CHAPTER 5

PART A

ENHANCED DNA HYBRIDIZATION ON NANOSTRUCTURED POROUS SILICON SURFACE

INTRODUCTION

The technological advances of the recent years in the genomic sequencing of many species have given a great impetus to the development of sensors for DNA diagnostics, forensic medicine and biowarfare. The recent advances have impacted three main areas of analytical tools: DNA biosensors, gene chips and miniaturized analyzers (“lab-on-a chip”) [1, 2]. High-density oligonucleotide arrays have emerged as a powerful and promising tool for genetic analysis with a lower cost, higher throughput and reproducibility. The so-called DNA chips or microarrays, have numerous applications in the studies related with gene expression or sequencing by hybridization. The specificity in the base pairing of DNA allows the identification of genetic mutations, pathogens and the quantification of gene expression levels in disease states. The key issue in any of these applications is to transduce the hybridization event into a useful signal with enhanced sensitivity, selectivity and speed. Commonly, optical [4], mechanical [5] and electrochemical [6] transduction have been used for detection. The DNA based sensor is usually functionalized with a probe that consists of a specific sequence of oligonucleotide. When the complementary oligonucleotide sequence binds to the probe, the hybridization is detected either by an optically active molecule (e.g., fluorescence indicator) or a change in resonance frequency or an electroactive reporter.

Several methods for the covalent attachment of oligonucleotides to functionalised solid support have been reported. The procedures to make functionalized substrates are well established and have developed on glass [7-9], oxidized silicon [10], silicon wafers [11], gold surfaces [12], filter membranes [13], optical fibers [14], polyacrylamide gel pads [15] and plastic (organic) polymers, and
other alternatives solid supports for making DNA microarrays [16].

Silicon which is routinely used in microfabrication and for mass production of integrated circuit devices, is ideal for the fabrication of optical [17] and field effect transistor based [18] biosensors. The modification of silicon to form the biorecognition interface is carried out via silane chemistry on the oxidized silicon surface. Silicon, in both bulk crystalline and nanostructured forms, has emerged as an interesting platform for tissue engineering [19], cell culture [20], and for interfacing cells with electronic devices [21]. The porous form of silicon shows significantly improved mammalian cell adhesion and viability [22], and improved implant stability in whole organisms [23] in comparison to flat crystalline silicon. The ability to tune both nanostructure and surface chemistry of electrochemically prepared porous silicon provides a means to adjust these parameters for successful DNA attachment and subsequent hybridization. Indeed, much research is underway to take advantage of the tunable porous nature of the material for controlled drug release [24] and the material is being assessed in clinical studies [25]. The ability of this nanostructured material to detect chemicals [26], biomolecules [27], enzymatic activity [28] and cells [29, 30] presents the possibility that porous silicon may play a role in \textit{in vitro} sensing or \textit{in vivo} diagnostic devices.

Porous silicon (PS) substrate has high surface area that can be prepared to have large variety of pore morphologies by chemical or electrochemical etching processes [31-33]. The hydride terminated freshly etched porous silicon surface can be modified by a number of methods such as oxidation, halogenation, nucleophilic substitution and hydrosilylation [34, 35]. Once oxidized, the porous silicon surface can be further functionalized using commonly used well established silane chemistry. Also, because of its unique electrical and optical properties, porous silicon has been used as the matrix and signal transducer in variety of biosensing applications [36-38].

In the present work, the immobilization of single stranded DNA and its hybridization on APTS modified silicon surface has been demonstrated. To show the enhanced DNA binding and hybridization on modified PS surface as compared to flat
silicon surface, the same protocol for DNA attachment was followed for both the cases where DNA is labelled with $^{\gamma}P^{32}$ and the surface probe concentration was detected using phosphoimager. The efficient adsorption of DNA on the flat silicon surface and porous silicon layer and the subsequent hybridization is confirmed using fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). The high fidelity of these nanostructured surfaces promises substantial benefit to the emerging technology of large-scale biological analysis using nucleic acid arrays.

**METHODOLOGY**

**Porous silicon formation**

Details of formation of porous silicon are given in Chapter 1 Part A (page no. 42-44). PS which is generally hydride-terminated was treated with SC2 to obtain a hydroxyl-terminated surface.

**Chemical modification of silicon surfaces**

The description of silanization process used for both flat silicon and PS surface has been given in Chapter 2 (page no. 79). The APTS functionalized flat silicon and PS samples were immersed in a solution of glutaraldehyde (2.5%, pH 7) which was used as a cross linker. The wafers were kept in the solution for 1 hr at room temperature. Subsequently, the samples were rinsed with DI water in order to remove any unbound glutaraldehyde.

