Biomolecule kinetics measurements in flow cell integrated porous silicon waveguides

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Abstract: A grating-coupled porous silicon (PSi) waveguide with an integrated polydimethylsiloxane (PDMS) flow cell is demonstrated as a platform for near real-time detection of chemical and biological molecules. This sensor platform not only allows for quantification of molecular binding events, but also provides a means to improve understanding of diffusion and binding mechanisms in constricted nanoscale geometries. Molecular binding events in the waveguide are monitored by angle-resolved reflectance measurements. Diffusion coefficients and adsorption and desorption rate constants of different sized chemical linkers and nucleic acid molecules are determined based on the rate of change of the measured resonance angle. Experimental results show that the diffusion coefficient in PSi is smaller than that in free solutions, and the PSi morphology slows the molecular adsorption rate constant by a factor of $10^2$–$10^4$ compared to that of flat surface interactions. Calculations based on simplified mass balance equations and COMSOL simulations give good agreement with experimental data.

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1. Introduction

Quantifying the kinetic properties of biomolecules is essential to understanding many biomolecular mechanisms, from primary processes in biomolecular recognition, to interaction networks in living cells [1]. Among the many existing techniques to measure kinetic parameters, surface plasmon resonance, resonant mirrors, dual polarization interferometry and resonant waveguide grating methods are commonly available commercial ones [2].

Label-free optical biosensors based on the resonant waveguide grating method have emerged over the last two decades as powerful tools that are capable of detecting the interactions of proteins and genes. They have received a great deal of attention for efficient, affordable, accurate, non-invasive and quantitative measurements in real-time [3–12]. These devices utilize gratings to couple light into/out of waveguides, which are functionalized with immobilized probe molecules. When selective molecules attach to the waveguides and bind with the probe molecules, the optical sensing elements can efficiently convert refractive index changes caused by the attachments to a measurable spectral shift. Conventional grating-waveguide biosensors generally rely on evanescent field sensing techniques, which only allow target analytes to bind to planar surface-bound probe molecules [2]. Recently, we introduced the all-PSi grating-waveguide biosensor, which allows molecules to infiltrate into the core of the waveguide where they interact with a guided mode, resulting in a 32-fold enhancement in detection sensitivity [13,14].

In this paper, we use the all-PSi grating waveguide biosensor in conjunction with a PDMS flow cell to facilitate near real-time monitoring of biomolecule attachment events and enable determination of the kinetics parameters of several kinds of biomolecules. Diffusion coefficients, as well as adsorption and desorption rate constants of different sized chemical linkers and nucleic acid molecules are determined based on the rate of change of the measured resonance angle. Both the magnitude of the waveguide resonance angle shift and kinetic parameters are observed to depend on molecule size. In section 2, the basic theory of grating coupled waveguides and kinetics models in porous media are presented. Next, materials,
fabrication and experimental procedures are described in section 3. Finally, experimental results and kinetics analysis of linker and oligo attachments are discussed in section 4.

2. Theory

2.1. Grating-coupled PSi waveguide sensor

The fundamental theory of the grating coupled PSi waveguide sensor, which is fully demonstrated in Ref. [13], can be summarized as follows. An incident light beam is reflected into several diffraction orders by the gratings. When the wave vector of one diffracted beam matches that of a waveguide mode, coupling occurs. The coupling condition can be calculated from the well-known grating equation

$$n_{eff} = n_c \cdot \sin \theta + m \cdot \frac{\lambda_0}{\Lambda}$$

where $n_{eff}$ is the effective refractive index of the waveguide mode, $n_c$ is the refractive index of the cover layer, $\theta$ is the coupling angle, $m$ is the diffraction order, $\lambda_0$ is the wavelength in free space, and $\Lambda$ is the grating period.

