Biosensors and Bioelectronics 41 (2013) 54–64

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Porous silicon biosensor: Current status

Saakshi Dhanekar [a,c,*,1], Swati Jain [b,c,1]

[a] Jamia Millia Islamia (Central University), Jamia Nagar, New Delhi 110025, India
[b] Indian Institute of Technology-Delhi, Hauz Khas, New Delhi 110016, India
[c] Amity Institute of Nanotechnology (AINT), Amity University, Sector-125, Noida, Uttar Pradesh, India

ARTICLE INFO

Article history:
Received 4 July 2012
Received in revised form
13 September 2012
Accepted 22 September 2012
Available online 9 October 2012

Keywords:
Porous silicon
Biosensor
Electrochemical etching
Surface functionalization
Antibodies
DNA

ABSTRACT

Biosensing technologies cater to modern day diagnostics and point of care multi-specialty clinics, hospitals and laboratories. Biosensors aggregate the sensitivity of detection methodologies and constitutional selectivity of biomolecules. Endeavors to develop highly sensitive, fast, stable and low cost biosensors have been made possible by extensive and arduous research. Immense research work is going on for detection of molecules using various materials as immobilization substrate and sensing elements. Amongst materials being used as bio-sensing substrates, nano-porous silicon (PS) has amassed attention and gained popularity in recent years. It has captivating and tunable features like ease of fabrication, special optico-physico properties, tailored morphological structure and versatile surface chemistry enhancing its prospects as transducer for fabricating biosensors. The present review describes the fabrication of PS and its biosensing capabilities for detection of various analytes including, but not limited to, glucose, DNA, antibodies, bacteria and viruses. Attention has been consecrated on the various methodologies such as electrical, electrochemical, optical and label free techniques along with the performances of these biosensors. It concludes with some future prospects and challenges of PS based biosensors.

Contents

Abbreviations: PS, Porous silicon; ss, Single strand; ds, Double strand; c-DNA, Complimentary DNA; Ab, Antibody; PL, Photoluminescence

* Corresponding author at: Jamia Millia Islamia (Central University), Jamia Nagar, New Delhi 110025, India.
E-mail addresses: saakshi.dhanekar@yahoo.com (S. Dhanekar), swatijainbiomed.iitd@gmail.com (S. Jain).

[1] These authors contributed equally in writing this manuscript.

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.09.045

1. Introduction

Nanotechnology is the science of materials which ranges in nano dimensions. The properties of a bulk material are quite dissimilar from a nanostructured material. Shrinking the size of any material causes tremendous (sometimes entire) change in its properties making it suitable for various applications. Nanostructured materials have not only paved the way for upcoming technologies but also invited numerous challenges for the research community. Porous silicon (PS) is a nanostructured material, which provides plentiful advantages to ace the present nano sized material community. As the name suggests PS is like a quantum sponge, containing a web like structure consisting of nanocrystallites and pores (Bisi et al., 2000). Not only PS but silicon itself is an attractive and a useful material. Any device application using silicon is bound to be a successful device as silicon is readily available, low cost and easily compatible with the modern IC industry. There are some unique properties of silicon which are noticeable when its structure reduces to nano-dimension. These changes in properties are the outcomes of Quantum Confinement Effect. Thus PS exhibits properties like, enhanced surface to volume ratio ($\sim$500 m^2 cm^{-3}), high surface reactivity and luminescent properties at room temperature. Because of its versatile nature, PS has a large number of applications in sensing, optoelectronics, micromachining, biotechnology, wafer technology etc. (Lehmann and Gösele, 1991; Föll et al., 2002). PS has been extensively exploited as sensor because of its fascinating aforementioned features.

Sensor is a device that as a result of a chemical interaction or process between the analyte and the sensor device, transforms chemical or biochemical information of a quantitative or qualitative type into an analytically useful signal (Stetter et al., 2003). Detection of an analyte or molecule is confirmed by a sensing material when any of the material properties show a considerable change, upon exposure to analyte or molecule. Biosensing is a small section of the wide term sensing, which is the recognition of a bioelement (enzyme, antibody, tissue, dead or live cell etc.) upon its exposure to a sensitive material. The change in material properties is fluxed with a transducer from where the actual output is obtained. This change in material properties can be detected by various scientific techniques thus forming a biosensing device (Mohanty and Kougianos, 2006).

PS demonstrating both optoelectronic properties and biocompatibility has attracted the attention of researchers in biosensing field. Many applications of PS being used as biosensor have been discussed in the recent past (Singh et al., 2009; Tembe et al., 2008; Stefano et al., 2007). PS is being used for detection of glucose, DNA, bacteria, viruses, proteins and many more biomedical treatments and diagnostics. The present comprehensive study reviews current status on the biosensors developed utilizing PS as a sensing matrix for the detection of various analytes. It also focuses on fabrication of PS, protocols followed for surface modification for biomedical applications on the PS surface and its eventual application in fabricating biosensors for the qualitative and quantitative estimation of relevant biomolecules.

2. Porous silicon (PS)

The captivating features of silicon like ease of availability, low cost and compatibility with advanced electronic industry make it an attractive, utile and suitable material for commercial industry. Its key parameters can further be enhanced by reducing bulk silicon to nano structured material. For example, silicon can be transformed from a poor emitter to an efficient emitter of light by making it porous using a simple electrochemical technique.

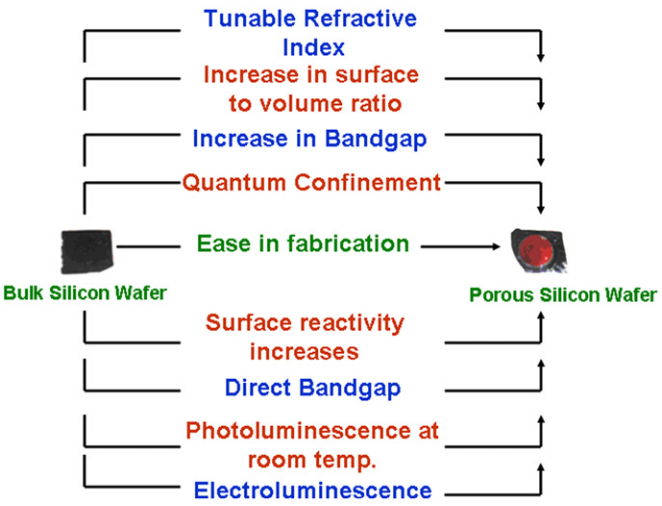

Fig. 1. Change of properties from bulk to PS.

Creation of porous layer where Quantum Confinement plays a dynamic role causes wide change in the material properties, such as optical and electrical, of silicon enhancing its capability of being used in industry of sensors, light emitting devices, transistors, solar cells, integrated circuits and many high end applications (Fig. 1). The pores on the surface of porous silicon (PS) act as binding sites attracting foreign species which get adsorbed on the spongy structure of PS. This forms the basis of sensing mechanism. PS surface can be easily subjected to modifications and chemical reactions to tailor their interface chemistry in view of realization of biosensors. The fascinating properties of PS have captured the industry's eye and proven not only its usefulness as optical devices but also as an expeditious sensor.

2.1. Fabrication of PS

One of the common, simple and efficient methods for fabrication of PS involves employment of electrochemical etching technique. The etching process requires a thorough pre-cleaning of silicon wafer by a chemical procedure for removal of any unwanted deposition on the silicon wafer. This cleaned wafer undergoes a galvanostatic electrochemical etching process which comprises of an etching cell, silicon wafer (anode), a constant current source, platinum wire (cathode) and an electrolyte (Sailor, 2012). The electrolyte consists of hydrofluoric acid (HF, 49%) and ethanol. The current is allowed to flow for a fixed duration called as etching time. The most accepted model for describing etching mechanism has been proposed by Lehmann and Gösele (1991) which is illustrated as Fig. 2 and the net reaction for silicon etching is given in Eq. (1): (Rauscher and Spohn, 2001), where h^+ and e^- represent hole and electron respectively

$$Si + 6HF + h^+ \rightarrow SiF_6^{2-} + H_2 + 4H^+ + e^- \quad (1)$$

Hydrogen gas evolution resulting from this reaction can be observed from the silicon surface during the etching process. It is evident from the reaction that silicon atoms are knocked out from the surface generating porous structure containing nanocrystallites of silicon.