**DNA immobilization and hybridization**

The sequence of the probes used for hybridization is shown in Table 1. The aldehyde terminated flat as well as PS samples were incubated with unlabelled amine-terminated oligo 1 (30 ngμl$^{-1}$) for 1 hr and then rinsed with 1X PBS. Oligo 2 (31ngμl$^{-1}$) and oligo 3 (31.2 ngμl$^{-1}$) were labelled by $^{\gamma}P^{32}$ (BRIT, India) by PNK method (41). The immobilized oligo 1 was incubated with labeled oligo 2 and oligo 3 for
hybridization at 65°C for 10 min. and ramping was done for about 1 hr. For control sample, unlabelled oligo 1 was hybridized with unlabelled complementary oligo 2. The samples were thoroughly rinsed with 1X PBS and then analysed using phosphoimager and quantification of the attached oligos on silicon surfaces was done by Nanodrop.

Table 1. *Probe sequence used for hybridization*

<table>
<thead>
<tr>
<th>Deoxyribonucleotide</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>Oligo 1</td>
<td>5’ NH₂-TTC AA GAC TCC TCC GCT GAC 3’ (20 mers)</td>
</tr>
<tr>
<td>Oligo 2 (complementary to</td>
<td>3’ AAGTTCTGAGGAGGCGACTG 5’ (19 mers)</td>
</tr>
<tr>
<td>oligo 1)</td>
<td></td>
</tr>
<tr>
<td>Oligo 3 (non complementary to oligo 1)</td>
<td>5’-AAT GTG CTC CCC CAA CTC CTC-3’ (21 mers)</td>
</tr>
</tbody>
</table>

**Sample characterization**

Samples were characterized using X-ray photoelectron spectroscopy (XPS), fourier transform infrared spectroscopy (FTIR), phosphoimager to detect the fluorescent signals and nanodrop technique as described earlier.

**RESULTS AND DISCUSSION**

Nanoporous silicon is a unique matrice for biomolecule immobilization compared to flat silicon surface or other conventional materials due to its large surface area and open pore structure that allow high biomolecule loadings and free access of the analytes. The formation process of PS is complex, that depends on many factors. The properties of PS such as porosity, thickness, pore diameter and microstructure depend on the anodization conditions. These conditions include HF concentration, current density, wafer type and resistivity, anodization duration, illumination (in case of n-type), temperature, ambient humidity, and drying conditions [42].
In the present study PS film formed after anodization at 50 mAc m\(^{-2}\) attain the pore size \(\sim 50–60\) nm as evident from SEM studies reported elsewhere [43]. The electrochemical etching procedure of silicon wafer was optimized in order to obtain porous silicon layers with pore diameters between 7.5 nm (the approximate diameter of a 21-mer double stranded DNA sequence) and 50 nm which coincide with the size of the oligonucleotides to ensure DNA stability inside the PS layer [44].

![Scheme 1 DNA binding and hybridization on modified silicon surfaces](image)

**Scheme 1** DNA binding and hybridization on modified silicon surfaces

Scheme 1 shows the DNA binding and hybridization on APTS-modified flat and nanostructured porous silicon surfaces. Freshly prepared PS with SiH\(_x\) terminated surface and flat silicon surface substrates are treated with SC2 to obtain hydroxyl-terminated surfaces. Initially, these hydroxyl-terminated oxidized surfaces are silanized with APTS in toluene to attach primary amines on the surface (a); secondly, when these amine-terminated surfaces treated with a cross-linker glutaraldehyde amine group of APTS binds with one of the aldehyde group of glutaraldehyde (b); the NH\(_2\)-DNA probes are subsequently reacted with the other aldehyde terminal of the cross-linker, establishing a covalent bond between the surface and the oligonucleotide probe (c); labelled complementary (d) and labelled non-complementary DNA (e) hybridizes with the probe DNA on both modified silicon surfaces.
**IR analysis**

FTIR spectra (Fig. 7.1 and Fig. 7.2) demonstrate the characteristics of the single stranded DNA (oligo1) and hybridized DNA molecule for both, flat and nanoporous silicon surfaces. In Fig. 7.1 (a) for oligo 1 binding on flat silicon surface indicates the appearance of both asymmetric CH$_2$ and CH$_3$ stretching at ~2920–2965 cm$^{-1}$, respectively. In plane nucleic base, vibrations are the features observed in the spectra at ~1720 and 1632 cm$^{-1}$ [stretching modes (C=O) of the nucleic bases and amide groups, respectively (C=C) and (C=N) of the rings] and the in plane ring vibrations are observed at ~1530 (ip ring, ip imidazole, pyrimidine ring vibration, respectively). The presence of these absorption bands indicates that most of the rings have a tilted orientation with respect to the surface thus allowing the dipole moments to be active [45, 46]. IR spectrum after hybridization with complementary DNA (oligo 2) on flat silicon surface is shown in Fig. 7.1 (b). The region from 2800-3000 cm$^{-1}$ corresponds to the CH$_x$ stretching modes. There is an appearance of both asymmetric and symmetric CH$_2$ stretching modes at ~2942 and 2832 cm$^{-1}$, respectively. Also an increase of the intensity of these bands observed with slight shift of the asymmetric and symmetric stretches observed towards lower wave-numbers might be similar to the previous study of the adsorbed DNA molecules after hybridization. The region from 1800 to 1500 cm$^{-1}$ corresponds to the in-plane double bond vibrations of the DNA bases; the absorptions in this region are sensitive to base-pairing and stacking effects. Peaks at ~1635 cm$^{-1}$ (C=O stretch) and 1539 cm$^{-1}$ (NH deformation) are observed for amide I and amide II, respectively. A small shift in the infrared frequencies of the nucleic bases of hybridized DNA as compared to single stranded DNA (oligo 1) is observed (stretching modes [C=O] of the nucleic bases and amide group respectively (C=C) and (C= N) of the rings] which could be related to the base pairing during hybridization.
Figure 7.1 FTIR spectra of (a) single stranded DNA (oligo 1) and (b) DNA hybridization with complementary (oligo 2) on flat silicon surface.

