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Improved surface sensing of DNA on gas-etched porous silicon

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Abstract

A new gas-based etching process has been used to produce porous silicon (PSi) samples as sensor substrates. The as-etched porous material demonstrated an intense photoluminescence signal at an average wavelength of 658 nm ($\sigma = 1\%$). The photoluminescence signal, with a peak wavelength in the range 530–540 nm, was found to increase with the length and concentration of unmodified oligonucleotide probes on surfaces coated with either amino-propyl trimethoxysilane (APTMS) or glycidoxy-propyl trimethoxysilane (GPTMS). The large surface area that characterized this material significantly increased the binding of DNA probes and led to increased sensitivity in target detection. Using fluorescently-labeled DNA, the target capture for sub-micromolar detection on this novel sensor substrate was demonstrated to be 100 times higher than that obtained on non-etched silicon. In a 1 h assay, we were able to detect specifically approximately 3 fmole of a 25mer target oligonucleotide per mm^2 of substrate.

Keywords: Porous silicon; Etching; Photoluminescence; DNA; Silane; Sensors

1. Introduction

Accurate and sensitive DNA detection tools have become pivotal to address fundamental questions relating to gene expression and regulatory biology [1–4], but also for disease diagnosis [5,6], drug discovery [7] and the identification of pathogens present in environmental samples [8]. This growing need to have access to specific DNA sequence information has prompted the development of faster, more sensitive and affordable nucleic acid biosensors [9].

Optical DNA detection methods include the use of various intercalating dyes [10,11] or rely on spectral interference of reflected white light [12,13]. Amperometric or electrochemical biosensors use a variety of electrode types functionalized with DNA to monitor enzymatic reactions occurring upon hybridization of the desired target [14,15]. Some electrical biosensors are based on monitoring the intrinsic molecular charge of DNA on field-effect sensors [16] or upon hybridization of a target molecule on PNA probes immobilized on silicon nanowires [17]. Other detection technologies utilize functionalized gold nanoparticles to detect hybridization events optically [18] or electrically following silver enhancement [19]. Many of the DNA detection strategies deployed today are based on their hybridization to probe molecules immobilized to solid surfaces [20]. The choice of the material and its chemical functionalization is of utmost importance to produce a sensitive and reliable biosensor.

Silicon has been the premier material driving the microelectronics revolution due to its unique electrical, chemical, and mechanical properties. Because of its indirect energy band-gap, bulk silicon is a poor light emitter and this has restricted its use in light-emitting devices and optoelectronics. Porous silicon (PSi), on the other hand, has been demonstrated to yield efficient visible light emission at room temperature [21–23]; such a behavior is primarily attributed to the electron confinement in the nanocrystals that constitute the porous structure [21,24]. Since 1990, the prospect of extending the use of silicon to light-emitting devices and optoelectronics has triggered numerous investigations on PSi and its photo- or electroluminescence properties [21,25]. Furthermore, the large fraction of voids inside PSi results in a very large specific surface area, on the order of a few hundred m^2/cm^3 [26,27]. Such considerable surface area, which provides a large number of potential binding sites, has generated significant interest in PSi for chemical and biosensing applications [21,28–33]. As such, PSi is used as a transducer whose photoluminescence, refractive index, or electrical conductivity

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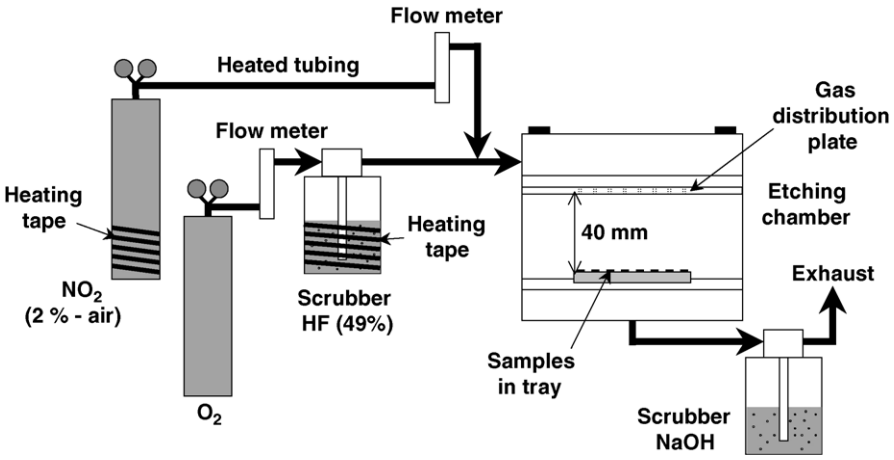

Fig. 1. Schematic of the gas etching setup.

properties, are modulated upon exposure to target molecules. Starodub et al. [34] were able to detect as little as 10 ng/ml of myoglobin using inherent properties of PSi both as a matrix and as a transducer of light.

