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Size-controllable quartz nanostructure for signal enhancement of DNA chip

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ABSTRACT

A mask-free, cost-effective dry-etching method for the fabrication of height- and spacing-controlled, pillar-like nanostructures was established in order to detect DNA molecules. The height and spacing of the quartz nanostructure were regulated by successive O_2 and CF_4 reactive ion etching times. The height and spacing of the nanostructures were tuned between 118 and 269 nm and between 107 and 161 nm, respectively. Probe DNA was immobilized on the structure and hybridized with fluorescently-labeled target DNA. Increases in the height and spacing of the nanopillar structure positively correlated with the fluorescence intensity of bound DNA. Usage of the nanostructure increased the DNA detection limit by up to 100-fold.

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1. Introduction

Recently, numerous studies on fabrication of nanostructures and their applications to biotechnology have been reported (Kaji et al., 2003; Ogawa et al., 2007; Oillic et al., 2007b; Rosi and Mirkin, 2005; Anandan et al., 2006). A nanopattern of well-defined height and spacing generally offers several advantages for improving the sensing ability of a biosensor with several reasons. The first is increased surface area with high aspect ratio for the immobilization of more sensing probes. Second, appropriate spacing between the immobilized probes on the nanostructure enhances the accessibility of target materials (Oillic et al., 2007b). Finally, in an optical sensing system, a patterned surface can reduce the quenching effect of fluorescent signal materials by controlling immobilization and spacing. The fabrication of silicon-based high-aspect-ratio nanostructures is performed either by nanolithography followed by deep RIE (Reactive Ion Etching) or nanomolding (Choi et al., 2009; Fu et al., 2009). However, such methods still remain costly and problematic in the point that those methods still require expensive masks or master molds, and the cost for fabricating a mask or a master mold increases exponentially as the required resolution gets smaller and smaller in nanometer scale. Therefore, production of a nanopattern with well-defined height and spacing via a simple low-cost method is a crucial requirement for the successful construction of a highly sensitive biosensor system.

We previously developed an effective method for fabricating high-aspect-ratio pillar-like nanostructures on a quartz surface (Lee et al., 2010). Our method finely controls the spacing and height of the resulting nanopattern in nanometer-scale resolution over several centimeters by simple two-step reactive ion etching (RIE) with O_2 and CF_4 plasma without any expensive mask, additional equipment or complicated technology. The spacing was controlled by the O_2 RIE time, and the height and shape of features in the nanopattern were mainly controlled by CF_4 RIE time.

DNA chips, also called as DNA microarrays, have been developed to analyze the concentration of specific DNA of which the sequences are related to genetic disease, pathogenic microorganism, or gene expression (Bittel et al., 2005; Cho et al., 2006; Ito et al., 2007; Wen et al., 2004). This technology using immobilized DNA oligonucleotides allows highly parallel analysis by hybridization process, after which the DNA chip is analyzed by various methods such as surface plasmon resonance, electrochemical signaling or fluorescence level (Ahmed et al., 2007; Bin Lim et al., 2008; Lao et al., 2009; Wakai et al., 2004). Although DNA chips have great potential as a high-throughput detection method, their sensitivity on planar substrates is not particularly high due to the limitation of mixing efficiency and probe immobilization capacity (Oillic et al., 2007a). Therefore, pillar-like nanostructures have been synthesized on solid substrate for the detection of biomolecules (Kuwabara et al., 2008; Murthy et al., 2008; Park et al., 2009). In this study, we controlled the height and spacing of pillar-like nanostructures and examined their impact on DNA detection sen-

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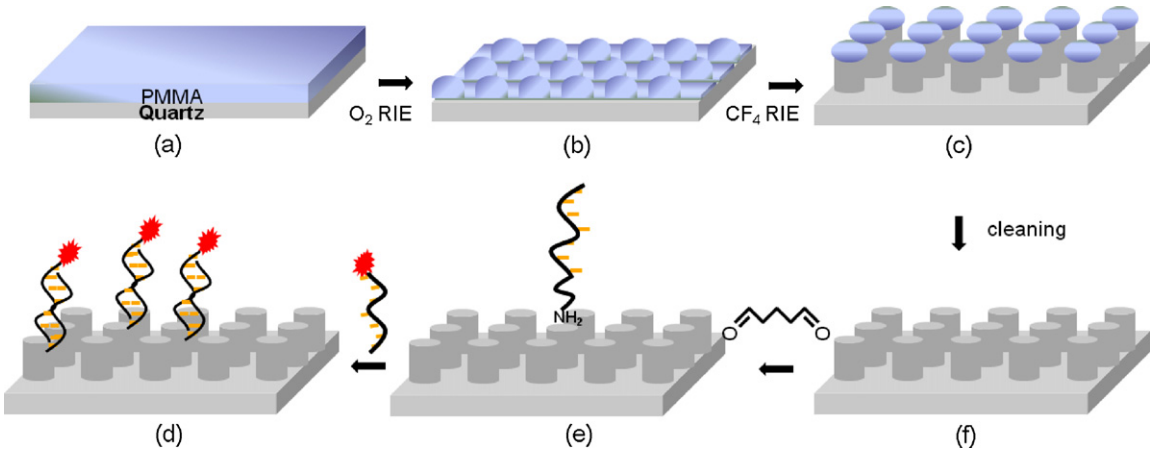

Fig. 1. Schematic diagram of the DNA chip experiment on nanopillar structure: (a)–(d) fabrication process of the nanopillar structure and (e)–(f) DNA chip experiment.

sitivity. By adjusting the O_2 and CF_4 plasma treatment time of the two-step RIE procedure, the morphology of the structure was successfully modified. After immobilization of probe DNA and hybridization with fluorescently-labeled target DNA, the effect of morphology changes on signal intensity and detection limit were examined.