Figure 7.2 IR spectra of (a) single stranded DNA (oligo 1) and (b) DNA hybridization with complementary (oligo 2) on porous silicon surface.
The region from 1500 to 1300 cm\(^{-1}\) is related to the base deformation motions coupled through the glycosidic linkage to sugar vibrations (base-sugar coupling entities). The appearance of new peaks at \(~1445\) and \(1330\) cm\(^{-1}\) are related to sugar vibrations. The band at \(1445\) cm\(^{-1}\) assigned to purine imidazolic ring vibrations. Peak appears at \(1330\) cm\(^{-1}\) is due to the glycosidic bond in the nucleoside. An antisymmetric phosphate stretching band in the Z-form appears at \(1218\) cm\(^{-1}\) [47] and infrared band at \(~1180\) cm\(^{-1}\) can be identified related to the deoxyribose presence arising from sugar–phosphate backbone vibrations [46].

The infrared spectra of single stranded DNA (oligo 1) and hybridized DNA on PS also shows some enhanced features and also the characteristic bands of DNA molecular groups as compared to flat silicon surface. Figure 2 (a) shows the IR spectrum of oligo 1 immobilized on the PS layer, in which the guanine bases show their characteristic absorption peaks at \(~1719, 1635\) and \(1535\) cm\(^{-1}\) characteristic of the vibration of C=O bond [48] and NH\(_2\) bending modes of guanine [49]. The both asymmetric and symmetric CH\(_2\) stretching modes are appeared at \(~2922\) cm\(^{-1}\) and \(2864\) cm\(^{-1}\), respectively.

Spectrum of the PS after hybridization of oligo 1 with oligo 2 displays the characteristic bands of the G-C and A-T pairs (Fig. 7.2 b). Peaks appears at \(~1736, 1647\) and \(1520\) cm\(^{-1}\) arise from the NH\(_2\) and N-H bending and carbonyl stretching of the G-C base pair. Sugar related vibration is present at \(1321\) cm\(^{-1}\) due to glycosidic bond adenosines [44]. The band at \(~1465\) cm\(^{-1}\) assigned to purine imidazolic ring vibrations. A broad peak around \(~3054-3152\) cm\(^{-1}\) indicates the N-H stretching vibration of amide bond. A comparison of these bands with the vibrational bands of the individual oligonucleotides indicates that a rearrangement of the bonding after hybridization takes place. These results clearly confirm the selectivity and efficiency of the hybridization reaction on the porous silicon layers. Also, an enhancement of the intensity of the IR bands is shown which is an unambiguous indication of the nucleic bases DNA contribution.
XPS studies

With a view to elucidate the composition of DNA molecule attached, the valence states of various element present and to determine the level of attachment of DNA on modified flat and nanoporous Si surfaces XPS experiments were performed. XPS survey spectra recorded for flat silicon surface and PS after DNA hybridization (oligo 1 and oligo 2) with complementary probe is shown in Fig. 7.3. The main signals for expected elements are Carbon (Cls), Oxygen (O1s), Nitrogen (N1s) and Silicon (Si 2s, 2p). From Fig. 3a, it is evident that the signal intensity of C (1s) peak of hybridized DNA is higher for flat Si surface than that of nanoporous surface while increased N (1s) signals are obtained in case of PS sample, Fig.7.3 (b). Table 2 shows the percentage elemental composition after hybridization of oligo 1 with oligo 2.