2.2. Porosity

PS fabrication forms pores ranging from macro, micro to nano sizes. Porosity is the fraction of total pore volume to the apparent

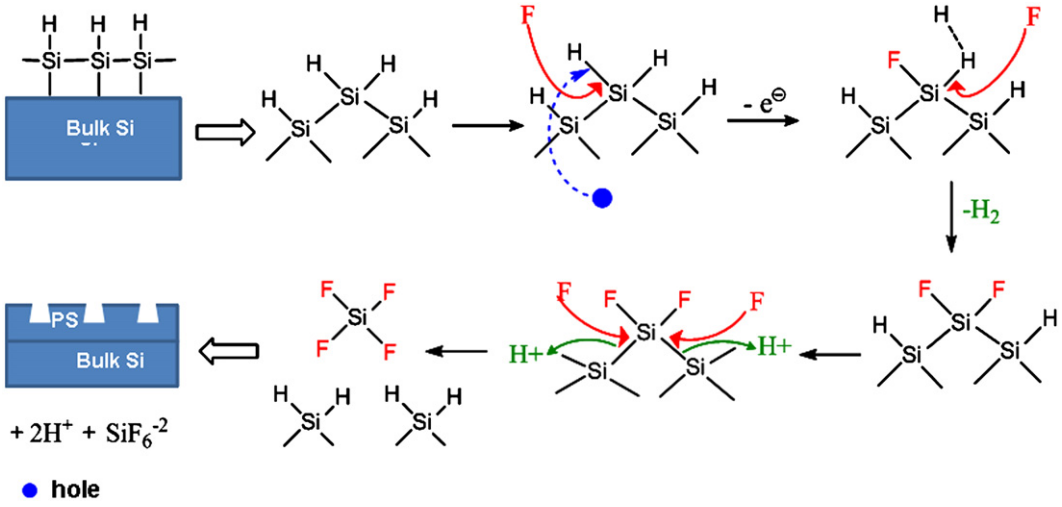

Fig. 2. Reaction mechanism of formation of porous structures on crystalline silicon.

Table 1
Different levels of porosity with their respective applications.

Void content (%)	Level of porosity	Potential applications of PS
0–30	Low	eg., Microcapacitors, wafer bonding, tissue boding
30–70	Medium	eg., Micromachining, sensors, silicon on insulator
70–100	High	eg., LED, anti reflection coating, non linear optics

volume (Sailor, 2012). Different ranges of porosity suitable for selective applications are mentioned in Table 1 (Canham, 1997).

Variant porosities can be obtained by tuning the etching parameters (Dhanekar et al., 2011). Certain factors which affect porosity are electrolyte concentration (HF concentration), current density, etching time, doping type, doping density, wafer resistivity, electrolyte composition, wafer crystallographic orientation, substrate type etc. Thus, etching silicon is very crucial as many parameters are to be mooted which depend upon individual cases and helps researchers decide the etching conditions beforehand.

2.3. Chemical modification of PS

The key feature of sensing matrix for the sensitive detection of chemical or biological analyte is the surface characteristics of the material itself. Large surface area, porosity, topography, morphology and surface functionality impact the sensing capabilities of sensing matrix and its interaction with the adsorbate. For sensing applications, biologically active species need to be chemically attached on the surface of PS. It has been noticed that for achievement of high selectivity chemical modification is required on PS surface (Buriak, 2006).

Freshly etched PS is hydrogen terminated having Si–H, Si–H_2 and Si–H_3 or Si–H_x hydrides on the surface which are very responsive, reactive and metastable (Anderson et al., 2003). During fabrication, storage in ambient or laboratory, air induces impurities in PS such as oxygen, carbon etc. Storage in air for long period of time also leads to substantial ageing of PS layer degrading its surface. (Beckmann, 1965). This was attributed to native oxidation of PS because of reaction of labile Si–H bonds with oxygen molecules due to environmental conditions of humidity, temperature etc. Therefore, substitution of Si–H with other functional groups renders stabilized PS. One of the most prominent ways of chemical modification of PS surface is its thermal oxidation (Salonen et al., 1997). Partial oxidation of PS causes back-bond oxidation and selective reaction of oxygen atoms with silicon atoms resulting in replacement of some of the H-atoms. This leads to formation of Si–O–Si, O–Si–H, O_3–Si–H groups and bridging of inter-atomic silicon layers with O atoms. In addition to stabilization, oxidation of PS also introduces hydrophilicity to the material which is an essential requirement in biological applications (Bragaru et al., 2007; Dhanekar et al., 2012).

PS can be functionalized by the replacement of hydride bonds with Si–C bonds which impart thermodynamic stabilization to PS surface (Salonen et al., 2008). Various chemistries are employed for Si–C bond formation when PS reacts under electrochemical conditions with aryl and alkyl lithium and methyl Grignard reagents (Kim and Laibinis, 1998). Hydrosilylation of alkenes and alkynes induced by Lewis acids ethyl aluminum chloride ($EtAlCl_2$) is also used for monolayer formation bound to PS. This results in alkyl or alkenyl termination instead of Si–H bonds. Long alkyl chain caps the hydrophobic PS surface while imparting stability under demanding basic chemical conditions as compared to unfunctionalized PS (Buriak et al., 1999; Smet et al., 2005). Hydrosilylation of undecylenic acid gives carboxy terminated architecture which further can be easily modified (Schwartz et al., 2006).

Linkers are utilized to covalently immobilize biomolecules on the surface of PS. Oxidized PS having Si–O–Si surface functionality reacts chemically with 3-aminopropyltriethoxysilane (APTS), 3-glycidoxypropyltrimethoxysilane (GOPS) etc. (Fernandez et al., 2008). In some cases linker attached PS film further reacts with a bifunctional reagent like glutaraldehyde (Meskini et al., 2007). Subsequently these linker tagged films covalently attach with amino group terminated biologically active moiety such as antibody or DNA. All kinds of chemical modifications are carried out, though linker attachment is more famous as their chemistry is easier and simple. Currently, due to effective chemical transformations culminating in sufficient functionalization, hydrosilylation is gaining importance and popularity. A schematic representation of different surface functionalization reactions of PS is given in Fig. 3 while Fig. 4 depicts covalent attachment of biomolecules such as amino group containing DNA, antibodies and enzymes onto functionalized PS surface.

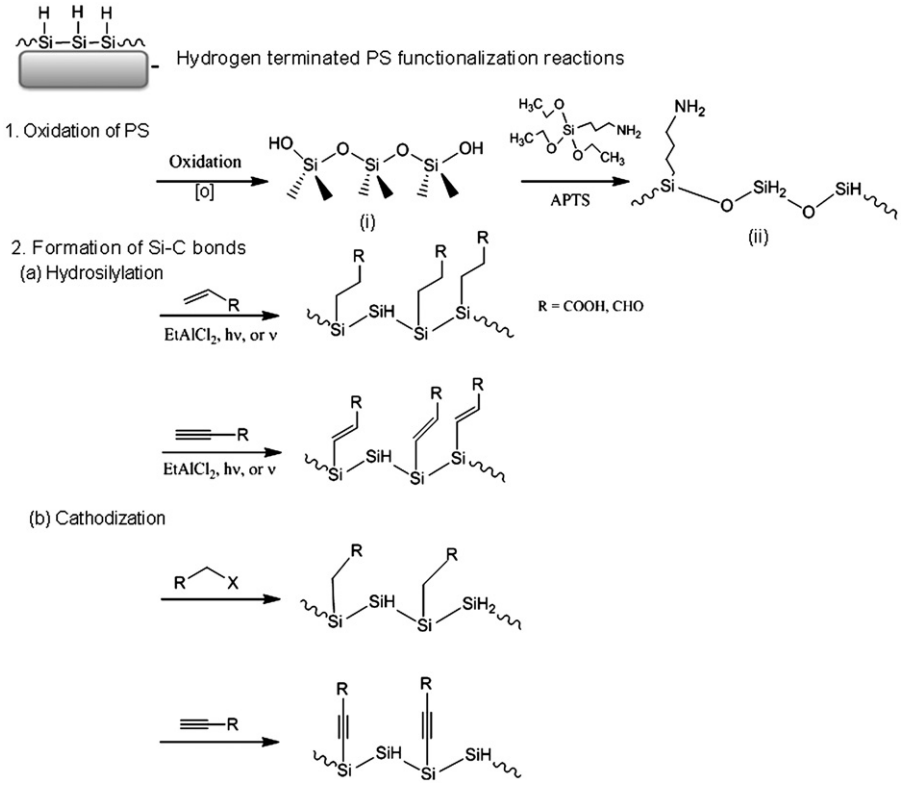

Fig. 3. Some of the surface chemical reactions on porous silicon. Hydrogen terminated silicon surface can be (1) oxidized to give (i) hydroxyl, silyloxy and alkoxy terminated bonds which can further react with APTS to generate (ii) amino groups on the surface. (2) Generation of Si–C bonds on PS surface by hydrosilylation and cathodization on reaction with various precursors like alkene, alkynes, Grignard reagents alkyl halides etc. (Buriak, 2006; Ciampi et al., 2010; Bragaru et al., 2007).