2. Materials and methods

2.1. Nanostructure fabrication

Various quartz nanostructures were fabricated according to previously reported procedures (Lee et al., 2010). Fig. 1(a)–(d) shows brief schemes of the nanostructure fabrication procedures. (a) A 0.5 mm thick quartz wafer (Buysemi, Seoul, Korea) was spin-coated with PMMA A8 (Microchem, USA) resist. The thickness of the PMMA layer was estimated to be about 500 nm. (b) The resist-coated quartz substrates were exposed to O_2 RIE for 1 min, 2 min or 3 min. During this process, dot-like nanostructures of PMMA resist was formed and served as seeds for the deposition and growth of C_xF_y polymer during the following CF_4 RIE, functioning as self-masks resistant to chemical dry-etching. The spacing of the dot-like nanostructure increased depending upon the exposure time to O_2 RIE. (c) CF_4 RIE was performed for 2 min, 5 min or 10 min. The height of the nanostructure increased as the exposure time to CF_4 RIE increased. (d) Finally, after soaking in ethyl acetate for 13 h and drying, the outermost organic layer was removed by heating at 900 °C for 1 h followed by a series of cleaning processes: cleaning with piranha solution, washing with deionized (DI) water, and blowing with nitrogen gas. The nanostructures were observed using field emission scanning electron microscopy (FE-SEM; Jeol JSM6701F, Japan).

2.2. Immobilization of probe

Clean nanostructure quartz or planar quartz slides were placed in a 12-well cell culture cluster (Corning, Corning, NY 14831, USA) and aminated by incubation with 1 mM 3-aminopropyldiethoxysilane (APS) (Gelest Inc., USA) in toluene for 1 h. The quartz nanostructure was then rinsed with pure toluene and dried with nitrogen gas. To induce aldehyde functional groups on the surface, each sample was soaked in 400 μl of 25% glutaraldehyde (Sigma–Aldrich, St. Louis, MO, USA) solution in 1 M phosphate buffer (pH 7.4). After 2 h reaction at room temperature, the substrates were washed twice with deionized water and phosphate buffered saline (PBS) solution (Sigma–Aldrich). One of the aldehyde functional groups of glutaraldehyde reacted with the aminated quartz nanostructure while the other was left open to the surface.

Amine-labeled probe DNAs were immobilized on the aldehyde functionalized substrates. Three DNA probes were synthesized for this purpose from Genotech, Inc. (Daejeon, Korea). Perfect matched (PM) probe was synthesized with a complementary sequence to part of the 5S rDNA of *Legionella pneumophila*. A three-base-pair mismatched (MM) probe and a non-matched probe were synthesized as well. The sequences of oligonucleotides were as follows.

Perfect matched (PM) probe: 5′-amino (C6)-ATCCTGGCGATGACCTACTTT-3′
Mismatched (MM) probe: 5′-amino (C6)-ATCCTGGCGTACACCTACTTT-3′
Non-matched (NM) probe: 5′-amino (C6)-TTGCAAAGCTTCTGTCCATTC-3′

The probe DNAs were dissolved in 1× TE buffer (1 M Tris and 0.5 M EDTA at pH 8.0) to concentrations of 100 nM, after which the substrates were soaked in 400 μl of probe solution for 2 h followed by washing with deionized water and PBS solution.

2.3. Hybridization

To decrease non-specific binding, each substrate was soaked for 1 h in 500 μl of 10 mg/ml bovine serum albumin (BSA) purchased from Sigma–Aldrich. Subsequently, the substrates were rinsed with deionized water and PBS solution. Then, various concentrations of target DNA (5′-Cy3-AAAGTAGGTCATCGCCAGGAT-3′) in TE buffer containing 50 mM sodium chloride were placed on the substrate. After 14 h incubation at 60 °C, the substrates were washed with deionized water and PBS solution. The substrates were then dried by nitrogen gas. The fluorescence intensities of substrates were detected and quantified using the microplate readers SpectraMax GeminiEM (Molecular devices, CA, USA). The intensity of the substrate was measured at nine different points and then averaged. Before calculating average intensity, any outlier data that had intensities outside of average ± standard deviation were removed.

3. Results

3.1. Preparation of nanopillar array

Fig. 2 shows the SEM images of the obtained nanostructure substrates with various heights and pillar densities (spacing). The

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