In this article, we report on the characterization of PSi substrates, fabricated using a new gas etching method, as an excellent surface enhancing material for the development of a sensitive DNA sensor. This new etching technique was developed as part of a novel biosensing platform, Bio-Alloy™[1], which attempted to make use of the unique photoluminescence properties of PSi to detect label-free target agents in real-time. Chemically functionalized PSi was validated as a substrate with increased surface area and sensitivity for DNA detection.

2. Experimental

2.1. Fabrication of porous silicon by gas etching

The gas etching technique consists of exposing silicon samples to a mixture of oxygen (O_2) and nitrogen dioxide (NO_2) gases and an acid vapor. The experimental setup is schematically presented in Fig. 1. 4 mm × 4 mm silicon samples were obtained from the dicing of ⟨1 0 0⟩ boron-doped p-type wafers (Shin-Etsu Handotai), whose electrical resistivity was 20 Ω cm. The samples were loaded onto a tray, which was mounted at the bottom of a chamber. After installing a gas distribution plate, whose role was to improve the uniformity of the gas flow, the chamber was hermetically sealed. The chamber, tray, and distribution plate were made of chemically inert Teflon™[2]. Pure oxygen (99.995%, Air Liquide) was flowed through a heated scrubber containing HF (47–51%, TraceMetal grade, Fisher Scientific), then merged with a flow of diluted nitrogen dioxide (2% in air, MEGS) before entering the etching chamber. The outlet of the chamber was connected to a scrubber containing a 2 N sodium hydroxide (NaOH) solution made from electrolytic pellets (certified ACS grade, Fisher Scientific). The role of the NaOH solution was to neutralize the HF. The O_2 and NO_2 gas flow rates could be varied in the range of 100–500 ml/min and 10–50 ml/min, respectively, using flowmeters (Cole-Parmer), while the HF scrubber could be kept at room temperature or heated up to 70 °C. The NO_2 cylinder was heated at its base to a temperature of 40 °C to avoid accumulation of nitrogen dioxide at its bottom and to enhance the mixing of NO_2 and air. Similarly, the stainless steel tubing connecting the NO_2 cylinder to the chamber was heated to a temperature of 30 °C to avoid condensation of NO_2 on the tubing wall. For all the investigations reported in this article, samples were etched for 30 min. Following the etching process, the samples were rinsed using ethyl alcohol (95%, undenatured grade, Commercial Alcohols); the substrates were dipped in ethyl alcohol for 5 min, then removed and left to dry in a nitrogen environment for about 30 min.

Prior to gas etching, the 4 mm × 4 mm silicon samples were first cleaned using RCA-type hydrogen peroxide mixtures [35], etched in a 5% HF solution for 2 min, rinsed in deionized water for 5 min, then oxidized at room temperature in a 3 SLM flow rate of ozone (O_3) gas (280 g/N m^3 concentration in nitrogen) for 5 min. At the end of the oxidation step, the samples were immediately loaded in the chamber and the etching was started. This cleaning sequence allowed a stringent control of the sample surface, yielding a hydrophilic surface. Spectroscopic ellipsometry measurements (Scientific Computing International, FilmTek 2000SE) revealed an oxide film thickness comparable to that of silicon native oxide (<2 nm).

The morphology of the porous layers was investigated by scanning electron microscopy (SEM, Hitachi, S-4700), while their photoluminescence properties were characterized using a photo-detection system (Fluorosense, PDS) whose excitation source was a diode laser (λ = 470 nm), with an on-sample spot size of about 2 mm. A 500 nm longpass filter was inserted at the entrance of the spectrometer to eliminate any contribu-

[1] Bio-Alloy is a registered trademark of IatroQuest Corporation.
[2] Teflon is a registered trademark of DuPont.