Influence of Biomolecule Size on Performance of Nanostructured Sensing Devices

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ABSTRACT

Porous silicon is an excellent material for biosensing because of its large surface area and ability to filter out large contaminant species. In order to characterize the sensitivity of porous silicon based biosensors for biomolecules of different sizes, a mesoporous silicon waveguide with average pore diameter of 20 nm is used to detect single strand DNA oligos with different numbers of base pairs at different concentrations. Experimental results indicate that 16-mer DNA is detected most sensitively with the mesoporous silicon waveguide.

Keywords: Porous silicon, waveguide, DNA detection, sensitivity, pore infiltration

1. INTRODUCTION

Porous silicon is widely considered to be a promising material for biosensing applications: it can immobilize a large number of biomolecules within its large surface area and it has advantages in selectivity due to its capability for size-selective infiltration. There have been many porous silicon thin film devices proposed for label-free biosensing applications, including a single porous silicon layer interferometer, a porous silicon rugate filter, a multi-layer porous silicon Bragg mirror, a porous silicon multi-layer microcavity, and a porous silicon waveguide. These label-free porous silicon biosensors are based on the refractive index change of porous silicon due to biomolecular binding events. In order to detect a range of analytes with different biomolecular sizes and weights, it is necessary to tune the pore size and morphology, for example, from mesoporous to macroporous silicon. The most sensitive detection is achieved only when the pore size is appropriate compared with the biomolecule size. If the pores are too small, the biomolecules will not infiltrate into the pores and the large surface area is not utilized for sensitive detection. On the other hand, if the pores are too large, the biomolecules will occupy such a small volume of the pores that the refractive index change of the porous silicon is not large enough to detect. A porous silicon biosensor with a given pore size will have very different sensitivities for the detection of biomolecules of different sizes.

To determine the size dependent sensitivities of porous silicon based biosensors, we use a mesoporous silicon resonant waveguide to detect the presence of different lengths of DNA. Mesoporous silicon is chosen due to its appropriate size range for sensitive DNA detection. The mesoporous silicon has an average pore diameter of approximately 20 nm and the DNA strands have lengths between 1.8 and 5.3 nm (for DNA between 8 and 24 base pairs). The use of probe and target DNA is a powerful model system: DNA molecules will bind only to its complementary strand and non-complementary strands result in minimum binding. DNA molecular length can be easily manipulated by adjusting the number of base pairs. The porous silicon waveguide is an excellent nanoscale sensing platform because it is a very thin structure of only a few hundred nanometers (fast response), and possesses strong field confinement (high sensitivity).

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2. FABRICATION OF THE POROUS SILICON WAVEGUIDE BIOSENSOR

2.1. Porous silicon waveguide structure

In general, an optical waveguide consists of three layers: a high refractive index layer sandwiched between two lower refractive index layers. Total internal reflection at the interfaces between the high and low refractive index layers enables light to be guided in the high index layer. The thickness of the high index layer determines the number of modes supported by the waveguide. As shown in Figure 1, the porous silicon waveguide consists of two porous silicon layers: a low porosity (high index) layer is the waveguiding layer, and a high porosity (low index) layer is the cladding layer. The air gap above the low porosity layer provides mode confinement at the top waveguide interface. A prism is used to evanescently couple a laser beam into the waveguide at a specific angle $\alpha$, at which the horizontal component of the incident beam’s wavevector matches that of the waveguide mode, and launch the 0th order TE mode. A detector placed at the output face of the prism detects a minimum in reflectance at this coupling angle due to the coupling of light into the waveguide. Biomolecules infiltrated into the pores of the waveguide change its refractive index and change the coupling angle at which the waveguide mode is launched. In this work, the coupling angle is experimentally measured by a Metricon 2010 prism coupler.

