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Label-free detection of DNA hybridization using nanopillar arrays based optical biosensor

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ABSTRACT

In this study we fabricated nanopillar arrays (NPLAs) of silicon, through a process involving very-large-scale integration and reactive ion etching, for use as two-dimensional periodic relief gratings on silicon surfaces. Oligonucleotides were successively immobilized on the pillar surface, allowing the system to be used as an optical detector specific for the targeted single-stranded DNAs (ssDNAs). The surfaces of the oligonucleotides-modified NPLAs underwent insignificant structural changes, but upon hybridizing with target ssDNA, the NPLAs underwent dramatic changes in terms of their pillar scale. Binding of the oligonucleotides to the NPLA occurred in a way that allowed them to retain their function and selectively bind the target ssDNA. We evaluated the performance of the sensor by capturing the target ssDNA on the NPLA and measuring the effective refractive index (n_{eff}). The binding of the target ssDNA species to the NPLA resulted in a color change from pure blue to red, observable by the naked eye at an angle of 15°. Moreover, we used effective medium theory to calculate the filling factors inside the NPLA and, thereby, examine the values of n_{eff} during the structural changes of the NPLA. Accordingly, these new films have potential applications as label-free optical biosensors.

1. Introduction

Recent advances and demands in the field of molecular diagnostics, molecular medicine, and forensics have propelled the development of highly selective and sensitive nucleotide sensors. Reduced sample volumes and low analyte concentrations have presented a challenge to the current detection technology. DNA hybridization-based detection is a major technique for the diagnosis of genetic disease, where clinical symptoms are linked to DNA alterations [1]. DNA hybridization is highly specific and becomes very sensitive when coupled with an optical detection scheme. Optical detection schemes usually require some kind of labeling such as the use of molecular beacons [2] or fluorophore tagging [3]. Typically, organic fluorophores and quantum dots are used for signaling the detection of the hybridization of DNA [4]. These fluorophores are usually excited at shorter wavelengths and emit at longer wavelengths. The labeling process could sometimes become very challenging, costly, and time-consuming, and may lead to occlusion of hybridization sites due to steric hindrance. Thus, label-free detection schemes would bring about the obvious benefits of being simple, quick, easy, and cost-effective [5]. There have been a number of label-free optical detections reported in the literature, such as well-known surface plasmon resonance (SPR), and conjugated polymers amplification with the use of intercalating dye, among others [6].

Among the many available biosensing platforms, optical diffraction-based biosensors are effective for recognizing the binding events of various biomolecules; these systems operate based on changes in effective height or refractive index on periodically patterned gratings [7,8]. Recently, several different types of optical biological sensors have been reported that function through the measurement of diffraction patterns as the assay readout; alternatively, these optical detection methods could employ surface plasmon resonance (SPR) [9], ordered structures [10], surface acoustic waves [11], and fiber optical techniques [12]. The sensor described in that earlier paper, however, the reproducibility of the inactivation of the scale-up of fabrication would be problematic when using that approach. Moreover, the change in refractive index across the interface as a result of protein adsorption was small. Although optical reflectometry, SPR, and ellipsometry base their measurements on detecting changes in refractive index and they can provide useful estimates of degrees of adsorption, they have little inherent sensitivity to the layer thickness or composition [13,14]. In many studies, the detection of small amounts of

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biomolecules require additional signal enhancement [15], which can be accomplished through either microfabrication of solid diffraction gratings or self-fabrication of in situ-assembled diffraction gratings from nano- or micro-size particles [16].

Nanopillar array (NPLA) surfaces are attractive components of new optical elements; indeed, many different elements presenting such surfaces have been developed previously. Through careful design of the NPLA structure, highly efficient diffraction gratings can be synthesized, even with a binary profile. When the NPLA element is a one-dimensional (1D) periodic surface or an unsymmetrical two-dimensional (2D) periodic surface, the effective refractive index depends upon the polarization states of the incident light (Fig. 1) [17,18]. Symmetrical 2D periodic surfaces are currently attracting great interest for their potential applications in gratings or photonics [19]. The 1D geometries of NPLAs can result in unique optical properties, including polarized photoresponses, and biosensing [20]. When the NPLAs interact specifically or nonspecifically with target molecules, changes typically occur to the geometrical parameters of the gratings and/or the refractive index contrast [21]. These materials have been exploited extensively in various technological applications ranging from sensitive chemical and biological sensing and nano-optical devices to magnetic memories and logic circuits [22]. Many top-down and bottom-up technologies have been developed for the fabrication of functional NPLAs [23,24]. Very-large-scale integration (VLSI) is currently a popular top-down technique for fabricating nanopillars possessing arbitrary structures [25]. Because the appearance of 1D periodic relief gratings (1DPRGs) can be quite sensitive to the observation angles α and β (Fig. 1) under transverse magnetic (TM) and transverse electric (TE) polarization, they always display composed color images [7,10,26]. Unlike 1DPRGs, the color of an NPLA, as observed by the naked eye, is dependent solely on the angle β, thereby providing improved stability and chromatic aberration without restricting the visual angles. The immobilization of oligonucleotides onto the surface of NPLAs and the subsequent capture of target single-stranded DNAs (ssDNAs) from sample solutions can change the geometrical parameters and refractive indices of the gratings [27]. In this present study, we used VLSI techniques to generate NPLAs of silica for use as 2D periodic relief gratings, thereby increasing the interface arising from biomacromoles and to enhancing the optical sensitivity. The detection of the oligonucleotides bound to the NPLA nanostructures has been achieved by illuminating the surface with visible light because the degree of diffraction is influenced by the binding of the biological material. Specifically, the target ssDNA-induced nanostructural variation in the NPLA system enables large-scale morphological reorganization, resulting in changes in the effective refractive index. This versatile process is particularly amenable to the creation of large-area uniform coatings on essentially any surface, with precise control over the pillar scale of silicon and the optical properties of the target ssDNA.

