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Fabrication of DNA extraction device with tethered poly(N-isopropylacrylamide) brushes on silicon surface for a specific DNA detection

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ABSTRACT

In this work, tethered poly(N-isopropylacrylamide) (PNIPAAm) with thermo responsive property brushes were grafted on a fluidic device by atom transfer radical polymerization (ATRP) as a extractor of DNA molecules from a specimen of human blood through temperature tuning. The thermo responsive property of temperature switching for tethered PNIPAAm was introduced to absorb DNA molecules at $T = 25\,^{\circ}C$ and release them at $T = 60\,^{\circ}C$. The specimen included a human genomic DNA (hgDNA584), a designed target DNA molecule with 2, 10, and 50 ng/μl concentrations, and biomarcomolecules from lysing human blood. A set of specific primers of polymerase chain reaction (PCR) process were designed to control the selectivity of specific DNA detection. We analyzed the specimen after amplification by agarose gel electrophoresis to recognize the existence of the hgDNA584. The results suggest that 2 ng/μl of hgDNA584 the fluid device could be extracted by tethered PNIPAAm brushes to be detected after PCR process, which could miniaturize cartridges of sample preparation for rapid disease diagnosis through fluid device to approach the lab-on-a-chip (LOC).

1. Introduction

"Lab-on-a-chip (LOC)" is named for devices that integrate multiple laboratory functions on a single chip within scale of several millimeters or centimeters. The LOC for DNA detection included DNA extraction, polymerase chain reaction (PCR) and DNA hybridization is designed to integrate the three main steps in a microfluidic chip for automating DNA sample detection. Recent advances in miniaturization of various components for chemical and biological assays have enabled their integration into lab-on-a-chip (LOC) systems [1–3]. One of the major bottlenecks in traditional bioanalytical methods involves sample preparation for subsequent detection and analysis, rather than detection itself. Therefore, development of microfluidic components for sample preparation, which is required for complete automation of diagnostics, has been receiving much attention [4–6]. The success of molecular approaches such as real-time PCR [7] and real-time NASBA [8] has greatly contributed to this. The sensitivity of these techniques and the low volumes required for the reaction make them a subject to miniaturization approaches in an attempt to create fully automated diagnostic cartridges [9]. A key challenge for such systems, however, remains the integration of automated sample preparation [10]. Sample pre-treatment includes the concentration of target species, lysis of cells and isolation of nucleic acid molecules. Current attempts to create miniaturized diagnostic cartridges use a large variety of cell lysis techniques, including electrical [10], mechanical [11], chemical [12] and thermal techniques [13]. Purification approaches of nucleic acids are largely restricted to solid phase adsorption techniques [14,15]. Particularly magnetic beads based solid phase adsorption is becoming popular for miniaturized nucleic acid purification [16]. However, the solid phase purification approach requires a great number of fluidic operations for processing, which lead to complicated actuation protocols and hamper miniaturization of the instrument. In addition, the absorption/desorption approach is accompanied by losses of nucleic acid material that become significant, especially when dealing with low volumes and concentrations. Therefore, simpler nucleic acid purification procedures are required to create faster, more efficient and cheaper cartridges.

"Smart surface" is an important factor to integrate these moduli in a device. Surfaces that can tune their properties when subjected to external stimuli have potential applications in a variety of fields including drug delivery [17], bioanalysis [18], protein separation [19] and microfluidics [20]. Wettability of a solid surface is an important property of a material as it controls its interaction with the liquid. It depends on various factors, among which surface roughness and surface energy are the dominant ones. For the fabrication of superhydrophobic surface, a combination of optimum surface roughness and low surface energy is required [21]. Responsive hydrogels have attracted the attention of scientists due to the large variety of possible applications in biology, medicine, and pharmaceutics, and as sensors and actuators [22–24]. The largest

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number of studies were performed on N-isopropylacrylamide (NIPAAm) based polymers in various modifications such as networks or surface grafted brushes [25]. The homopolymer exhibits a lower critical solution temperature (LCST) close to body temperature (32 °C), above which the polymer aggregates and expels bound water, while at T < LCST, water is a good solvent for the polymer. Hydrogels, being cross-linked polymer networks, swell upon incorporation of water to a multiple of their initial volume. By temperature increase above the LCST, the polymer and the water molecules phase separate, leading to a macroscopic network collapse of the hydrogel with incorporated water being released. This phase separation originates from a shift of the balance between the hydrophilic and hydrophobic interactions with respect to temperature [25–27] or, in thermodynamic terms, between the mixing free energy and the rubber elasticity free energy, and in the presence of ionizable groups the osmotic effect of all charged species [28]. Therefore, the polymer chains hydrate and stay in extended structures when the solution temperature is below the LCST. In contrast, the polymer chains form intramolecular hydrogen bonds and dehydrate when the solution temperature increases above the LCST, resulting in compact hydrophobic structures. By grafting PNIPAAm onto a rough surface, a surface that could change its wettability from superhydrophilicity to superhydrophobicity by increasing the ambient temperature was reported [29]. With the advent of nanotechnology, thin surface-attached films of responsive polymers receive increasing attention due to the prospect of novel applications. For the efficient introduction of foreign DNA into cells, a DNA extractor is required. In this work, our primary attention was focused on a fluid device with tethered NIPAAm brushes as a media on the silicon surface to capture and release DNA molecules from human blood for clinical disease diagnosis. At a temperature of 25 °C, the tethered PNIPAAm stretched its polymer chains to form a water boundary layer on the hydrophilic surface as the specimens flowed through the channels, because of the water-solubility of the tethered PNIPAAm. Furthermore, DNA molecules are extremely water-soluble because of their ability to hydrogen bond with water. Water molecules could be regarded as connectors between the gDNA molecules and the PNIPAAm brushes at 25 °C. Therefore, the gDNA molecules were captured by the hydrophilic surface, mediated by hydrogen bonding through the water molecules to the tethered PNIPAAm at the water boundary layer. Meanwhile, other biomacromolecules flowed out from the channels. This system can, therefore, be regarded as a DNA capture process. Upon heating to 60 °C, the tethered PNIPAAm collapsed because of its resulting enhanced hydrophobicity, decreasing the thickness of the water boundary layer. The previously bound gDNA molecules were released from the tethered PNIPAAm at 60 °C because of diminished hydrogen bonding between water molecules and the amido groups of the grafted PNIPAAm, causing the bound water and gDNA molecules to separate from the surface—a DNA release process. The released DNA molecules from the tethered PNIPAAm at T > 40 °C were amplified with specific primers by polymerase chain reaction (PCR) to detect the target DNA through agarose gel electrophoresis. In addition, hgDNA584 is considered probably one kind of DNA, a genomic disease index of breast cancer. It has a specific sequence in human genomic DNA molecules, so we chosen hgDNA584 to be the target DNA in our work.