When the diffracted light is coupled into the waveguide, the zeroth-order transmitted intensity will drop. In reflection, however, a narrow peak in intensity is concurrently observed. If molecules are attached inside the waveguide and change the effective refractive index of the waveguide mode, the coupling angle $\theta$ will be changed according to Eq. (1). The angular position of the reflection peak will shift, accordingly. Therefore, molecular interactions can be monitored quantitatively by measuring the magnitude of this angular shift.

2.2. Adsorption kinetics in PSi

The two-dimensional model used in this work to analyze adsorption kinetics in PSi takes into account mass transportation and binding reactions between molecules in bulk solution and immobilized groups at the pore walls. The PSi waveguide with flow cell geometry is shown in Fig. 1. For the PSi waveguide biosensor used in this paper, the upper PSi layer (waveguide layer) has a porosity of approximately 64% and is 200 nm deep, while the lower layer (substrate layer) has a porosity of ~87% and is 1500 nm in depth. Measurements were taken in a static state, so the flow velocity in the fluid channel is zero, and only diffusion in the $y$-direction is considered for the mass transportation. The diffusion equation is given as

$$\frac{\partial c(y, t)}{\partial t} = D \frac{\partial^2 c(y, t)}{\partial y^2}, \quad 0 \leq y \leq b$$

with the following initial and boundary conditions:

at $t = 0$, $c = c_b$ for all $0 \leq y \leq b$,

for all $t$, $c\left(y = \frac{b}{2}\right) = c_b$,

at $y = 0$, $\frac{d\Gamma(t)}{dt} = D \frac{\partial c(0, t)}{\partial y} = k_{ads}c(0, t)(\Gamma_{max} - \Gamma(t)) - k_{des} \Gamma(t)$,

where $c(y, t)$ is the concentration of the molecule, $D$ is the diffusion coefficient of the molecule, $c_b$ is the bulk concentration of the molecule in the flow cell, $b$ is the thickness of flow cell, $\Gamma(y, t)$ denotes molecule surface concentration, $\Gamma_{max}$ is the maximum surface concentration, $k_{ads}$ is the adsorption rate constant and $k_{des}$ is the desorption rate constant of the molecule. Since the volume of the flow cell is much larger than that of the PSi waveguide, the diffusion and adsorption of biomolecules into the PSi layers will not significantly affect the solution concentration in the flow cell. Therefore, the boundary condition of Eq. (4) is chosen.
as a reasonable approximation. The boundary condition given in Eq. (5) assumes first order Langmuir kinetics at the PSi top surface [15,16].

Equation (2), together with boundary conditions (3), (4) and (5), can be solved by various numerical methods. Here, since the numerical solutions are complex and time-consuming, we first simplify these equations to yield useful analytical results.

First, consider the assumption that the rate of molecules entering into PSi is unlimited, which suggests that when a molecule arrives at the inlet of PSi, it is absorbed inside the pores immediately. This can be treated as the perfect sink condition, e.g. $c_b(y = 0, t) = 0$. Combining the perfect sink boundary condition with Eqs. (2)–(5), and assuming uniform molecule distribution inside the pores, the molecule surface concentration $\Gamma$, is found to be

$$\Gamma(t) = 2c_b \cdot \sqrt{D \pi} \frac{S_{\text{inlet}}}{S_{\text{PSi}}}$$

where $S_{\text{inlet}}$ is the pore entry area, and $S_{\text{PSi}}$ is the overall internal surface area of a single pore. From Eq. (6), it is apparent that under the perfect sink assumption diffusion dominates the mass balance process.

Next, we consider a second circumstance when molecular binding in the PSi is very slow and the molecules inside the pores are nearly saturated, the concentration within the pores is now approximately equal to the concentration outside, resulting in zero diffusion. Therefore, a stationary layer of molecules with concentration $c_b$ is established near the top of the PSi, such
that \( c(y=0,t) = c_b \). With this boundary condition, the molecule surface concentration \( \Gamma \) can be solved as

\[
\Gamma(t) = \Gamma_{\text{max}} \cdot \frac{k_{\text{ad}} \cdot c_b}{k_{\text{ad}} \cdot c_b + k_{\text{des}}} \cdot \left[ 1 - \exp\left( -\left( k_{\text{ad}} \cdot c_b + k_{\text{des}} \right) t \right) \right] \cdot \frac{S_{\text{PSi}}}{S_{\text{PSi}}}
\]

(7)

For this circumstance, the adsorption and desorption of molecules on the pores’ surface dominate the mass balance process. Again, Eq. (7) also assumes a uniform distribution inside the pore.