Figure 1. Schematic of porous silicon waveguide biosensor, consisting of a low porosity (high refractive index) layer, a high porosity (low refractive index) layer, and air gap. Total internal reflection enables waveguiding in the low porosity porous silicon layer. A prism is used to couple light at a specific angle $\alpha$ into the waveguide mode through an evanescent wave. Biomolecules are infiltrated into the porous silicon waveguide, increasing its effective refractive index and changing the angle at which light is coupled into the waveguide.
2.2. Etching of a porous silicon waveguide

Porous silicon is a nanostructured material consisting of air pores in a silicon matrix. It can be fabricated in a straightforward manner by electrochemical etching of a silicon wafer in hydrofluoric acid electrolyte. The resulting porous silicon characteristics, such as porosity and pore morphology, depend on the wafer doping, the electrolyte composition, the current density applied during etching, and illumination conditions. Porous silicon can have pore sizes ranging from a few nanometers to several microns. The IUPAC (International Union of Pure and Applied Chemistry) guidelines define ranges of pore size as micropore ($\leq 2$ nm), mesopore (2-50 nm), and macropore (>50 nm). In this work, mesoporous silicon is used due to its appropriate size range for DNA detection. Mesoporous silicon can be fabricated by electrochemical etching of boron doped $p^+$ silicon with a 0.01Ω·cm resistivity and $<100>$ growth direction. The electrolyte is 15% hydrofluoric (HF) acid, which is composed of 175 mL 99% ethanol and 75 mL 50% aqueous HF. Before anodization, the silicon wafer samples were first cleaned by rinsing with 15% HF solution to remove the native oxide. The samples were then oxidized at 800°C in ambient air (20% oxygen) for 30 minutes. The oxide formed was then removed by soaking the wafer in 15% HF for 1 min, followed by ethanol rinsing. Then the waveguide structure was etched, using the conditions specified in Table 1. Also given in Table 1 are the porosities of the two porous silicon layers measured by weight, their 2D Maxwell Garnett effective refractive indices, and their thicknesses measured by Scanning Electron Microscopy (SEM).

<table>
<thead>
<tr>
<th>Layer</th>
<th>Current Density (mA/cm²)</th>
<th>Porosity (%)</th>
<th>2D Maxwell Garnett Effective Refractive Index</th>
<th>Etching Time (seconds)</th>
<th>Thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waveguide Layer</td>
<td>5</td>
<td>60%</td>
<td>1.98</td>
<td>42</td>
<td>240 nm</td>
</tr>
<tr>
<td>Cladding Layer</td>
<td>48</td>
<td>84%</td>
<td>1.41</td>
<td>60</td>
<td>1500 nm</td>
</tr>
</tbody>
</table>

After etching, the samples were soaked in 1.5 mM KOH solution (1:5 by volume of 9 mM aqueous KOH and ethanol) for 30 minutes to open up the pores by 15-20% in order to ease the infiltration of DNA molecules. Table 2 gives the porosity, the 2D Maxwell Garnett effective refractive index, and thickness for each porous silicon layer after KOH soaking, which results in larger pores as shown by the larger porosities for the two porous silicon layers. The resonance spectra measured by a Metricon 2010 prism coupler after anodization and after KOH soaking are shown in Figure 2. The KOH soaking results in a shallower resonance due to oxidation of the porous silicon waveguide by KOH and, thus, the change of the porous silicon waveguide parameters away from optimum.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Porosity (%)</th>
<th>2D Maxwell Garnett Effective Refractive Index</th>
<th>Thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waveguiding Layer</td>
<td>69%</td>
<td>1.77</td>
<td>240 nm</td>
</tr>
<tr>
<td>Cladding Layer</td>
<td>88%</td>
<td>1.32</td>
<td>1500 nm</td>
</tr>
</tbody>
</table>
2.3. Porous silicon waveguide functionalization