2. 2.Experimental

2.1. Materials

2.1.1. Fabrication of the substrate with multi-layer thin film

Single-crystal Si wafers, Si(1 0 0), polished on one side (diameter: 6 in.) were supplied by Hitachi, Inc. (Japan). The Si substrates were immersed in HF solution (50 wt%) for 5 min at room temperature to remove the native oxide film. The HF-treated substrates were immersed in a mixture of HNO_3 and H_2O_2 (2:1, mol%) for 5 min and then rinsed with doubly distilled water al least five times to oxidize the Si. The schematic in Fig. 1 illustrates the specific DNA capture and release mechanism by tethered PNIPAAm through temperature tuning. After soft-etching at 200 W in Ar plasma, the aluminum thin film was deposited through radio frequency (RF) reactive sputtering (ULVAC Sputter SBH-3308RDE) by using Ar as the gases under 1500W of RF power for 20 min. The base pressure in the PVD chamber was 5×10^{-7} Torr and the target-to-substrate distance 150 mm. The thickness of the aluminum layer on the silicon surface was about 20 μm. A nitride thin film was deposited by Plasma-enhanced chemical vapor deposition (PECVD, 100 PECVD cassette system) under the mixture gases of SiH_4 and N_2 on the aluminum thin film surface to be a buffer layer. Silicon oxide thin film was sequentially deposited on the nitride layer by PECVD under tetraethyl orthosilicate (TEOS) for reacting with the initiator of atom transfer radical polymerization (ATRP). The thicknesses of the nitride and silicon oxide were about 150 and 180 nm, respectively. Finally, the channels of fluidic device were fabricated by semiconductor processes as shown in Fig. 2.

2.1.2. Synthesis of the tethered PNIPAAm

3-Aminopropyltriethoxysilane (AS) and 2-bromo-2-methylpropionyl bromide (BB), N-isopropylacrylamide (NIPAAm), copper(I) bromide, copper(II) bromide, triethylamine (TA), and 1,1,4,7,7-pentamethyldiethylenetriamine (PMDET), were purchased from Acros Organics. Styrene, PMDET, AS, and BB were purified through vacuum distillation prior to use. All other chemicals and solvents were of reagent grade and purchased from Aldrich Chemical. Scheme 1 illustrates synthetic route of tethered PNIPAAm brushes on silicon surface with fluidic channels through ATRP. To immobilize the ATRP initiator, the Si substrate was immersed in a 0.5% (w/v) solution of AS in toluene for 2 h at 50 °C. The sample was immersed in a solution of BB and TA (both 2%, v/v) in tetrahydrofuran (THF) for 8 h at 2 °C. After completing the reaction, the wafer was placed in a soxhlet apparatus to remove any non-grafted material. This procedure resulted in a surface of AS–BB for ATRP. The functionalized Si substrates were removed from the solution, washed with toluene for 15 min to remove any unreacted materials, dried under a stream of N_2, and subjected to surface-initiated polymerization reactions. Finally, the surfaces were dried under vacuum and stored under a dry N_2 atmosphere. For the preparation of tethered PNIPAAm brushes on the Si–AS–BB surface, NIPAAm, Cu(I)Br, $CuBr_2$, and PMDET were added to dimethylformamide (DMF) [30]. The solution was stirred and degassed with Ar for 15 min at 90 °C. The Si–AS–BB substrate was then added to the solution. After various polymerization times, the wafers were placed in a soxhlet apparatus to remove any unreacted monomer, catalyst, and non-grafted materials. The surfaces were then dried under vacuum at 80 °C for 20 min. The polymer-modified Si surfaces were analyzed using ellipsometry (SOPRA SE-5, France) and X-ray photoelectron spectroscopy (XPS; Scientific Theta Probe, UK). In addition, samples of "free" PNIPAAm were synthesized in solution under the same conditions as those used for grafting polymerization to provide polymers having the same molecular weights of PNIPAAm as the brushes grafted on the Si substrate. The free PNIPAAm was analyzed using gel permeation chromatography (GPC), performed using a VISCOTEK-DM400 instrument and an LR 40 refractive index detector. Monodisperse polystyrene standards (Polymer Lab, Agilent) were used to generate the calibration curve. The monomer conversion was determined gravimetrically. The morphology of the tethered PNIPAAm brushes were measured using atomic force microscopy (AFM; Veeco Dimension 5000 scanning probe microscope) and high-resolution scanning electron microscopy (HR-SEM; JEOL