During the actual mass transportation process in PSi, molecular dynamics cannot be simply classified into one of the two extreme circumstances. At the beginning of the process, however, molecular transport by diffusion is very rapid, and the molecules within the pores are adsorbed to the surface quickly, which can be approximated by the first, diffusion-dominated circumstance. As time progresses, the diffusion of molecules into the pore slows. The process therefore tends towards the second circumstance. Thus, the above two analytical results are suitable to describe the initial and long-time processes, and allow for preliminary estimation of the diffusion coefficient, adsorption rate constant, and desorption rate constant.

To fill the gap between these two circumstances, we used the finite element method software COMSOL to set up an axially symmetric model (Fig. 1(d)) to simulate the diffusion and attachment process of biomolecules in a single pore: the upper region of the pore (waveguide layer) is 10.0 nm in radius, 200 nm thick and the lower region (substrate layer) has a radius of 11.7 nm and thickness of 1500 nm. A fluidic domain outside the pore (modeling the flow cell, not shown in Fig. 1(d)) is 200 \( \mu \)m in height. In section 4, we will show that the parameters calculated from Eqs. (6) and (7) give good agreement with the results from numerical simulation using COMSOL.

3. Experimental

3.1. Fabrication

The fabricated biosensor is composed of two components. One is a PSi waveguide sample, which is similar to the all-PSi grating-coupled waveguide structure introduced in Ref. [13]. PSi films were fabricated by electrochemical etching of silicon (p-type, 0.01 \( \Omega \cdot \text{cm} \)) in a 15% ethanolic hydrofluoric acid based electrolyte. The two-layer PSi waveguide structure was produced by applying current densities of 5 mA/cm^2 for 80 s and 48 mA/cm^2 for 53 s in sequence, followed by a 30 min soak in 1.5 mmol·L\(^{-1}\) KOH to widen the pore opening and 500\(^\circ\)C oxidation for 5 min to form a thin SiO\(_2\) layer on the surface. A 300 nm film of PMMA 950 photosist was spun onto each PSi waveguide and exposed by a Raith eLINE electron beam lithography tool to form a diffraction grating with a grating period of 1350 nm. After development, the PSi waveguide with PMMA gratings was reactive ion etched (Trion Phantom II) with 30 sccm SF\(_6\) under 100 W RF power and 30 mTorr chamber pressure for 70 s. The remaining PMMA was removed by acetone.

The second component of the device is a PDMS flow cell. It is fabricated using standard soft-lithography techniques [17] with replica molding PDMS (Ellsworth Adhesives, Germantown, WI). The mold was created using a photo-sensitive material (SU-8 2100, MicroChem, MA) patterned through a transparent photomask and positioned over a silicon wafer [18]. A pre-polymer solution of PDMS was then mixed with a curing agent at a 10:1 weight ratio and poured over the mold. After degassing, the PDMS layer was allowed to solidify over the mold at 70\(^\circ\)C for 2 h. The solidified layer of PDMS was then peeled from its mold, and a sharp metal puncher was used to generate holes for the media wells. As shown in Fig. 1(a), the dimensions of the flow channel are 10 × 4 × 0.2 mm.

