ABSTRACT

Nanostructured porous silicon has properties that make it a very promising biomaterial, particularly for sensing devices that need to be linked to the biological system. The unique feature of the material is its large surface area within small volume, controllable pore size, convenient surface chemistry and its compatibility with conventional silicon microfabrication technologies. Porous silicon (PS) can be prepared by chemical or electrochemical etching processes and consists of nano- and microcrystalline domains with defined pore morphologies. The diameter, geometric shape and direction of the pores depend on surface orientation, doping level and type, temperature, the composition of the etching solution and the current density.

Porous silicon is also a biocompatible material and could prove to be the bridge that allows signals and informations to be transmitted between a semiconductor device and a biological system. It is a luminescent semiconductor that could play the roles of both transducer and the matrix. The PS matrix is suitable for biomolecule immobilization, provides a protective environment for the biomolecule and can transduce biorecognition events such as antigen-antibody binding, DNA, protein, enzymatic reactions, etc.

An objective of the proposal is to study the growth of porous silicon on the silicon wafer by electrochemical method. PS surface was amino-silanised by a simple three-step method using 3-aminopropyltriethoxysilane (APTS) that provides amine terminated surface. The primary amine terminated PS surface reacted with either of two cross-linkers, glutaraldehyde and sulfo-NHS-biotin. The biofunctionalised
nanostructured surface was used to attach immunoglobulin and DNA using glutaraldehyde. Sulfo-NHS-biotin was used to immobilize streptavidin. Similar protocol has been adopted for DNA hybridization and streptavidin-biotin interaction on silicon nanowires. The work presented in this thesis has been discussed in five chapters. General Introduction is followed by experimental techniques used such as photoluminescence (PL), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), contact angle and nanodrop method, etc.

Chapter 1 comprises of two parts; Part A deals with the process adopted for the formation of porous silicon surface and its characterization. Boron doped, single crystalline, p-type silicon (100) wafers were used for preparing PS by the electrochemical anodisation in an aqueous hydrofluoric acid and isopropyl alcohol solution at different current densities ranging from 20–70 mAcm$^{-2}$. In this work, porosity calculated by gravimetric method is $\sim$ 68-92% and pore-size $\sim$20–100 nm, for PS films formed at different $I_d$ (20–70 mAcm$^{-2}$). Different elements that form this system have been presented and their influences on the porous silicon characteristics discussed. Various measurement techniques used for the characterization of the fabricated porous silicon layers have been explained. These techniques are Scanning Electron Microscopy, photoluminescence and current-voltage. The present study can be attributed to the ease and speed of fabrication, remarkable optical and morphological properties of the porous silicon including tuneable pore size and porosity which make this nanostructured material poised to take centre stage in the biosensor development efforts.
Part B of Chapter 1 deals with the process adopted for the formation of silicon nanowires and its characterization. A simple, cheap and rapid growth process of vertically aligned SiNW arrays of controlled length on p-type (100) silicon substrates using electroless wet chemical etching of Si in aqueous HF–AgNO$_3$ solution at room temperature has been demonstrated. Structural analyses of the as grown SiNWs revealed that Si nanowires were the single crystalline having axial orientation identical to the silicon wafer used. SiNWs are formed due to the selective etching of the silicon surface directly in contact with Ag nanoclusters via self assembled nano-electrochemical process wherein the Ag nanoclusters which are formed due to the reduction of Ag$^+$ over the silicon surface act as the catalyst during etching process. The length of the SiNWs was found to be linearly dependent on etching time at room temperature. The simplicity of the present process may lead to the advancements and applications of SiNWs in opto-electronics and nano-electronic devices. Nanowire assembly and integration with microchip technology is emphasized as a key step towards the ultimate goal of biomolecules detection at the point of care using portable and low power electronic biosensor chips.