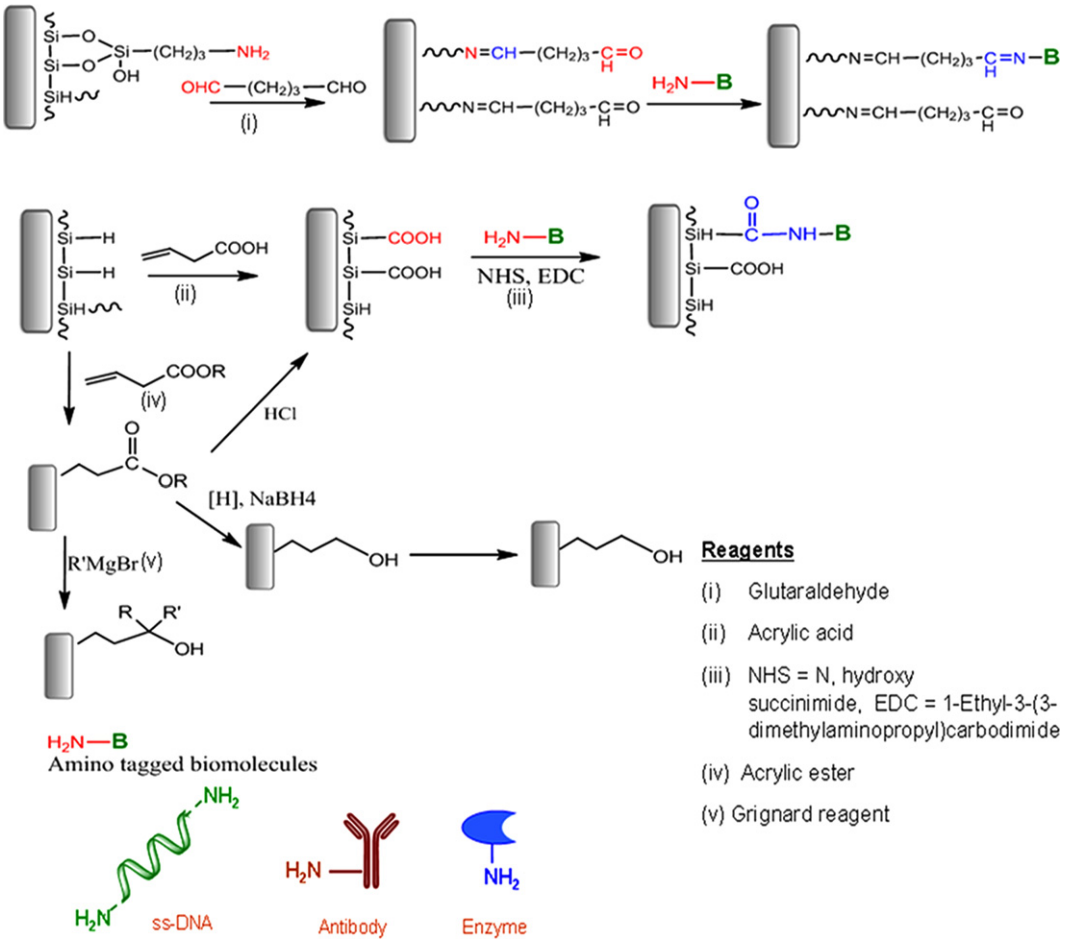

Fig. 4. Covalent attachment of amino group containing biomolecules onto functionalized PS.

3. Biomolecule detection on PS

The field of biosensors sprang up in 1962 when Leland C. Clark developed enzyme electrodes (Mohanty and Kougianos, 2006) that measured dissolved oxygen in the blood of patients undergoing surgery. PS based biosensors comprises of four main components: bio-element, sensing material, transducer and signal processor. The bio-element bounds to the surface of PS inducing a change in its material properties. These changes are recorded and conveyed to a transducer. The transducer converts signal from one form to another and further directs them to a signal processor delivering a readable signal for users. The combinatorial effect of PS, a sensitive matrix and its surface modification make it a selective and sensitive substrate for sensing biomolecules. The change in material properties of such functionalized PS surfaces upon exposure to bio-elements forms a fundamental subject for sensing purposes. These changes can be in the form of electrical (conductance, resistance, capacitance etc.), electrochemical (conductimetric, amperometric, impedimetric and potentiometric), optical (reflectance, photoluminescence, fluorescence etc.) and thermal (temperature change) signals. Biosensors have a wide variety of applications including glucose monitoring, pesticide detection, pathogen detection, quantitative measurements of toxicity, determination of drug residues in food, protein engineering, drug delivery and evaluation of biological activity of new compounds (Mohanty and Kougianos, 2006; Marquette and Blum, 2006). Fig. 5 depicts the sensor components for biosensing applications by PS using various techniques. The following sections discuss the detection of bio-elements by involving changes in PS properties in detail and are also summarized in Table 2.

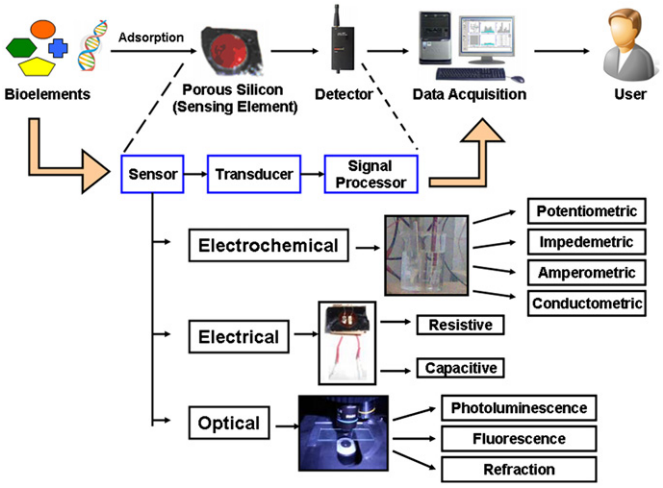

Fig. 5. Various techniques for PS based biosensing.

3.1. Glucose

Diabetes is one of the most prominent lifestyle diseases in present scenario with each year millions of cases being reported across the globe. Regulation of sugar in the human blood is the most crucial criteria for health benefits. Hence, close control and monitoring of glucose has become essential to avoid severe adverse effects of hypoglycaemia and hyperglycemia (Diabetes) which leads to blindness, neuropathies, coma and to an extreme, even death (Gavin, 2007). A regular check of glucose helps avert many emergencies and thus such a check should be available in the form of an easy process or a hand held device, in short called a glucose biosensor. Mammoth research work is devoted towards the development of non-invasive sensor for glucose which not

Table 2
Summary of detection of various biomolecules using PS as sensor (where nd–not defined, Aq.–Aqueous solutions, H.s.–humic solutions).

