt% potassium hydroxide solution to generate Ni mold.

The hot embossing technique (HEX03, Jenoptics, Germany) was applied to construct the micropillar arrays in PMMA sheet (Rohm, Germany). During the process, the PMMA sheet was sandwiched between a flat plate and the fabricated Ni mold. While heating the PMMA sheet at 150 °C, an embossing force (35 kN) was applied for 800 s. The mold was subsequently cooled to 26 °C with the force maintained to preserve the microstructures, and then demolding was performed. Afterwards, Cr (200 Å) was sputtered, and SiO_2 (5000 Å) was successively deposited on the replicated PMMA sheet through e-beam evaporation (Korea Vacuum, Korea) at room temperature. Finally, the organosilane layer (tridecafluoro-1,1,2,2-tetrahydrooctyl trimethoxysilane) was coated on the deposited SiO_2 layer at the wafer level.

2.3. PDMS-interface bonding with TiO_2/UV treatment

TiO_2 sol solution was prepared by mixing 6 mL of titanium (IV) isopropoxide (Ti{OCH(CH_3)$_2$}$_4$, Sigma–Aldrich, USA) and 0.8 mL of 0.1 N HCl in isopropanol (85 mL). The mixed solution was mildly stirred to form TiO_2 sol overnight and was spin-coated onto a transparent Pyrex wafer with 0.5 mm-thickness (Corning, USA) at the speed of 4000 rpm for 40 s. The processed glass wafer was calcined (500 °C, 1 h) in a furnace, and the measured thickness of TiO_2 was *ca.* 400 Å. The organosilane-coated pillar arrays (Si or PMMA) were irradiated for 10 min using TiO_2/Pyrex as a photomask with an UV aligner (Shinu, Korea) at the wafer level. O_2 plasma treatment of PDMS sheet with thickness of 0.7 mm (HT-6200, Rogers, USA) was