Table 2 Elemental composition after hybridization

<table>
<thead>
<tr>
<th>Elements</th>
<th>Flat Silicon</th>
<th>Porous Silicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>66.7</td>
<td>56.2</td>
</tr>
<tr>
<td>Oxygen</td>
<td>20.4</td>
<td>17.1</td>
</tr>
<tr>
<td>Silicon</td>
<td>9.2</td>
<td>17.4</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>4.7</td>
<td>9.2</td>
</tr>
</tbody>
</table>
To identify the different types of covalent bond formation and quantify the nitrogen and carbon contributions after hybridization on both silicon surfaces, a detailed deconvolution analysis of the C (1s) and N (1s) core-level spectra were performed. C (1s) core level spectra for flat silicon and PS surfaces are shown in Fig. 7.4 and Fig. 7.5. The deconvolution of C (1s) spectra revealed that it consist of three peaks in both the cases. The peak at 284.7 eV appeared due the presence of hydrocarbon moieties on flat silicon surface, is much higher ~ 56% as compared to PS surface (38.5%) (Fig. 7.4a and Fig. 7.5a). C1s spectra showed a second component consisting of contributions from C–N and C–O at 286.1 and 285.8 eV for flat silicon (Fig. 7.4b) and PS film (Fig. 7.5b), respectively. At the same time, a new species appeared in the C1s spectrum (Fig. 7.4c and Fig. 7.5c) which can be attributed to highly oxygenated carbon species (C=O, O–C–O). Table 3 explains the binding energies and percentage area of the deconvoluted C (1s) core level peak for different chemical states of carbon involved in the DNA hybridization.
Table 3 Binding energies and percentage area of C (1s) core level spectra of after hybridization

<table>
<thead>
<tr>
<th>Species</th>
<th>PS Surface</th>
<th>Flat Silicon Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Binding Energy (eV)</td>
<td>FWH M % composition</td>
</tr>
<tr>
<td>Hydrocarbon</td>
<td>284.7</td>
<td>1.8 38</td>
</tr>
<tr>
<td>C-N, C-O</td>
<td>285.8</td>
<td>2 47</td>
</tr>
<tr>
<td>C=O, O-C-O</td>
<td>287.8</td>
<td>2 13</td>
</tr>
</tbody>
</table>

Figure 7.4 XPS core level spectra of C (1s) after DNA hybridization on flat silicon surface
The N (1s) peakfit has been performed by using three curve components, which are shaped as a convolution of Gaussian curve. Fitting of N (1s) spectra revealed the occurrence of several species (Fig. 7.6 and Fig. 7.7) and higher nitrogen content in PS. The nitrogen component with the lowest binding energy, 399.1 eV, in Fig. 7.6(a) and Fig. 7.7(a) are assigned to unsaturated chemical bonds (-N=). Fig. 6(b) and Fig 7 (b) spectra show that the nitrogen (-NH-) contribution from amide group and nucleic bases rings. Peaks at 401.8 and 401.9 eV (Fig. 7.6c and Fig. 7.7c), respectively) correspond to -NH$_2$ nitrogen from cysteine and nucleic base rings. N (1s) spectrum of PS shows a shifts towards higher binding energies with respect to that of flat silicon as shown in Table 4. Previous XPS experiments performed for different amides have shown that the N (1s) asymmetric signals could be deconvoluted into two peaks due to the coexistence of free and hydrogen bonded species. Thus, when the amide was protonated a chemical shift of the N (1s) signal to higher binding energies was observed due to the increase of the net positive charge at the nitrogen atom [50] as observed in PS sample. The shift of the N (1s) peak to
higher energy values is indeed related to the hybridization and, therefore, a good corroboration of the process [46].

**Table 4**  
*N (1s) core level peak for different chemical states of nitrogen after hybridization*

<table>
<thead>
<tr>
<th>Species</th>
<th>Flat Si Surface</th>
<th></th>
<th>PS Surface</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Binding Energy (eV)</td>
<td>FWHM</td>
<td>% composition</td>
<td>Binding Energy (eV)</td>
</tr>
<tr>
<td>-N=</td>
<td>399.10</td>
<td>1.8</td>
<td>56</td>
<td>399.11</td>
</tr>
<tr>
<td>-NH-</td>
<td>400.32</td>
<td>1.7</td>
<td>27</td>
<td>400.47</td>
</tr>
<tr>
<td>NH₂</td>
<td>401.80</td>
<td>1.7</td>
<td>15</td>
<td>401.91</td>
</tr>
</tbody>
</table>

**Figure 7.6**  
*XPS core level spectra of N (1s) after DNA hybridization on flat silicon surface*
Figure 7.7 XPS core level spectra of N (1s) after DNA hybridization on porous silicon

Phosphoimager and nanodrop analysis

To achieve hybridization (i.e., specificity) and surface effect (i.e., signal intensity), two types of labeled DNA sequences oligo 2 (complementary) and oligo 3 (non-complementary, shown in Table 1) were used for modified flat silicon and PS film. As fluorescence measurements are not quantitative, $^3\text{P}^{32}$ labelling is used to compare the two substrates. Fig. 7.8 shows the fluorescent signal obtained after hybridization of oligo 1 with labelled oligo 2 (complementary) Fig. 7.8 (a) and oligo 3 (non-complementary) Fig. 7.8 (a) on flat silicon surface. The signal obtained in Fig. 7.8(a) with oligo 3 is because of its background noise which is similar to control sample.