Analyte	Transducer	Detection range	Sensitivity	Response time	Reference
DNA	Electrochemical	0.5×10^{-10}–500×10^{-10} M	0.5×10^{-10} M	15 min	Lugo et al. (2007)
	Electrical: impedance	$>10^{-7}$ M	0.5 μM	30 min	Archer et al. (2004)
	Optical: fluorescence	6–80 μM	90 nM	nd	Stefano et al. (2007)
	Electrical: impedance	nd	nd	15 min	Vamvakaki and Chaniotakis, (2008)
Glucose	Electrical: current–voltage (I–V)	3–7 g/L	nd	30 min	Melikjanyan and Martirosyan (2011)
	Electrical: (I–V)	1–1000 μg/L	nd	25 min	Sánchez et al. (2010)
Bacteria	Electrical: (I–V)	2.5–100 μg/mL	nd	30 min	Sánchez et al. (2010)
	Electrical: (I–V)	2.5–100 μg/mL	nd	30 min	Sánchez et al. (2011)
	Optical: photo-luminescence (PL)	10–1000 cells/Ml	20 cells/mL	nd	Li et al. (2011)
Virus Lambda	Optical: PL	nd	nd	1 h	Chan et al. (2001b)
Virus MS2	Optical: fluorescence	1×10^{6}–1×10^{12} pfu/mL	2×10^{7} pfu/mL	45 min	Rossi et al. (2007)
Catechol	Current	1.0×10^{-5}–3.0×10^{-4} M	5.0×10^{-5} M	120 s	Tembe et al. (2008)
Triglycerides	Potentiometric	nd	30 mV/pH unit (5–40 mM)	nd	Reddy et al. (2003)
Antibodies—rabbit IgG	Optical: PL (Microcavity)	1–2 μM	0.3 ng/mm^{-2}	nd	Ouyang et al. (2005)
Penicillin	Potentiometeric	10^{-4}–10^{-1} M	40 mV	nd	Thust et al. (1996)
Alanine aminotransferase (ALT)	Electrochemical	1.3–250 U/L	0.145 μA/(U/L)	20 s	Song et al. (2009)
	Electrochemical	1.3–250 U/L	0.13698 μA/(U/L)	20 s	Song et al. (2007)
Aspartate aminotransferase (AST)	Electrochemical	1.3–250 U/L	0.463 μA/(U/L)	20 s	Song et al. (2009)
	Electrochemical	1.3–250 U/L	0.45439 μA/(U/L)	20 s	Song et al. (2007)
Cholesterol	Electrochemical	$1–50\times10^{-3}$ M	0.2656 μA/mM	20 s	Song et al. (2007)
Bilirubin	Electrochemical	0.002–0.020 mM	0.15354 mA/mM	20 s	Song et al. (2007)
Pesticide Atrazine	Optical: distributed Bragg reflectors	0–5 ppm	1.19 nm/ppm (Aq.); 0.51 nm/ppm (H.s.)	nd	Rotiroti et al. (2005)
Triadimenol	Optical microcavities: refrective index	nd	0.878 nm/ppm (Aq.); 0.644 nm/ppm (H.s.)	nd	Stefano et al. (2005)

only is accurate, sensitive and is less painful also (Newman and Turner, 2005). Glucose sensor should selectively respond to glucose and broadly reject any other analyte. The basic concept of the glucose biosensor is based on the oxidation of β-D-glucose by molecular oxygen producing gluconic acid and hydrogen peroxide. Hydrogen peroxide is further oxidized at the electrode which can easily recognize the number of electrons transferred. This electron flow is proportional to the number of glucose molecules present in the blood (Rahman et al., 2010). Various research groups have attempted glucose detection based on aforementioned principle. Incubation of PS samples in varying glucose solutions have been done for electrical measurements performed for both longitudinal and transversal conduction through PS structure for glucose detection (García and Palma, 2007). Performance of glucose sensing has been ameliorated by surface treating PS with glucose oxidase (GOx) which is most efficient in attracting glucose molecules. The complete reaction is shown in Eqs. (2) and (3). Three strategies used for electrochemical sensing of glucose are by (a) measuring oxygen consumption, (b) measuring amount of H_2O_2 production or (c) using diffusible/immobilized mediator to transfer the electrons from GOx to the electrode

$$D\text{-Glucose} + O_2 + H_2O \xrightarrow{GOx} D\text{-Gluconic acid} + H_2O_2 \quad (2)$$

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^- \quad (3)$$

GOx functionalized PS sensors have been very successful in detecting glucose selectively (Fig. 6). It has already been shown that the activity of enzyme glucose oxidase increases by a factor of 100 when it is immobilized on PS surface (Melikjanyan and Martirosyan, 2011). PS microcavities on functionalization with GOx can detect upto 0.3% change in glucose concentration (Rong et al., 2011).

3.2. Deoxyribose nucleic acid (DNA) hybridization

DNA is the programmable long polymeric network constituting the genetic material of cell and has garnered popularity in biotechnology, diagnostics and clinical analysis owing to its inherent molecular recognition property. New age devices based on DNA sequence detection rely on nucleic acid hybridization which is designed with the immobilization of short single stranded DNA probe (ss-DNA) which forms double stranded (ds-DNA) helical duplex with the complimentary target nucleic acid fragment (Kerman et al. 2004; Tichoniuk et al. 2008). The probe is associated with the transducer translating this hybridization event into measurable value. Variety of solid substrate such as gold, carbon nanotubes (CNTs), colloidal particles, polymers, porous alumina, crystalline silicon and PS have been examined for immobilization of ss-DNA (Singh et al., 2011). Few of these advanced nanoscale materials such as CNTs, nanocrystals, quantum dots etc. are difficult to synthesize in high yield having appreciable biocompatibility. However, PS proved to be advantageous as its large number of pores have the ability to accommodate and attach the active biomolecule like ss-DNA on them. Various methodologies have been applied for DNA/oligonucleotide sequence attachment on PS surface. Surface modification involving oxidation of PS surface forms a thin layer of SiO_2 creating hydrophilic channels for efficient DNA penetration. Silanization of oxidized PS with different silanes 3-glycidoxypropyltrimethoxy silane (GOPS) and 3-aminopropyltriethoxysilane (APTS) provides electrophilic reactive sites for the covalent binding with amino terminated oligonucleotide (Sotiropoulou et al., 2005). Further, hybridization with complimentary DNA (c-DNA) sequence results in observable changes in PS material properties depicting sensing as represented in Fig. 7.

In early 21st century reports suggest that PS surface immobilized with oligonucleotides responds electrically, portraying DNA detection (Archer et al., 2004,2005). Macroporous PS surface was functionalized with poly-L-lysine and immobilized with DNA via electrostatic interactions. Their research concluded that the hybridization with c-DNA was detected due to localized enhancement in charges, shift in phase angle and reduction in impedance of PS. This study was described by space charge region modulation model which states that both the space charge region of crystalline silicon columns and dielectric constant of porous structure change which is attributed to the formation of electrical double-layer when PS is exposed to a liquid containing charged species. Neutral species did not respond to this phenomenon as proved by uncharged DNA analog peptide nucleic acid (PNA) which did not give rise to any impedance change.

Electrochemical biosensor for the detection of DNA involves conversion of some electroactive compound where the single strand of oligonucleotide is attached on PS surface. Duplex formation has been detected by employing reversible redox reaction of guanine base present in DNA with ruthenium bipyridine in PS. This reaction shows the catalytic effect on the anodic peak current directly related to the concentration of target DNA. Report (Lugo et al., 2007) confirms higher DNA sensitivity of PS (5×10^{-11} M) than gold substrate (9.0×10^{-11}) as summarized in Table 2. Vamvakaki and Chaniotakis (2008) devised electrochemical impedance based DNA

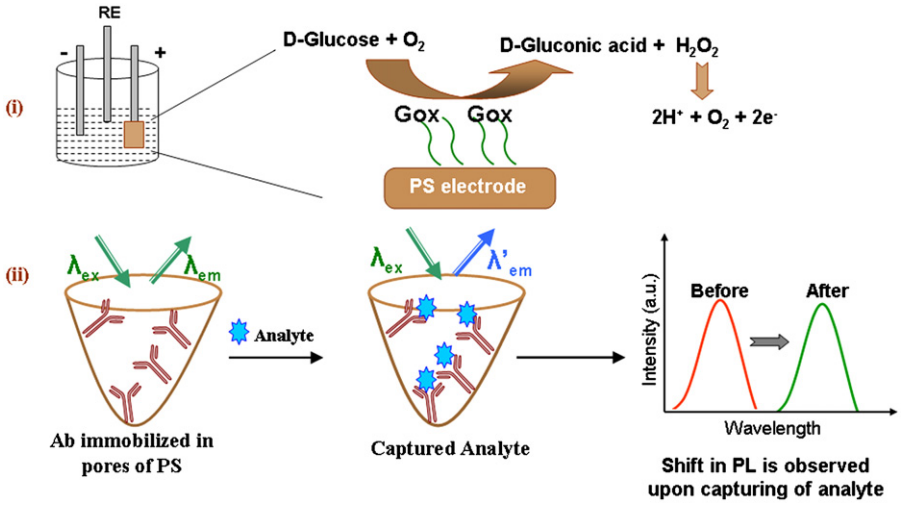

Fig. 6. Schematic representation of detection of (i) glucose (ii) any analyte attached through antibodies on PS.