2. Experimental

2.1. Materials

Single-crystal Si wafers, Si(1 0 0), polished on one side (diameter: 6 inch) were supplied by Hitachi (Japan) and cut into 1.5 cm × 1.5 cm samples. To remove dust particles and organic contaminants, the Si surfaces were ultrasonically rinsed sequentially with methanol, acetone, and dichloromethane for 10 min each and subsequently dried under vacuum. The materials used for fabrication and characterization of the oligonucleotides modified NPLAs were purchased from Acros Organics and used without further purification. Fresh 3-(mercaptopropyl)trimethoxysilane (MPTES), purchased from Acros Organics, was used since it is subject to oxidation of the SH groups and crosslinking of the molecules due the hydrolysis of silanol groups when humidity condenses inside the bottle. Thiolated oligonucleotides were purchased from Synthetic Genetics (San Diego, CA). Two kinds of oligonucleotides that were immobilized onto the MPTES layer possessed the sequence 5′-HS-C_{18}-ATGACTAAGCTGTTACTACGG-3′ (FS) and 5′-HS-C_{18}-TTATTCAGCTCCAATTATTATAT-3′ (RS), forward and reverse complementary sequences of *S. aureus* panC gene (GenBank accession number YP_501370.1), respectively [28]. All other chemicals and solvents were of reagent grade and purchased from Aldrich Chemical.

2.2. Oligonucleotides immobilization on NPLA

The basic strategy for the fabrication of the oligonucleotides-modified NPLA using the VLSI process and reactive ion etching (RIE) is depicted in Fig. 2. (A) The Si wafer was treated with hexamethyldisilazane in a thermal evaporator (Track MK-8) at 90 °C for 30 s to transform the OH groups on the surface of wafer into an inert film of $Si(CH_3)_3$ groups for photoresist coating [29]. Negative photoresist was spun onto the HMDS-treated Si wafer; advanced lithography was then used to pattern the photoresist as an NPLA after development. (B) RIE (Plasma Etcher, TEL TE5000) was used for the dry etching process, with the photoresist NPLA on the silicon wafer employed as a protection mask. Only the exposed regions of silicon were dry-etched by supplying a gas mixture of CHF_3 and CF_4 under RIE processing conditions at an etching rate of 475.2 nm/min. (C) Subsequently, the remaining photoresist hard mask was removed from the surface through immersion in solvents, leaving behind an NPLA of silicon. (D) The silicon NPLAs were then immersed in the mixture of HNO_3 and H_2O_2 (2:1, mol%) for 10 min and subsequently rinsed with doubly distilled water a minimum of five times to oxidize the silicon NPLA [30]. (E) The NPLA of silicon oxide was immersed in a solution of MPTES (1.5 wt%) in toluene to assemble the thiol groups on the surface [31]. (F) Thiol-terminated oligonucleotide probes FS and RS were diluted in sodium chloride/sodium citrate (SSC) buffer (3 M NaCl, 0.3 M Na citrate–$2H_2O$, pH 4.5, Aldrich) to a final concentration of between 50 and 200 nM. The oligonucleotide buffered solution was dropped onto freshly MPTES-modified NPLAs using a pipette. These surfaces were subsequently incubated for 16 h at 20 °C in a humidified chamber (54% relative humidity) to prevent spot drying (which can lead to uneven distribution) and then washed three times with water and buffer solution. Finally, the surfaces were dried with nitrogen gas. Commercially available recombinant oligonucleotides were covalently immobilized on the MPTES-modified NPLA surface through disulfide bridges to give oligonucleotides-NPLAs. The sample was cleaned ultrasonically for 90 min to separate any unbounded oligonucleotide, placed in PBS, and then the cleaning process was repeated until no oligonucleotide was recovered in the supernatant. Finally, the resulting NPLA was kept wetted until required for further use, thereby yielding a promising general platform for the specific and sensitive detection of the target ssDNA in terms of the effective refractive index. Target ssDNA of *S. aureus* panC gene and fully immobilized surfaces of FS and RS were subsequently incubated for 6 h at 10 °C in a hybridization buffer solution (consisting of 1.8 M SSC pH 7, Denhardt's solution (1%, w/v BSA (bovine serum albumin), 2% Ficoll 400, and 2% polyvinylpyrrolidone (PVP)), 0.5% sodium dodecyl sulfate (SDS), and sheared salmon sperm DNA (55 μg/mL) to a final concentration of 400 nM). Control ssDNA of *E. coli* cells was also tested in the same condition to evaluate the selectivity of the oligonucleotides-NPLAs.