The surface modifications of porous silicon using APTS, its stability studies and characterization by PL, FTIR and XPS are discussed in Chapter 2. A simple silanization reaction to modify/functionalise PS surface prepared at two current densities $I_d\sim20$ and 50 mA cm$^{-2}$ is presented. This reaction proceeds by hydrolysis of the surface silicon–hydrogen groups that generated hydroxyl-terminated surfaces from freshly prepared PS. The functionalization of the hydroxyl-terminated PS surface with silanization reagents proceeds by abstraction of SiO$_2$ to form an organic
monolayer. The resulting monolayer is stable under a variety of humid conditions and retains the intrinsic structural properties of the PS layers. Surface functionalisation of nanostructured PS films and their stability under humid conditions are characterised using PL, FTIR and XPS techniques. PS films prepared at $I_d \sim 50 \text{ mA cm}^{-2}$ having high PL intensity and stable surface bond configuration as compared to PS film prepared at $I_d \sim 20 \text{ mAcm}^{-2}$ conforms its viability for the effective biofunctionalisation using APTS as precursor which can ensure covalent linking between the surface and biomolecules. The ease of this method of biofunctionalisation and low cost technique opens the possibility of using biofunctionalised PS in biosensing devices, microarray technology, organic semiconductors, and many other biotechnology and physics applications.

In Chapter 3 immunoglobulin (Human IgG and Goat anti Human IgG) immobilisation to APTS functionalised porous silicon and surface characterization have been described. This study demonstrates that antibody (Human IgG) binds to the APTS derived PS surface by covalent bonds between the reactive amine group of the antibody and aldehyde group of the glutaraldehyde. This also demonstrates that antibody immobilised on PS surface binds selectively to its complimentary antigen (goat anti-human IgG). The results from XPS, FTIR and flouroscope microscopy have convincingly proved the antibody-antigen binding on PS surface. Therefore, it can be concluded that PS surface can be used for bionanotechnology applications in general and immunosensing in particular.
Chapter 4 has two parts; Part A deals with the streptavidin-biotin interaction on modified porous silicon surface and in Part B modified silicon nanowire surface is utilized for streptavidin-biotin interaction. Nanostructured PS surfaces with pore size of ~50-60 nm provide large and biocompatible surface areas for biospecific bonding of streptavidin. APTS functionalized PS reacts with biotin-NHS at its amine group and the biotinylated surface subsequently binds with streptavidin. The ability to monitor these important chemical and biochemical reactions, and obtain a positive measure of streptavidin in porous silicon makes it possible to develop the use of PS for a broad range of applications in the field of biosensors. In addition, it can be expected that a tailored porous structure could also act as a matrix for a large variety of biological and chemical molecules. Immobilization of proteins on functionalized PS surfaces constitutes a research area of considerable importance in emerging technologies employing biocatalytic and biorecognition events.

Silicon nanowires have been utilized for the protein immobilization to demonstrate its biological selectivity and specificity. In the present study, APTS modified flat and nanostructured SiNW show streptavidin-biotin interaction. The binding capacity and efficiency of streptavidin on biotinylated surfaces were experimentally measured by the use of FTIR and XPS. SiNW showed the enhanced protein attachment as compared to flat silicon surface due to its large surface area and good molecular penetration to its surface. The methodology developed herein could be generalized to a wide range of protein-ligand interactions that relatively easy to conjugate biotin with diverse biomolecules such as antibodies, enzymes, peptides and nucleotides.
Chapter 5 consists of two parts; Part A deals with DNA hybridization on functionalised porous silicon. 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 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.

Part B of this chapter deals with DNA hybridization on functionalised silicon nanowire surface. Silicon nanowire has been used for the DNA immobilization and hybridization to demonstrate its biological selectivity/specificity. Present study indicates that hybridization of DNA oligonucleotides on nanostructured SiNW surface by complementary oligo with probe attached to the surface also exhibited the enhanced fluorescence intensity as compared to non-complementary oligo. SiNW after surface modification and hybridization have been characterized by FTIR, XPS, and Nanodrop supporting the enhanced DNA binding and hybridization on SiNW. The results of this study reveal that controlled SiNW is very good substrate 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.