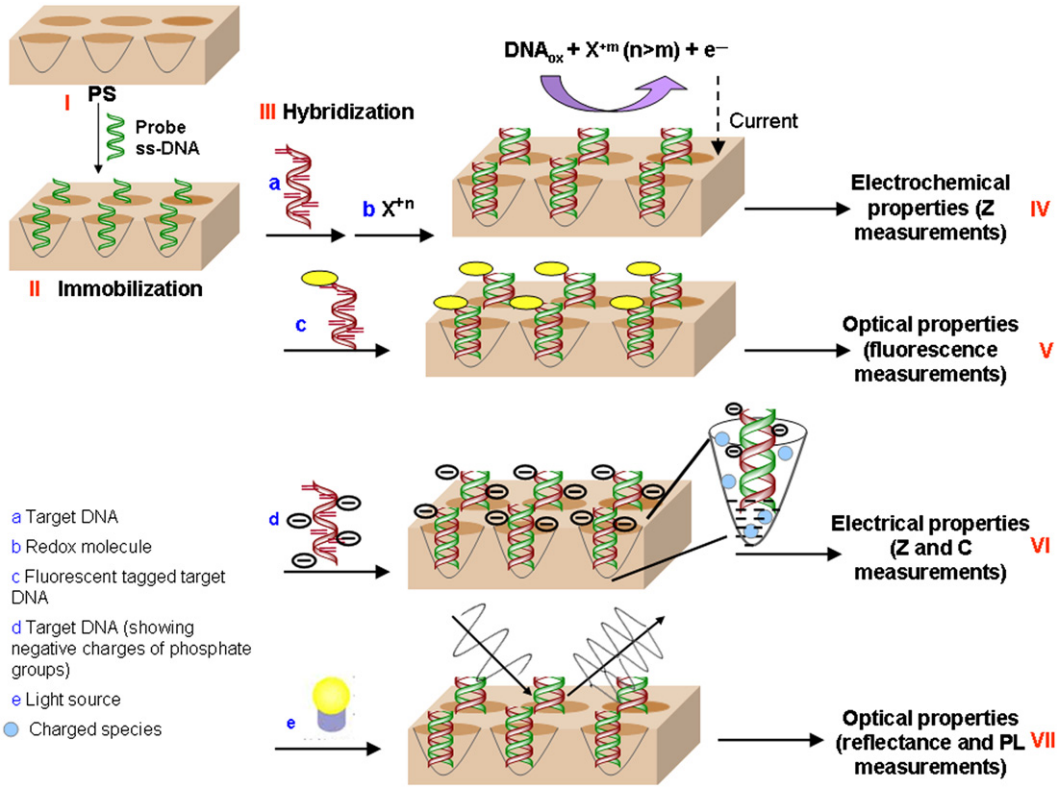

Fig. 7. Descriptive representation of DNA detection using (I) PS, (II) upon immobilization of ss-DNA and (III) hybridization resulting in ds-DNA, via change in (IV) electrochemical properties such as impedance (*Z*) measurements, (*V*) optical properties like fluorescence, (VI) electrical properties viz. impedance (*Z*), conductance (*C*) and (VII) optical properties like reflectance and photoluminescence (PL).

hybridization PS biosensor. Pores ranging from 20–40 nm size on p-type boron doped silicon wafers were created using electrochemical etching. PS surface was functionalized by anodic oxidation and 21-mer oligonucleotide (5′-GAG GAG TTG GGG GAG CAC ATT-3′) was attached on it thereafter. It was observed that the dielectric constant of PS increased on attachment of such highly negative nucleotides on the surface. This increase in dielectric constant led to decrease in impedance of the designed system. Complimentary DNA sequence (5′-AAT GTG CTC CCC CAA CTC CTC-3′) hybridization forms double helix leading to a decrease in hydrated sphere and thus the amount of electrolyte surrounding the initial ss-DNA. It has been proposed that formation of duplex on hybridization results in lesser interface charge density following decrease in capacitance and increase in impedance measurements.

Pioneering work of Sailor demonstrated integration of PS with some biological moiety allows the realization of advantageous label free biosensors. He and his coworkers proposed interferometeric (Fabry–Pèrot) PS sensor based on changes in refractive index offering label free analyte sensing (Kochergin and Föll, 2009). Binding of c-DNA strand with functionalized PS surface modifies the refractive index or optical thickness of the PS layer. The binding of molecules on pore walls brought forth red shift in Fabry–Pèrot fringes interference and those were monitored spectrally via reflection spectroscopy (Lin et al., 1997; Sailor, 2012).

Attachment of ss-DNA chain and its further hybridization duplex formation also results in variation of optical properties of PS. In one of the articles, specially designed linker tri-methoxy-3-bromoacetamidopropyl silane was found to react through silane group of the APTS silanized PS surface (Francia et al., 2005). Single chain 5-phosphothionate oligonucleotide reacted with bromoacetoamido moiety of the linker and covalently bound to PS surface and further hybridized with c-DNA sequence. PL spectra of every step of chemical reaction revealed the mechanism of linker and DNA binding to PS. They showed an increase in integrated PL on DNA duplex formation with c-DNA and null effect with non c-DNA. The linker coverage stabilized the capping layer, improved PS passivation and enhanced its PL.

PS optical biosensor for the qualitative detection of DNA hybridization based on reflectivity measurements of native, functionalized, ss-DNA bioconjugated, c-DNA hybridized PS surface has been realized (Stefano et al., 2007). Porous layer can infiltrate oligonulcleotides or ss-DNAs which get adsorbed on the layer due to electrostatic interactions. These probes hybridize the c-DNA sequence producing an increase in the overall charges, hence each step of reaction increases the optical path in reflectivity spectrum due to air in the pores in PS layer being substituted by organic and biological species. ss-DNA interacted with c-DNA sequence and detection has been realized as fringes shift in wavelengths corresponding to change in optical path.

3.3. *Immunoglobulins (Igs)*

Immunoglobulins or antibodies (Abs) are groups of proteinaceous macromolecules, defending against “foreign” invaders such as microbes, toxins, etc. Determination of Abs or immunological assays, give information on immune system functioning and level of immunity. Antibody attachment does not cause any appreciable change in functionalized PS surface characteristics for the development of assays (Singh et al., 2009; Schwartz et al., 2007). Label free biosensor for the detection of rabbit immunoglobulins G in whole blood and dilute serum samples have been established by Bonanno and DeLouise (2007) utilizing PS microcavity system. The sensing set up used PS microcavities as intrinsic size exclusion filters which enhanced signal differentiation. Covalent linkages of receptor antibodies with PS surface were formed by employing Biotin/Streptavidin chemistry which captured target

analyte antibodies upon filtration, detected by change in wavelength. Linear detection range of 2–10 mg/mL, high specificity and good sensitivity were demonstrated. Another sensor based on highly doped n-type macroporous silicon microcavity system for the detection of large molecules is also reported (Ouyang et al., 2005). The article illustrated the systematic dependence of sensor sensitivity on pore size of silicon. Biotin/Streptavidin coupling involved in the filtration device for the detection of rabbit IgGs (150 kDa; 1 Da = 1 g/mol) which displayed sensitivity of 1–2 μM (equivalent to 0.3 ng/mm) of Abs.

PS based immunosensor has been developed for detection of antigen to as low as nanogram levels (Prabhakar et al., 2012). Electrochemically etched silicon wafers upon chemical treatments immobilized Human IgG (antibody). The antibody attached PS surface was exposed to Goat anti-human IgG (taken as antigen). Cyclic voltammetry (*C–V*) studies were performed for confirming the binding of antibody and antigen. The anodic current was found to increase upon attachment of antibody on PS surface and it decreased subsequently when this functionalized surface was exposed to antigen. Immunosensing study was established when the surface reacted to attachment of antigen/analyte to the functionalized PS. Due to dual nature of PS surface serving as sensing element and filtration device, it illustrates high possibilities for Ab detection especially in whole blood. Systematic studies are requisite before fabrication of PS sensor for Ab detection.