Figure 7.8 DNA hybridization on flat silicon surface with $^3\text{P}^{32}$ labelled (a) complementary (b) non-complementary DNA and (c) control sample
The PS substrate shows hybridization areas with more intense fluorescence as compared to flat silicon surface whereas the radioactive measurements detect homogeneous areas as shown in Fig. 7.9 (a). The fact that the signal is higher for the porous substrate is thus a substrate effect due solely to its large specific surface and good molecular diffusion into its pores. Fig. 7.9 (b) explicit that porous surface also shows the specificity for DNA binding as labelled non complementary oligo 3 gives only background noise and no hybridization takes place with oligo 3.

![DNA hybridization on PS surface with $^{32}P$ labelled (a) complementary and (b) non complementary DNA](image)

**Figure 7.9** DNA hybridization on PS surface with $^{32}P$ labelled (a) complementary and (b) non complementary DNA

Figure 7.10 explains the binding of oligo 1 and its hybridization with complementary oligo 2 probe on functionalised flat and PS surface. It was observed that 10.5 and 25.4 ngμl$^{-1}$ of oligo 1 was bound to the flat and PS surfaces, respectively. After hybridization with complementary oligo 2, it was found that 5.8 and 24.1 ngμl$^{-1}$ of hybridized DNA attached to the flat and PS surface, respectively. More than 70% of DNA hybridization was achieved on the porous silicon surface in comparison to less than 20% of DNA hybridization on flat silicon surface. The above results support the enhanced single stranded DNA binding and also, its hybridization on nanostructured PS as compared to the flat silicon surface.
SUMMARY

Silicon substrate has been used for the oligodeoxynucleotide immobilization and hybridization to demonstrate its biological selectivity/specificity. Present study shows that DNA oligonucleotides on flat and nanostructured porous silicon surfaces hybridized with the complementary probe attached to the flat and PS surfaces. However, PS surface exhibited the enhanced fluorescence intensity as compared to flat silicon surface due to its large surface area and good molecular penetration into its pores. Both silicon surfaces have been characterised by FTIR, XPS and Nanodrop, support the enhanced DNA binding and hybridization on PS film. The results of this study reveal that controlled porous silicon layers are very good substrates for the absorption, stabilization and detection of DNA sequences. Based on these results it is concluded that porous silicon can be successfully employed for the development of DNA microarrays and microfabricated DNA sensors.

REFERENCES


PART B

DNA HYBRIDIZATION ON SILICON NANOWIRES
INTRODUCTION

Nano-scale bioelectronic devices have been demonstrated to possess unique characteristics for ultrasensitive, miniaturized molecular sensing applications. The sensing mechanism of semiconductor nanostructures, such as carbon nanotubes [1-3], silicon nanowires [4, 5], SnO$_2$ nanowires [6] and In$_2$O$_3$ nanowires [7] is generally believed to be related to change of surface charge of nanostructures in presence or absence of analyte species [8]. Nanowire-based detection strategies provide promising new routes to bioanalysis that could one day revolutionize the healthcare industry. Nanowires show promise in a number of different sensing strategies, including optical [9], electrical [10], electrochemical [11], and mass-based [12] approaches. They are attractive because of their small size, high surface-to-volume ratios, and/or electronic, optical and magnetic properties which can differ markedly from those observed for bulk or thin film materials as the nanowire cross-sectional diameter decreases [13].

Silicon nanowires (SiNWs) are particularly attractive because of the well known semiconducting properties of silicon. Silicon nanowires (SiNWs) have been proven to be highly sensitive and selective sensors for nucleic acids [14-18] proteins [5, 19, 20], virus [21] and cells [20, 22]. SiNW surface is in nature covered with a native oxide grown in air. The native silicon oxide coating on the SiNW surface is an effective layer in most cases which can usually be functionalized with bioaffinitive moiety. A number of SiNW biosensors are based on the functionalization of the SiNW with the above-mentioned native oxide [5, 14, 15, 17, 19, 21-23].

DNA sensing strategies have recently been varieted with the number of attempts at the development of different biosensor devices based on nanomaterials, which will further become DNA microchip systems. DNA hybridization is a process in which probe with specific organization of nucleotide bases exhibiting complementary pairing with each other under specific given reaction conditions, thus forms a stable duplex molecule. This phenomenon is possible because of the biochemical property of base pairing, which allows fragments of known sequences to
find complementary matching sequences in an unknown DNA sample [24]. An increasing interest have appeared in the development of simple, rapid and user-friendly electrochemical detection systems based on DNA sequence and mutant gene analysis, for instance early and precise diagnosis of infectious agents, for routine clinical tests [25–31]. Thus, DNA hybridization biosensors can be employed for determining early diagnoses of infectious agents in various environments [32, 33] and these devices can be exploited for monitoring sequence-specific hybridization.

DNA biosensors are attractive devices especially for converting DNA hybridization event into an analytical signal for obtaining sequence-specific information in connection with clinical, environmental or forensic investigations. Such fast on-site monitoring schemes are required for quick preventive action and early diagnosis [34].