In order for the porous silicon waveguide to detect biomolecules, functionalization steps are carried out to link organic molecules to the inorganic silicon surface. Probe molecules must be immobilized inside the pores by means of bio-crosslinking procedures in order for the waveguide biosensor to selectively detect only the target biomolecules. Here we describe the functionalization method often used for the detection of DNA oligos. First, the porous silicon waveguide was oxidized in order to lower the waveguide loss and to prepare the surface for subsequent chemical functionalization. The samples were placed in the furnace and heated from room temperature to 800°C under ambient air (20% O₂) in a process of 50 min. The samples were then oxidized at 800°C for 30 min. After the furnace was cooled down to below 700°C, the samples were taken out. A shift in the resonance to lower angle is observed after oxidation (see Figure 3 and compare with Figure 2). This is due to the lower effective refractive index of the waveguide layers when part of the silicon pore walls is converted to silicon dioxide. A lower effective index waveguide mode can be supported and light couples into the mode at a smaller angle of incidence.
After thermal oxidation, the porous silicon waveguide samples were silanized with 3-aminopropyltriethoxysilane (3-APTES) in (1:1) DI water and methanol for 20 minutes (see Figure 4 for reaction). The samples were then rinsed with DI water and baked at 100°C for 10 min. The resonance shift after silanization can be seen in Figure 3. Figure 5 shows the angular resonance shifts after exposing the oxidized waveguide to 100 µL of different concentrations of 3-APTES. As seen from the figure, the porous silicon waveguide sensor distinguishes between the different concentrations. Saturation of the resonance shift at greater than approximately 2% 3-APTES suggests a monolayer coating of 3-APTES is formed on the pore walls.

Next, the amine-derivatized porous silicon waveguide was exposed to 2.5 mg/ml sulfo-SMCC in HEPES buffer (20 mM HEPES, 150 mM NaCl, 5 mM EDTA, pH 7.4) for 2 hours, followed by a 1 hour soaking in HEPES buffer, rinsing with HEPES buffer, and drying with nitrogen. The NHS-ester group of sulfo-SMCC reacts with the amine group on porous silicon, and the porous silicon is maleimide derivatized (see Figure 4 for reaction). Because 2.5 mg/ml is the solubility of sulfo-SMCC in water, no concentration optimization for sulfo-SMCC was carried out.

After the porous silicon waveguide samples have been maleimide activated with sulfo-SMCC, the thiol modified probe DNA can be attached. For simplicity, the thiol modifiers on DNA oligos were used as received from MWG Biotech, without performing a reducing procedure that would likely give higher binding efficiency. The porous silicon waveguide samples were exposed to 100 µL of probe DNA oligos of different lengths at different concentrations in HEPES buffer and incubated for 11 hours, followed by a 1 hour soak in buffer, rinsing with buffer, and drying with nitrogen. At this point, the porous silicon waveguide samples were ready to detect only the complementary DNA strand. The experimental detection of complementary DNA strand is in progress.
In order to achieve the highest sensitivity for the mesoporous silicon waveguide biosensors, experiments were performed to determine the relationship between biosensor sensitivity and DNA size. The length of DNA is important since different DNA lengths will have different infiltration efficiencies into the pores and different strengths of transduction. Out of the total 20 nm pore diameter, considering the length of 3-APTES (7-8 Å) and sulfo-SMCC (19 Å) obtained by spectroscopic ellipsometry measurements of derivatized flat silicon wafers, the space left for DNA is approximately 7 nm. Here we test the attachment of 8, 16 and 24 base pair DNA molecules. Given the base to base distance of 2.2 Å for single strand DNA, their length are 1.76, 3.52, and 5.28 nm, respectively, assuming that each strand is fully extended. The sequences are:

Probe 24, 5’-TAGC TATG GAAT TCCT CGTA GGCC-3’
Target 24, 5’-GGCC TACG AGGA ATTC CATA GCTA-3’

Probe 16: 5’-TAGC TATG GTCC TCGT-3’
Target 16: 5’-ACGA GGAC CATA GCTA-3’