3.4. Bacteria

Sensitive biosensing of DNA, proteins and other molecules stimulated research to examine the suitability of PS structures as a detector for complex living organisms. Biorecognition properties of both genetic and proteinaceous material have been exploited in large complex structures including disease causing bacteria and viruses amounting to death of large population globally. Evaluating contamination of bacteria is a daunting task but essential for preventive and treatment measures. Nanoporous PS offers advantages of variable chemical and biological designing of its surface for the determination of various bacterial strains.

Gram positive and negative bacteria can be easily differentiated by biosensors relying on their compositional difference. This was executed by fabricating a biosensor using an organic receptor tetratryptophan ter-cyclopentane (TWTCP) chemically bonded to 3-glycidoxy propyl trimethoxy silane modified PS surface acted as the probe (Chan et al., 2001a). It recognized highly abundant lipid A, a lipopolysaccharide (LPS) specific to gram −ve bacteria by exhibiting a red shift in the photoluminescence of PS microcavity. This generated sensor was able to detect lipid A with an appreciable detection limit of ~1.7 μg.

Biosensors based on recognition of specific antibodies immobilized on PS have also been studied for bacterial detection (Sánchez et al., 2010,2011). Metal/nanoPS/Metal devices fabricated by making metallic contacts on PS silicon wafer were functionalized with APTS and immobilized with *E. coli* antibodies which further attached bacterial fragments empirically. This resulted in enhanced conductivity thus, forming an electrical biosensor.

Zhang and Alocilja (2008) fabricated a label free DNA electrochemical biosensor on nanoporous silicon chips for the determination of food borne bacteria, *Salmonella Enteritidis*. The nano PS chips silanized by GOPS before immobilization with DNA probe hybridized with c-DNA resulted in variation of current of PS system. Their quality control experiments revealed that the PS had 4 times higher surface area than crystalline silicon and higher DNA probe selectivity and affinity to the target DNA with an impressive ~1 mg/mL detection limit. Most of these detection procedures involve non-specific fragments of bacteria, more work is imperatively needed for further establishing correlation between bacterial counts with fragments.

3.5. Viruses

Detection of viruses is considerably challenging owing to their very small size as compared to bacteria but is essential for pharmaceutical research, disease prognosis, agriculture and homeland security. Utilization of PS for virus detection is very recent but promising. Rabbit-anti Bacteriophage MS2 antibody has been successfully immobilized on surface functionalized PS film covalently, which resulted in selective capturing of dye-labeled MS2 viruses from solution. The detection limit of 2×10^7 plaque-forming units per mL (pfu/mL) was measured by fluorescence from exposed PS film (Rossi et al., 2007).

State of the art technique has been devised for the detection of *E. coli* bacteriophage lambda based on its DNA hybridization phenomenon (Chan et al., 2001b). It has been demonstrated that specific heating of PS chip reduced the problems of steric hindrance of phage due to its 3-D supercoiled structural conformation. Heating uncoiled the DNA strands providing thermodynamic assistance in penetration of the target into the pores of PS. After oligonucleotide immobilization the temperature was reduced for c-DNA hybridization which caused a red shift in PL spectrum of the surface. Vashpanov et al. (2008) investigated changes in electrical parameters of mesoporous silicon after adsorption of plant viruses called Nematode transmitted Polyhedral such as TORSV (Tomato Ringspot Virus), GFLV (Grapevine Fan Leaf Virus). Geometrical morphology, sizes and shapes of viruses influences their penetration in the pores of PS elucidating their selective incorporation and indicating effective detection of viruses with 50 nm size.

3.6. Other small analytes

3.6.1. Triglycerides

PS is used as electrode of electrochemical cell in potentiometric biosensors, where the modified PS wafer both immobilizes biomolecule and forms the pathway for transducing electrolytic solution in a detectable electric signal. Potential difference between cathode usually platinum electrode and anode PS electrode of the electrochemical cell is determined. A hypothesis was formulated by Thust et al. (1996) that PS electrode can detect analyte which produces change in pH of the electrolyte solution in contact with electrode surface. He and his coworkers investigated PS for the very first time as a substrate material for potentiometric biosensor operating in aqueous solutions. Penicillinase enzyme, physically immobilized on oxidized PS surface acted as catalyst for the hydrolysis of penicillin G leading to variation in pH of the solution and this was monitored by Capacitance–Voltage measurements. Following this work, researchers developed potentiometric biosensor for the detection of triglycerides. These molecules are ester derivatives of glycerol with fatty acids which aid in fat metabolism working as energy source in human body and also assist in transporting fats. They are related to artherosclerosis, coronary diseases, heart stroke etc. when present in high levels in the blood stream. p or n-type PS immobilized with lipase enzyme catalysis conversion of triglycerides into glycerol and fatty acid promoting the pH shift that is detected by the electrochemical cell and read as potential change (Setzu et al., 2007; Reddy et al., 2003). Reports suggest quantification of triglycerides by PS sensors compare well with other sensing set ups utilizing different substrate material (Salis et al., 2011). The fact that high surface area of PS increases the biosensing capability of the device, Song et al. (2006) studied

the development of cholesterol biosensor on PS surface and compared the results with planar flat silicon surface. It was demonstrated that effective surface area and biosensing ability of porous matrix was 3.1 fold times than that of the planar surface of the silicon electrode. Electrochemical biosensor array system was further fabricated for the diagnosis and monitoring of liver diseases. The array consisted of cholesterol, bilirubin and glutamate sensors for the sensitive determination of four different biomarkers- cholesterol, bilirubin, alanine aminotranferase (ALT) and aspartate aminotransferase (AST) levels in aqueous fluid samples (Song et al., 2009).

3.6.2. Phenolics, organophosphates and warfare agents

Biosensing technology using PS has been extended to the detection of smaller analytes such as pesticides, explosives, organophosphate, nitro-aromatics etc. which pose health and environmental threats. Phenolics and their derivatives like catechol, used in paper, dye, plastics and drug manufacturing industry, are common pollutants. Catechol is readily absorbed from the gastrointestinal tract, causing vasoconstriction, renal tube degeneration, liver function decrease, cancers, and neurodegenerative diseases, apart from accumulating in bone marrow (Irnos et al., 1985). A conductivity based sensor was formulated by immobilizing tyrosinase enzyme on oxidized PS (Tembe et al., 2008). High sensitivity of 5×10^{-5} M concentration of catechol was achieved on enzyme immobilized PS which can be attributed to possibility of adjustment of PS pore size with enzyme molecules. Also immobilization inside the pores of PS resulted in better mechanical stability against leaching of enzyme over time.

Pesticides are generally chemical or biological agents which are used for preventing, repelling, extenuating insects, pathogens, weeds, rodents and microbes that destroy property, cause nuisance, spread disease or are vectors for diseases. But, if overused they may become detrimental to human and society lead to a variety of health risks like irritation of skin and eyes, damage to nervous system, reproductive problems and cancer. It is therefore essential to detect these pesticides in the environment before its exposure exceeds its permissible limit. The commonly used methods for such detections are very complicated and time consuming. Hence, there is a need of simple and quick detection of pesticides. Considering the core benefits of PS as sensing element, Rotiroti et al. (2005) detected and quantified the presence of atrazine (2-chloride-4-ethylamine-6-isopropyl amino-1, 3, 5-triazine) in water and humic acid solutions using PS microcavities. Arrangement of distributed Bragg reflectors (DBRs) with a Fabry–Pèrot film aid in efficient detection of molecules. Upon exposure of atrazine solutions, a marked red shift of PS microcavity characteristics peak was observed showing appreciable sensitivity and limit of detection. In addition to this research, Stefano et al. (2005) also sensitively detected the presence of pesticide-triadimenol in water and humic solutions (Table 2). These studies concluded that sensing pesticides by PS can be done by using simple and less time consuming techniques.

L-glutamine is a non-essential amino acid and plays an important role in biochemical functions including protein synthesis, regulation of acid–base balance in kidney, nitrogen donation, and nontoxic transportation of ammonia and is also used in culturing of eukaryotic cells. Monitoring of glutamine is essential throughout the microbial culturing process since catabolism of glutamine leads to ammonia formation which might result in increase in toxicity and inhibition of growth process. PS having Fabry–Perot interferometers structure has been employed for detection of L-glutamine (Stefano et al., 2004). L-Glutamine binding protein (GlnBP) of *E. coli* interacted with hydrophobic PS surface showing fluorescence signal because of adsorbed dye labeled protein whereas no fluorescence was marked in absence of protein. The ligand binding was detected by fringes shift in wavelength, corresponding to a refractive index change. One shift was noticed when protein adsorbed on PS surface and consecutively, when glutamine was attached to this protein adsorbed PS surface.