**METHODOLGY**

**Silicon nanowire (SiNW) formation**

Details pertaining to growth of silicon nanowire have been given in Chapter 1 Part B (page no. 69-71).

**Chemical modification of silicon surface**

The description of silanization process used is similar as given in Chapter 2 (page no. 79) and procedure for glutaraldehyde treatment and DNA hybridization is explained in Chapter 5 Part A (page no.144-145).

**Sample characterization**
X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), phosphoimager and nanodrop techniques are used for sample characterization details of these have been described earlier.

RESULTS AND DISCUSSION

Nanowires are defined as metallic or semiconducting particles having a high aspect ratio with cross-sectional diameters << 1 µm and lengths as long as tens of microns. Systematic SEM of SiNWs array is shown in Chapter 1 (page 71-72). SEM studies reveal that SiNWs are smooth and uniform with an average diameter of ~100-300 nm and average length of ~1-1.15 µm [32]. Optical photograph shows that silicon sample with SiNW arrays surface looks black in color after Ag removal whereas polished silicon wafer looks grey in color (Fig. 7.11)

![Figure 7.11 Optical images of SiNWs (a) and (b) polished Si wafer](image)

**Figure 7.11** *Optical images of SiNWs (a) and (b) polished Si wafer*

IR analysis

The Fig. 7.12 (a) shows the IR spectra of APTS functionalized PS. The IR absorption bands are observed at approximately 1551 cm\(^{-1}\), assigned to the NH\(_2\) scissoring vibration of APTS amine groups, 1450 cm\(^{-1}\) assigned to the symmetric NH\(^3+\) deformation mode [24]. The 900-1290 cm\(^{-1}\) spectral region involves the Si-O-Si stretching modes, including those created by attachment of APTS to the oxide surface [37]. The presence of CH\(_x\) stretching mode is observed between 2824 and 2858 cm\(^{-1}\): the CH\(_2\) asymmetric and symmetric stretching modes are observed at 2858 and 2824 cm\(^{-1}\), respectively [37, 38]. The spectrum of the surface obtained following exposure
of the APTS layer to a glutaraldehyde solution (Fig. 7.12 b) contains a band at 1736 cm\(^{-1}\) assigned to the C=O stretching vibration of the aldehyde groups of glutaraldehyde. A relatively weaker band is also present at 1641 cm\(^{-1}\) and can be assigned to the imine bond resulting from the reaction between the amine groups of APTS and the aldehyde groups of glutaraldehyde [39].

![FTIR spectra](image)

**Figure 7.12** FTIR spectra of SiNW surface obtained by sequential exposure to (a) 3-APTS and (b) glutaraldehyde

FTIR spectra (Fig. 7.13) demonstrate the characteristics of the single stranded DNA (oligo1) and hybridized DNA molecule. Figure 7.13 (a) for oligo 1 binding on silicon nanowire surface indicates the appearance of both, symmetric and asymmetric CH\(_2\) stretching at ~2865–2940 cm\(^{-1}\), respectively [36]. The broad peaks at ~1728, 1643 and ~1551 cm\(^{-1}\) are mainly due to the C=O, C=C, C=N stretching vibration modes [40, 41] and NH\(_2\) bending of the DNA bases [42-46]. In Fig. 7.13 (b) the spectrum after hybridization of oligo 1 with oligo 2 shows some enhanced features and also the characteristic bands of DNA molecular groups. The region from 2800-3000 cm\(^{-1}\) corresponds to the CH\(_x\) stretching modes and it indicates the
appearances of both asymmetric and symmetric CH₂ stretching modes at ~2933 and 2874 cm⁻¹, respectively. Also, an increase of the intensity of these bands is observed. The region from 1800 cm⁻¹ to 1500 cm⁻¹ corresponds to the double bond vibrations of the DNA bases. The absorptions in this region are sensitive to base-pairing and stacking effects. Peaks at ~1650 cm⁻¹ (C=O stretch) and 1559 cm⁻¹ (NH deformation) are observed for amide I and amide II, respectively. A small shift in the infrared frequencies of the nucleic bases of hybridized DNA as compared to single stranded DNA (oligo 1) are observed stretching modes (C=O) of the nucleic bases and amide group, respectively (C=C) and (C= N) of the rings, which could be related to the base pairing during hybridization. The region from 1500 to 1300 cm⁻¹ is related to the base deformation motions coupled through the glycosidic linkage to sugar vibrations (base-sugar coupling entities). The appearance of new peaks at ~1478 cm⁻¹ and 1323 cm⁻¹, are related to sugar vibrations. The band at 1478 cm⁻¹ is assigned to purine imidazolic ring vibrations. Appearance of peak at 1323 cm⁻¹ is due to the glycosidic bond in the nucleoside. An antisymmetric phosphate stretching band in the Z-form appears at 1230 cm⁻¹ [47]. IR bands of oligonucleotides indicate the rearrangements of the bonding after DNA hybridization on silicon nanowires.
Figure 7.13 IR spectra of (a) single stranded DNA (oligo 1) and (b) DNA hybridization with complementary (oligo 2) on modified SiNW surface

XPS studies

XPS is a highly sensitive diagnostic tool for the assessment of the chemical state of elements and degree of transformation of the surface after different chemical treatments. Figure 7.14 shows the general scan XPS survey spectra of carbon, oxygen, nitrogen, and silicon after silanization, glutaraldehyde treatment and DNA hybridization. Table 2 shows the percentage elemental composition present after chemical treatments and DNA hybridization.