A thin layer of curing agent as glue was used to bond the PDMS flow cell to the silicon wafer [19]. A glass coverslip as the carrier substrate was spin coated with the curing agent at 5000 rpm. After the surfaces were treated with oxygen plasma, the PDMS was stamped onto the curing agent coated coverslip. Subsequently, PDMS was aligned and transferred onto the
silicon wafer with the PSi. The integrated sample was secured by a mechanical clamp and then baked at 70°C overnight to generate a permanent bonding between the PDMS flow cell and the silicon wafer. The biosensor system was completed by attaching two microbore tubes (Cole-Parmer, Vernon Hills, IL) to the punched holes (Fig. 1(b)).

3.2. NaCl solution test

For an initial performance test, different concentrations of NaCl solutions were injected into the flow cell integrated PSI biosensor. The biosensor was mounted in a Metricon Model 2010/M prism coupler (Metricon Corp., USA) with the prism removed to monitor the angular reflectance in near real-time [13]. Deionized water (DI water, representing the 0% NaCl solution) was first injected into the flow cell to be used for reference. Then, 1%, 2%, 3%, 5%, 7.5% and 10% NaCl solutions were injected in sequence. Reflectance spectra were measured every 90 s. The time resolution was limited by the speed of the rotation stage in scanning from 0 to 45°. DI water was re-injected after the 10% NaCl solution to verify that the system returned to the original, solute-free status.

3.3. DNA hybridization

Complete details of the process used to functionalize the grating-coupled waveguides can be found in Ref. [13]. In this work, all solutions were injected into the flow cell to achieve PSI functionalization. In order to attach probe DNA oligos to oxidized PSi waveguides, two linker molecules are used to modify the SiO2 surface. The first one is 3-aminopropyltriethoxysilane (3-APTES, ACROS, 4%), an organofunctional silane that has a terminal amine group. The other linker is Sulfo-succinimidyl 4-[(N-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC, Pierce, 2.5mg·L⁻¹), which was attached to the aminosilane to provide the necessary maleimide derivatization for subsequent immobilization of thiol-tagged DNA oligonucleotides. After attaching the linker molecules, the integrated PSI biosensor is heated by a back-attached silicone rubber fiberglass insulated flexible heater (OMEGALUX®). The solution is injected and bound to the modified surface. Complementary peptide nucleic acid (PNA, ACG AGG ACC ATA GCT A, BioSynthesis) and non-complementary PNA (GGT TTC TGA TGC TGA C, BioSynthesis) are chosen as the target and mismatch molecules and are used with the same concentration as the probe DNA. A solution of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer and DI water is injected between each molecular step to clean the flow cell and the sample surface. Angular reflectance spectra are measured by the Metricon tool during the binding processes every 75 s, which was the time required to scan the rotation stage from 0 to 37.5°.

4. Results and Discussion

4.1. NaCl solution test measurements

Figure 2(a) shows the resonance angle shifts of the PSi biosensor during the NaCl solution test. Because the higher concentration solutions have larger refractive indices, the resonance angle shift becomes larger as the concentration increases. The return of the resonance angle to the initial position at the end of the experiment after reintroducing a pure DI water solution demonstrates that there is no permanent adsorption of NaCl on the pore walls and that the biosensor is stable during the ~2 hour process. Since the diffusion of NaCl is very rapid immediately after injection of a different concentration NaCl solution, the response time of the biosensor is less than the interval time of the measurement, resulting in discontinuous resonance angles changes after each injection. Figure 2(b) gives the relation between the resonance angle shifts and the concentration. The sensitivity of the biosensor is calculated to be approximately 37° per refractive index unit (37°/RIU) based on this linear relationship.
4.2. Biomolecule kinetics in PSi