Warfare agents, dangerous chemicals such as organophosphorous compounds, nitro derivates pesticides attack the nervous system of body blocking transmission of signal at synaptic junction by inhibiting acetylcholinetrase enzyme (AChE). Auria et al. (2006) have reported optical biosensor technology for the detection of common explosives on PS substrate immobilized with AChE based on fluorescence.

3.6.3. Cancer detection

PS is studied for its potential use in making various formats for the detection of variety of substances. Ressine et al. (2007) introduced PS as a substrate for 3-D microarray applications helpful in cancer diagnostics, where cancer is known to be one of the most potent and lethal diseases. A reverse phase protein microarray in cancer biomarker detection on PS was designed. Cyclin E which shows over-expression and poor prognosis in different types of cancers, for example: breast, brain, gastrointestinal, colorectal and lung cancer (Kirla et al., 2003; Goto et al., 2003; Takahashi et al., 2002) and others, is detected specifically and sensitively on PS based microarray. PS surface offers advantages of high spot density, spot quality, enlarged area, superhydrophobic zones, confined microarray spots, high contact efficiency, linear range and sensitivity as compared with other types of materials (Finnskog et al., 2004; Steinhauer et al., 2005; Ressine et al., 2005, 2006).

In case of in vivo diagnostics and in vitro sensing PS needs to be surface modified for direct contact with the live cells to improve cell viability. Alvarez et al. (2009) grafted different chemical species on PS—silicon oxide (via ozone oxidation), dodecyl (via hydrosilylation with dodecene), undecanoic acid (via hydrosilylation with undecylenic acid), and oligo(ethylene) glycol (via hydrosilylation with undecylenic acid followed by an oligo(ethylene) glycol coupling reaction). The surfaces adhere primary rat hepatocytes with and without pre-attachment of collagen and studied their adhesion efficacy and cell viability. They demonstrated that surface functionalization does not exert any deleterious effect on hepatocytes and hydrosilylation chemistry increases the stability and adhesion coverage of live rat cells which compares well with standard tissue culture polystyrene preparations. This research can direct the use of PS over polystyrene plates in culturing techniques.

4. Challenges of PS based biosensors and their future scope

Apart from the numerous advantages provided by PS, there are few challenges that are still to be dealt with. The challenges include formation and functionalization of PS layer. Forming a uniform porous layer on silicon wafer using electrochemical etching technique is complicated and perplexing as it is dependent on multiple etching parameters. A slight alteration in any of the parameters would bring a vast change in the porous layer. It is therefore, imperative to predefine the etching parameters to make sure that desired porous layer is fabricated. Another challenge is to address the chemical nature of the formed porous layer, as prepared PS is covered with hydrogen bonds which make the surface very unstable. Silicon surface is also labile to formation of an oxide layer when exposed to ambient air. Hence, it is indispensable to functionalize the PS surface for making it stable and selective for particular analytes. From device point of view, placing ohmic contacts for electrical studies on PS based devices

is very crucial. Formation of contacts includes protocols which bring about many modifications in the surface chemistry of PS. Ageing effect is also a major issue in using PS devices as the surface is prone to significant changes in properties, if exposed to environment for a longer time. This can be tackled if the surface properties are locked so that it is not affected by the environment. Medical analysis, central laboratory testing and point-of-care testing handle blood plasma samples and whole blood where red blood cells may pose some challenges while sensing. They cause optical and chemical interference leading to difference in analyte concentration determination. Though this problem is being worked on where porous structure of PS work as filters. The future of PS as inexpensive, commercial and hand-held biosensor device will be decided upon overcoming the challenges so as to conquer the industry share among other material devices.

5. Conclusion

In this paper, we have overviewed recent progress in biosensing by porous silicon (PS). The working capabilities of PS as a physical sensing element, has been successfully extended to biological world. The perspectives of PS, its usefulness as sensor and detection of numerous biomolecules have been discoursed. The sensor parameters have been quantified to realize the capacity of PS as biosensor. Interaction of biological recognition element with PS depends critically on pore morphology and surface chemistry of PS which are tunable. Detection of biomolecules like glucose, DNA, immunoglobulins, bacteria, viruses, triglycerides, phenolics, organophosphates, warfare agents and cancer were thoroughly studied and incited. In vitro diagnosis of biomolecules was depicted to satisfy validating sensor parameters such as selectivity, specificity, reproducibility, etc. From an unconventional point of view, research is under progress for in-vivo testing on PS but still these proof-of concept models can further initiate development of advance bio-chips for the determination of medical anomilities. Thus PS has high capabilities as immobilization substrate and sensing element and will pave the way for new age diagnostics.

Acknowledgment

The authors express sincere gratitude to Prof. S. S. Islam, Nano-Sensor Research Laboratory, Department of Applied Sciences & Humanities, Faculty of Engineering and Technology, Jamia Millia Islamia, New Delhi, India for his guidance in understanding the concepts of porous silicon. We also extend our gratitude to Dr. Sruti Chattopadhyay, postdoctoral fellow at IIT Delhi, New Delhi, India for reviewing this manuscript. The authors are earnestly thankful Dr. Ashok K. Chauhan, Founder President of Amity University, Noida, Uttar Pradesh, India, for his continual motivation and incitation for completion of this manuscript.