Probe 8: 5’-GGGG GGGG-3’
Target 8: 5’-CCCC CCCC-3’

The resonance shifts of the porous silicon waveguide for 8-, 16-, and 24-mer probe DNA at different concentrations were measured using a prism coupler. We also simulated the shift for different lengths of probe DNA at different coverages on the pore walls, using the same method as described in reference 17. The only difference is that the maximum probe DNA density was assumed to be $2.5 \times 10^{13}$ probes/cm$^2$ instead of $4 \times 10^{13}$ probes/cm$^2$ since sulfo-SMCC is a larger cross-linker than the glutaraldehyde used in reference 17. By comparing the experimental and simulated shift, an estimate of DNA coverage for a given resonance shift can be obtained.
Figure 6 shows the porous silicon waveguide resonance shift for different concentrations of 8-mer DNA. It saturates at about 100 μM and the maximum resonance shift is 0.33°, corresponding to coverage of 20%. The sensitivity is found approximately as the steepest slope of the linearly fitted curve below the saturation level, which is 0.003276°/μM. Figure 7 shows the resonance shift for different concentrations of 16-mer DNA. It saturates at 100 μM and the maximum resonance shift is 0.38°, corresponding to coverage of 13%, smaller than 8-mer due to larger probe-to-probe spacing for larger probe DNA molecules. The sensitivity is 0.048930°/μM, more than one order of magnitude higher than 8-mer sensitivity. The negative shift at 10 μM is likely due to corrosion/oxidation of the porous silicon matrix catalyzed by negatively charged DNA molecules, which reduces the refractive index of the porous silicon waveguide and the magnitude of the angular resonance shift upon DNA attachment. We note that DNA is immobilized in the pores even though a negative shift is measured. Figure 8 shows the resonance shift for different concentrations of 24-mer DNA, where all resonance shifts are negative and the shift has not been saturated. At higher 24-mer concentrations, more DNA is attached and a larger resonance shift is measured. The positive shift from DNA attachment is small due to inefficient infiltration and binding of 24-mer DNA and negative shifts due to corrosion/oxidation dominate. The data points can be fitted by a linear curve, with a slope of 0.001300°/μM, which is the sensitivity of detection.

Figure 6. Mesoporous silicon waveguide resonance shifts for different concentrations of 8-mer probe DNA. The smoothed curve is a guide to the eye.
Figure 7. Mesoporous silicon waveguide resonance shifts for different concentrations of 16-mer probe DNA. The smoothed curve is a guide to the eye.

Figure 8. Mesoporous silicon waveguide resonance shifts for different concentrations of 24-mer probe DNA. A linear fit is shown.

The above results suggest that 16-mer DNA is the most sensitive to detect with the current porous silicon waveguide biosensor. While the 8-mer DNA has the largest surface coverage in the pores, the smaller refractive index change induced upon binding allows the lower coverage 16-mer DNA to yield a larger resonance shift. The 24-mer DNA with the smallest resonance shift clearly does not infiltrate into the pores as efficiently as the smaller molecules. During infiltration and attachment, the DNA likely coils and the backbone may lie down on the pore walls, which will result in
lower DNA density in the pores for longer DNA strands. Our simulation did not take into account either the effect of DNA coiling on packing density or the effect of porous silicon corrosion/oxidation. Thus, the actual DNA coverage may be higher than the estimates provided. Target DNA detection experiments are in progress to verify that the mesoporous silicon waveguide is most sensitive for detection of 16-mer target DNA.

4. CONCLUSION

Mesoporous silicon waveguides with 20 nm average pore diameter were used to detect the presence of single strand DNA oligos with different number of base pairs. After comparing the detection sensitivities for different lengths of DNA, we found that the mesoporous silicon waveguide has highest sensitivity for 16-mer DNA detection. The total length of the 16-mer DNA and the molecules used to immobilize the DNA is approximately 6 nm.

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