In the DNA hybridization experiments, angular reflectance measurements were taken throughout each step in the functionalization process of the PSi waveguide biosensor in order to monitor the binding processes of the biomolecules described in section 3.3. Figures 3 and 4 show the entire dynamic process of the infiltration and attachment of chemical and biological molecules. The figures clearly show the effects of both volume infiltration and molecular surface attachment on the waveguide resonance angle. The molecular attachments are confirmed by the resonance angle shifts measured after the rinsing step that follows each molecular infiltration. In the data set presented, the functionalized PSi waveguide was first exposed to a mismatched PNA sequence before introducing the complementary PNA target sequence to demonstrate the selectivity of the sensing system. Figure 3 shows the resonance angle shift during the attachment processes of 3-APTES and Sulfo-SMCC, which were carried out at room temperature. Figure 4 shows the continued resonance angle shift during exposure of the sensor to probe DNA, mismatch PNA, and antisense PNA molecules at 37°C. The increases in resonance angle following the infiltration of 3-APTES and Sulfo-SMCC, 0.32° and 0.59°, respectively, suggest that both molecules form stable layers on the surface of pore walls after rinsing. The probe DNA attachment resulted in a 0.29° increase in resonance angle, suggesting that fractional coverage on the pores is achieved [20]. A noticeably smaller resonance angle shift of approximately 0.06° is measured following the mismatch PNA infiltration. This non-zero value is attributed to non-specific binding events. It is clear that not all of the probe DNA sites are hybridized after exposure to the mismatch sequence both because of the much smaller mismatch-induced resonance shift compared to the probe-induced resonance shift and because subsequent exposure to complimentary antisense PNA molecules resulted in an additional 0.17° resonance shift. For waveguide sample for which antisense PNA molecules were infiltrated directly after probe DNA attachment, typical resonance shifts were approximately 0.28°. Hence, we anticipate the selectivity of the flow cell integrated PSi waveguide sensors toward the target PNA sequence is approximately 5:1.

In order to extract biomolecule kinetics information, the time-dependent resonance angle change following each molecular infiltration was carefully analyzed. The time-dependent change in the waveguide resonance angle during injection of the 3-APTES solution is shown in Fig. 5(a). Within the time period of the first measured data point, the resonance angle increases significantly as the methanol/DI water mixture inside the PSi is rapidly replaced by the 4% 3-APTES solution, which has a greater refractive index. Diffusion dominates the mass balance process during this interval, and the perfect sink condition can therefore be assumed.
Accordingly, the diffusion coefficient of 3-APTES in the PSi environment is calculated to be $5.41 \times 10^{-9}$ m$^2$s$^{-1}$ from Eq. (6). After this initial short period, the rate of change of the resonance angle decreases in magnitude. At long time periods, slow adsorption on the PSi pore walls dictates the reflectance changes, and, therefore, Eq. (7) provides an appropriate fit to the experimental data during this time. The adsorption and desorption rate constants of 3-APTES in PSi were calculated to be 0.02958 M$^{-1}$s$^{-1}$ and $7.652 \times 10^{-5}$ s$^{-1}$, respectively. For comparison, an experiment was conducted to monitor the 3-APTES binding process on a flat silicon surface using a laser reflectometry approach that is fully described in Ref. [21].
experimental result is shown in Fig. 5(b), in which the surface coverage \( \Gamma / \Gamma_{\text{max}} \) change denotes the 3-APTES binding process. With the same concentration of 3-APTES solution as was introduced to the PSi waveguide, nearly complete 3-APTES coverage on the flat wafer surface is achieved in approximately 10 s, which translates into an adsorption rate constant \( k_{\text{ad}} = 2.826 \text{ M}^{-1} \cdot \text{s}^{-1} \) and desorption rate constant \( k_{\text{des}} = 8.5 \times 10^{-4} \text{ s}^{-1} \). The adsorption rate constant of 3-APTES on the flat surface is about 100 times greater than that in PSi, which is consistent with a previous report of avidin adsorption in anodic aluminum oxide [15]. We believe that the difference in rate constants between a flat surface and porous material may be due in part to the steric and electrostatic hindrance present in biomolecule films on the pores walls, as well as conformational restriction of the surface-tethered strands suppressing biomolecule interactions inside the pores [22,23].