References

Alvarez, S.D., Derfus, A.M., Schwartz, M.P., Bhatia, S.N., Sailor, M.J., 2009. Biomaterials 30, 26–34.
Anderson, S.H.C., Elliot, H., Wallis, D.J., Canham, L.T., Powell, J.J., 2003. Physica Status Solidi A 197, 331–335.
Archer, M., Christophereson, M., Fauchet, P.M., 2004. Biomedical Microdevices 6 (3), 203–211.
Archer, M., Christophereson, M., Fauchet, P.M., 2005. Sensors and Actuators B 106, 347–357.
Auria, S.D'., de Champdor, M., Aurilia, V., Parracino, A., Staiano, M., Vitale, A., Rossi, M., Rea, I., Rotiroti, L., Rossi, A.M., Borini, St., Rendina, I., Stefano, L.D., 2006. Journal of Physics: Condensed Matter 18, S2019–S2028.
Beckmann, K.H., 1965. Surface Science 3, 314–319.
Bisi, O., Ossicini, S., Pavesi, L., 2000. Surface Science Reports 38, 1–126.
Bonanno, L.M., DeLouise, L.A., 2007. Biosensors and Bioelectronics 23, 444–448.
Bragaru, A., Simion, M., Miu, M., Ignat, T., Kleps, I., Schiopu, V., Avram, A., Craciunoiu, F., 2007. IEEE 11, 147–151.
Buriak, J.M., Geders, T.W., Allen, M.J., Choi, H.C., Smith, J., Raftery, D., Canham, L.T., 1999. Journal of the American Chemical Society 121 (49), 11491–11502.
Buriak, J.M., 2006. Philosophical Transactions of the Royal Society A 364, 217–225.
Canham, L., 1997. Properties of Porous Silicon, first ed. INSPEC, The Institution of Electrical Engineers, London, United Kingdom.
Chan, S., Horner, S.R., Fauchet, P.M., Miller, B.L., 2001a. Journal of the American Chemical Society 123 (47), 11797–11798.
Chan, S., Li, Y., Rothberg, L.J., Miller, B.L., Fauchet, P.M., 2001b. Materials Science and Engineering C 15, 277–282.
Ciampi, S., Harper, J.B., Gooding, J.J., 2010. Chemical Society Reviews 39, 2158–2183.
Dhanekar, S., Islam, S.S., Harsh, 2011. International Journal of NanoScience 10, 777–781.
Dhanekar, S., Islam, S.S., Harsh, 2012. Nanotechnology 23, 235501–235509.
Fernandez, R.E., Bhattacharya, E., Chadha, A., 2008. Applied Surface Science 254, 4512–4519.
Finnskog, D., Ressine, A., Laurell, T., Marko-Varga, G., 2004. Journal of Proteome Research, 988–994.
Föll, H., Christophersen, M., Carstensen, J., Hasse, G., 2002. Materials Science and Engineering: R 280, 1–49.
Francia, G.D., Ferrara, V.L., Manzo, S., Chiavarini, S., 2005. Biosensors and Bioelectronics 21, 661–665.
García, L.J., Palma, M.R.J., 2007. Sensors and Actuators B 126, 82–90.
Gavin, J.R., 2007. US Endocrine Disease. Touch Briefings, Atlanta, GA, U.S.A.. (pp. 1–3).
Goto, Y., Kawauchi, S., Ihara, K., Ikemoto, K., 2003. Cancer Science 94, 412–417.
Irnos, R.D., 1985. Journal of Toxicology and Environmental Health 16, 673–678.
Kerman, K., Kobayashi, M., Tamiya, E., 2004. Measurement Science and Technology 15, R1–R11.
Kim, N.Y., Laibinis, P.E., 1998. MRS Proceedings 536, 167–173.
Kirla, R.M., Haapasalo, H.K., Kalimo, H., Salminen, E.K., 2003. Cancer 97, 644–648.
Kochergin V., Föll H., 2009. Porous Semiconductors: Optical Properties and Applications-Engineering Materials and Processes. SpringerLink, pp. 197–206.
Lehmann, V., Gösele, U., 1991. Applied Physics Letters 58, 856–858.
Li, S., Huang, J., Cai, L., 2011. Nanotechnology 22 (425502), 1–6.
Lin, V.S., Motesharei, K., Dancil, K.P., Sailor, M.J., Ghadiri, M.R., 1997. Science 278 (5339), 840–843.
Lugo, J.E., Ocampo, M., Kirk, A.G., Plant, D.V., Fauchet, P.M., 2007. Journal of New Materials for Electrochemical Systems 10, 113–116.
Marquette, C.A., Blum, L.J., 2006. Biosensors and Bioelectronics 21 (8), 1424–1433.
Melikjanyan, G.A., Martirosyan, K.S., 2011. Armenian Journal of Physics 4 (4), 225–227.
Meskini, O., Abdelghani, A., Tlili, A., Mgaieth, R., Jaffrezic-Renault, N., Martelet, C., 2007. Talanta 71 (3), 1430–1433.
Mohanty, S.P., Kougianos, E., 2006. IEEE Potentials 25, 35–40.
Newman, J.D., Turner, A.P.F., 2005. Biosensors and Bioelectronics 20, 2435–2453.
Ouyang, H., Christophersen, M., Viard, R., Miller, B., Fauchet, P., 2005. Advanced Functional Materials 15, 1851–1859.
Prabhakar, D., Kumari, V., Islam, S.S., 2012. Science of Advance Materials 4 (5), 121–125.
Rahman, M.Md., Ahammad, S.A.J., Jin, J.H., Ahn, S.J., Lee, J.J., 2010. Sensors 10, 4855–4886.
Rauscher, M., Spohn, H., 2001. Physical Review E 64, 031604–031614.
Reddy, R.R.K., Basu, I., Bhattacharya, E., Chadha, A., 2003. Current Applied Physics 3, 155–161.
Ressine, A., Finnskog, D., Malm, J., Becker, C., 2005. Nano Biotechnology 1, 93–104.
Ressine, A., Auzelyte, V., Kristiansson, P., Marko-Varga, G., 2006. Nuclear Instruments and Methods B 249, 715–718.
Ressine, A., Corin, I., Järås, K., Guanti, G., Simone, C., Marko-Varga, G., Laurell, T., 2007. Electrophoresis 28, 4407–4415.
Rong, G., Wu, C., Pan, S., Tang, Y., Xing, X., 2011. Proceedings of the IEEE, Symposium on Photonics and Optoelectronics (SOPO), pp. 1–4.
Rossi, A.M., Wang, L., Reipa, V., Murphy, T.E., 2007. Biosensors and Bioelectronics 23 (5), 741–745.
Rotiroti, L., Stefano, L.D., Rendina, I., Moretti, L., Rossi, A.M., Oiccolo, A., 2005. Biosensors and Bioelectronics 20, 2136–2139.
Sailor, M.J., 2012. Porous Silicon in Practice: Preparation, Characterization and Application, first ed. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.
Salis, A., Setzu, S., Monduzzi, M., Mula, G., 2011. In: Serra P.A. (Ed.) Biosensors—Emerging Materials and Applications. InTech Publishers, Croatia, pp. 333–352.
Salonen, J., Lehto, V.P., Laine, E., 1997. Applied Physics Letters 70, 637–639.
Salonen, J., Lehto, V.P., 2008. Chemical Engineering Journal 137, 162–172.
Sánchez, G.R., Costa, V.T., Manso, M., Gallach, D., García, J.L., Palma, R.J.M., 2010. Materials 3, 755–763.
Sánchez, R., Cañizares, G.D., Manso, M., Preda, I., Costa, V.T., Gutiérrez, A., Soriano, L., Palma, R.J.M., 2011. Current Nanoscience 7, 178–182.
Schwartz, M.P., Derfus, A.M., Alvarez, S.D., Bhatia, S.N., Sailor, M.J., 2006. Langmuir 22, 7084–7090.
Schwartz, M.P., Yu, C., Alvarez, S.D., Migilori, B., Godin, D., Chao, L., Sailor, M.J., 2007. Physica Status Solidi A 204, 1444–1448.
Setzu, S., Salis, S., Demontis, V., Salis, A., Monduzzi, M., Mula, G., 2007. Physica Status Solidi A 204, 1434–1438.

Singh, S., Sharma, S.N., Govind, Shivaprasad, S.M., Lal, M., Khan, M.A., 2009. Journal of Materials Science: Materials in Medicine 20, S181–S187.
Singh, A., Eksiri, H., Yingling, Y.G., 2011. Journal of Polymer Science Part B: Polymer Physics 49, 1563–1568.
Smet, L.C.P.M., Zuilhof, H., Sudhölter, E.J.R., Lie, L.H., Houlton, A., Horrocks, B.R., 2005. Journal of Physical Chemistry B 109 (24), 12020–12031.
Song, M.J., Yun, D.H., Jin, J.H., Min, N.K., Hong, S.I., 2006. Japanese Journal of Applied Physics 45, 7197–7202.
Song, M.J., Yun, D.H., Min, N.K., Hong, S.I., 2007. Journal of Bioscience and Bioengineering 103, 32–37.
Song, M.J., Yun, D.H., Hong, S.I., 2009. Bioscience, Biotechnology, and Biochemistry 73, 474–478.
Sotiropoulou, S., Vamvakaki, V., Chaniotakis, N.A., 2005. Biosensors and Bioelectronics 20, 1674–1679.
Stefano, L.D., Randina, I., Moretti, L., Scognamiglio, D., Rossi, M., Auria, S.D.', 2004. Proceedings of IEEE Sensors. vol. 2, pp. 1018–1019.
Stefano, L.D., Moretti, L., Randina, I., Rotiroti, L., 2005. Sensors and Actuators B 111/112, 522–525.
Stefano, L.D., Arcari, P., Lamberti, A., Sanges, C., Rotiroti, L., Rea, I., Rendina, I., 2007. Sensors 7, 214–221.
Steinhauer, C., Ressine, A., Marko-Varga, G., Laurell, T., 2005. Analytical Biochemistry 341, 204–213.
Stetter, J.R., Penrose, W.R., Yao, S., 2003. Journal of The Electrochemical Society 150, S11–S16.
Takahashi, S., Kamata, Y., Tamo, W., Koyanagi, M., 2002. International Journal of Clinical Oncology 7, 349–355.
Tembe, S., Chaudhari, P.S., Bhoraskar, S.V., D'Souza, S.F., Karve, M.S., 2008. IEEE Sensors Journal 8, 1593–1597.
Thust, M., Schöning, M.J., Frohnhoff, S., Arens-Fischer, R., Kordos, P., Lüth, H., 1996. Measurement Science and Technology 7, 26–29.
Tichoniuk, M., Ligaj, M., Filipiak, M., 2008. Sensors 8, 2118–2135.
Vamvakaki, V., Chaniotakis, N.A., 2008. Electroanalysis 20 (17), 1845–1850.
Vashpanov, Y., Son, J.Y., Kwack, K.D., 2008. Sensors 8, 6225–6234.
Zhang, D., Alocilja, E.C., 2008. IEEE Sensors Journal 8 (6), 775–780.