Figure 7.14 XPS spectra of (a) APTS modified, (b) glutaraldehyde treated, and (c) DNA hybridized DNA SiNW surface

Table 2 Atomic concentration of elements C, N, O and Si
To identify the different types of covalent bond formation and quantify the nitrogen and carbon contributions after surface functionalisation and DNA hybridization on SiNW, a detailed deconvolution analyses of the C(1s) and N(1s) core-level spectra was performed. C1s spectra of APTS modified SiNW surface show a hydrocarbon species (C1), due to adventitious contamination is much higher (~80%) as compared to glutaraldehyde treated and DNA hybridized samples (Fig. 7.15 b and c). A second carbon component (C2) consists of contributions from C–N and C–O. Different C2/N values (Table 4) revealed that a significant part of anchored 3-APTS molecules still contained one ethoxy group during silanization, possibly because of the dry solvents used to perform silanization [48]. After glutaraldehyde treatment a new carbon species (C3) appeared in the C1s spectrum (Fig.7.15 b) which can be attributed to highly oxygenated carbon species (C=O, O–C–O) thus confirming reaction with glutaraldehyde [49].

<table>
<thead>
<tr>
<th>Element (%)</th>
<th>APTS modified SiNW</th>
<th>Glutaraldehyde treated SiNW</th>
<th>DNA hybridized SiNW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>45.9</td>
<td>45.8</td>
<td>52.5</td>
</tr>
<tr>
<td>Oxygen</td>
<td>38.2</td>
<td>41</td>
<td>32.4</td>
</tr>
<tr>
<td>Silicon</td>
<td>12.1</td>
<td>9.3</td>
<td>5.7</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>3.8</td>
<td>3.9</td>
<td>9.4</td>
</tr>
</tbody>
</table>
XPS Intensity (a.u.)

Binding Energy (eV)

(a)

(b)
**Figure 7.15** C (1s) XPS spectra of (a) APTS modified (b) glutaraldehyde treated and (c) DNA hybridized SiNW surface

**Table 3** Binding energies and (%) area of the deconvoluted C (1s) core level peak for different chemical states of carbon involved in the DNA hybridization

<table>
<thead>
<tr>
<th>Species</th>
<th>C1 (C-C, C-H) %</th>
<th>C2 (C-N, C-O) %</th>
<th>C3 (C=O, O-C-O) %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Binding Energy (eV)</td>
<td>Binding Energy (eV)</td>
<td>Binding Energy (eV)</td>
</tr>
<tr>
<td>APTS</td>
<td>80.2 (284.4)</td>
<td>19.8 (285.5)</td>
<td>-</td>
</tr>
<tr>
<td>GA</td>
<td>66 (284.6)</td>
<td>24 (285.6)</td>
<td>9 (286.6)</td>
</tr>
<tr>
<td>DNA</td>
<td>56 (284.6)</td>
<td>34 (285.8)</td>
<td>12 (287.6)</td>
</tr>
</tbody>
</table>

The XPS data indicate that the surface of SiNW modified by silanization is covered with carbon and nitrogen compounds. Appearance of the N1s signal was the evidence that the first step silanized the substrate anchoring NH$_2$ groups to the surface (Fig.7.12 a). The average thickness of the silane films was calculated from the attenuation of Si2p (as already done for APTS films [50, 51] using formula $I_{Si}/I'^{Si}_{Si} = \exp(-d/\lambda^{APTS}_{Si})$, where $I_{Si}/I'^{Si}_{Si}$ is the Si2p intensity ratio for silylated and blank substrates, respectively, d is the film thickness, and $\lambda^{APTS}_{Si}$ is the attenuation length,
assumed to be equal to 43 Å [52] (density for APTS=0.946 g cm$^{-3}$ [54]). $d$ was found to be equal to 17 Å (1.7 nm) which corresponds on average to a monolayer [49, 50].