Using the same method, the diffusion coefficients, adsorption rate constants, and desorption rate constants of Sulfo-SMCC, probe DNA and antisense PNA were calculated by Eqs. (6) and (7). The results are summarized in Table 1. In porous media, the diffusion coefficient is affected by the morphology of the porous material and is lower than the free solution diffusion coefficient because of the constricted, elongated or tortuous solute flow paths [24,25]. For the small linker molecules, the diffusion coefficient is on the order of \( 10^{-9} \text{ m}^2 \cdot \text{s}^{-1} \). For the larger oligos, the diffusion coefficient in PSi is on the order of \( 10^{-11} \text{ m}^2 \cdot \text{s}^{-1} \), which is nearly 10-fold smaller than that in free solution [26,27]. The PSi structure also has similar impact on the adsorption rate constants in the nanoscale pores. The adsorption rate constant of the small linkers is in the range of \( 10^{-4}-10^{-2} \text{ M}^{-1} \cdot \text{s}^{-1} \). The adsorption rate constant of DNA hybridization is near \( 10^{7} \text{ M}^{-1} \cdot \text{s}^{-1} \), which is \( 10^{2}-10^{4} \) times smaller than that on flat surfaces [22,28]. Applying these values as inputs into COMSOL simulations, we simulated the biomolecule attachments. Figure 6 shows both the COMSOL simulation and the experimentally normalized resonance angle changes during 3-APTES, Sulfo-SMCC, probe DNA and antisense PNA binding. The resonance angle changes proportionally with both the surface coverage of the molecules bound in the pores and the refractive index change of the free solution in the pore. As expected, the resonance angle changes

|                | 3-APTES          | Sulfo-SMCC       | Probe DNA        | Antisense PNA    |
|----------------|------------------|------------------|------------------|------------------|
| \( D (\text{m}^2 \cdot \text{s}^{-1}) \) | \( 5.41 \times 10^{-9} \) | \( 2.442 \times 10^{-9} \) | \( 9.542 \times 10^{-11} \) | \( 9.576 \times 10^{-11} \) |
| \( k_{\text{ad}} (\text{M}^{-1} \cdot \text{s}^{-1}) \) | 0.02958          | 0.1274           | 5.641            | 4.442            |
| \( k_{\text{des}} (\text{s}^{-1}) \) | \( 7.652 \times 10^{-4} \) | \( 1.748 \times 10^{-5} \) | \( 7.4 \times 10^{-4} \) | \( 1.422 \times 10^{-4} \) |
Fig. 6. Experimental (blue circle) and COMSOL simulated (red line) normalized resonance angle changes during (a) 3-APTES, (b) Sulfo-SMCC, (c) probe DNA and (d) antisense PNA binding in the PSi waveguide.

increases in all cases over time. The experimental and simulation results show comparatively good agreement with each other, which suggests that the assumptions of Eqs. (6) and (7) are valid and can be applied for deriving kinetic parameters of molecules in nanoscale pores.

5. Conclusion

A PDMS integrated grating-coupled PSi waveguide biosensor is demonstrated as a platform for near real-time detection of chemical and biological molecule binding events. Molecular attachments in the PSi waveguide are monitored by real-time angle-resolved reflectance measurements. Selectivity of the biosensor towards the target oligomer is estimated to be 5:1. Precise and simplified mass balance equations, which reveal the binding events inside the pores, are given. Diffusion coefficient, adsorption and desorption rate constants of different sized chemical linkers and nucleic acid molecules are determined based on the changes of the measured resonance angle. For small linkers, the diffusion coefficient is on the order of $10^{-9}$ m$^2$s$^{-1}$, and the adsorption rate constant is in the range of $10^{-1}$-$10^{-2}$ M$^{-1}$s$^{-1}$, while for the larger oligos, the diffusion coefficient in PSi is on the order of $10^{-11}$ m$^2$s$^{-1}$, and the adsorption rate constant of DNA hybridization is near $10^{1}$ M$^{-1}$s$^{-1}$. Based on the experimental and calculated results, the PDMS integrated grating-coupled PSi waveguide shows great promise for sensitive, real-time detection of small chemical and biological molecules.
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