The N (1s) peakfit has been performed by using three curve components, which are shaped as a convolution of Gaussian curve. Fitting of N (1s) spectra revealed the occurrence of several species (Fig.7.16) and higher nitrogen content in DNA hybridized SiNW. The nitrogen component with the lowest binding energy, $\sim$399.1 eV, in Fig.7.16 (N1) are assigned to unsaturated chemical bonds (-N=). Figure 7.16 (N2) spectra show the nitrogen (-NH-) contribution from amide group and nucleic bases rings. Peaks at $\sim$ 401.9 eV (N3) correspond to -NH$_2$ nitrogen from cysteine and nucleic base rings. N (1s) spectrum of NA hybridized SiNW shows a shifts towards higher binding energies with respect to that of APTS and glutaraldehyde treated SiNW surface as shown in Table 5. Previous XPS experiments performed for different amides showed that the N (1s) asymmetric signals could be deconvoluted into two peaks due to the coexistence of free and hydrogen bonded species. Thus, when the amide was protonated, a chemical shift of the N (1s) signal to higher binding energies was observed due to the increase of the net positive charge at the nitrogen atom [54] as observed in PS sample. The shift of the N (1s) peak to higher energy value is indeed related to the hybridization and, therefore, a good corroboration of the process [44].

**Table 4** (\%) area of the deconvoluted C(1s) and N (1s) core level for different chemical states involved in the DNA hybridization

<table>
<thead>
<tr>
<th>Species</th>
<th>C/N</th>
<th>C2/N</th>
<th>C3/N</th>
<th>N/Si</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTS</td>
<td>12</td>
<td>5.2</td>
<td>-</td>
<td>0.25</td>
</tr>
<tr>
<td>GA</td>
<td>11.7</td>
<td>6.1</td>
<td>2.3</td>
<td>0.41</td>
</tr>
<tr>
<td>DNA</td>
<td>5.6</td>
<td>3.6</td>
<td>1.2</td>
<td>1.6</td>
</tr>
</tbody>
</table>
**Figure 7.16** $N\ (1s)$ core level XPS spectra of (a) APTS modified (b) glutaraldehyde treated and (c) DNA hybridized SiNW surface

**Table 5** Deconvolution of $N\ (1s)$ spectra after surface modification and DNA hybridization

<table>
<thead>
<tr>
<th>Species</th>
<th>N1 (-N=) %</th>
<th>N2 (-NH-) %</th>
<th>N3 (NH$_2$) %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Binding Energy (eV)</td>
<td>Binding Energy (eV)</td>
<td>Binding Energy (eV)</td>
</tr>
<tr>
<td>APTS</td>
<td>83.7 (399.1)</td>
<td>16.3 (400.1)</td>
<td>-</td>
</tr>
<tr>
<td>GA</td>
<td>69.5 (399.4)</td>
<td>22.2 (400.2)</td>
<td>8.3 (401.1)</td>
</tr>
<tr>
<td>DNA</td>
<td>47 (399.1)</td>
<td>37 (400.3)</td>
<td>16 (401.9)</td>
</tr>
</tbody>
</table>

**Phosphoimager and nanodrop analysis**

To achieve hybridization (i.e., specificity) and surface effect (i.e., signal intensity), two types of labeled DNA sequences oligo 2 (complementary) and oligo 3 (non-complementary, shown in Table 1) were used for modified SiNW. Fig.7.17 shows the fluorescent signal obtained for control sample and after hybridization of oligo 1 with labelled oligo 3 (non-complementary) Fig.7.17 (b) and oligo 2 (complementary)
Fig.7.17 (c). The SiNW substrate shows hybridization areas with more intense fluorescence with complementary sequence as compared to non-complementary. The fact that the signal is higher for the complementary sequence is due to its large specific surface and good molecular attachment. Fig.7.17 (c) explicits that SiNW surface also shows the specificity for DNA binding as labelled non complementary oligo 3 gives only background noise and no hybridization takes place with oligo 3.

![Images](a) (b) (c)

**Figure 7.17** DNA hybridization on SiNW surface (a) control (b) $^{32}$P labelled non-complementary and (c) complementary DNA.

Figure 7.18 explains the binding of oligo 1 (30 ngμl$^{-1}$) and its hybridization with complementary oligo 2 (31 ngμl$^{-1}$) and oligo 3 probes (31.2 ngμl$^{-1}$) on functionalised SiNW surface. It is observed that 27 ngμl$^{-1}$ of oligo 1 binds to the SiNW surface. After hybridization with complementary oligo 2 and non-complementary oligo 3 the concentration of hybridized DNA was 25.7 and 3.2 ngμl$^{-1}$, respectively. More than 80% of DNA binding was achieved with complementary oligo in comparison to less than 12% of DNA binding with non-complementary oligo. The above results support the enhanced single stranded DNA binding and also its hybridization on modified SiNW.
SUMMARY

Silicon nanowire has been used for the DNA immobilization and hybridization to demonstrate its biological selectivity/specificity. Present study shows that hybridization of DNA oligonucleotides on nanostructured SiNW surface by complementary oligo with probe attached to the surface showed the enhanced fluorescence intensity, as compared to non-complementary oligo. SiNW after surface modification and hybridization have been characterized by FTIR, XPS, and nanodrop, support the enhanced DNA binding and hybridization on SiNW. The results of this study reveal that controlled SiNW is very good substrates for the absorption, stabilization and detection of DNA sequences. Based on these results it is concluded that SiNW can be successfully employed for the development of DNA microarrays and microfabricated DNA sensors.

REFERENCES


