Porous Silicon Electrochemical Biosensors: Performance and Commercial Prospects

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Contents

Abstract

This chapter reviews the performance of electrochemical biosensors based on porous silicon (PS) substrates and their prospects for commercialization. Different transduction mechanisms have been discussed with emphasis on various issues like nature of PS–metal contacts, orientation of the molecules within the pores, equivalent circuit models, and modulation of electric field lines through the pores and its interface with the biomolecules and ions of the analytes. Critical assessment indicates that sensitivity, reproducibility, and longevity are significantly affected by these factors and not only by the surface-area-to-volume ratio. The mechanisms of metal contact fabrication and surface derivatization have also been briefly discussed. For enhancing the commercial prospects of such sensors,

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the reliability aspects and recent attempts of integration with microfluidics platform have been outlined. Finally, the existing challenges and the future prospects of such devices for commercial use are highlighted.

Keywords
Amperometric biosensors · Antibody immobilization · Conductance biosensor · DNA hybridization detection · DNA–cDNA interactions · Electrolyte insulator semiconductor-based capacitive (EISCAP) structure · Glucose sensing · Glutaraldehyde and maleimidobutyric acid *N*-succinimidyl ester (GMBS) · Impedance biosensors · Microfluidics platform · Porous silicon electrochemical biosensors

Introduction

There is an ever-increasing requirement of efficient bioanalytical system for quantification and detection of wide range of biomolecules and microorganisms in food and water (Luo and Davis 2013; Cai et al. 2013; Singh et al. 2014; Tang et al. 2014; Veeramani et al. 2014). The sensitivity and specificity of the available exhaustive microbiological techniques and ELISA are dependent on high-quality instrumentation (Esteves et al. 2008; Li et al. 2014; Jan and Patel 2013). The rational for the thrust into the research area focusing on commercial development of biosensors is a combination of several factors like sensitivity, specificity, economic, and less manual intervention (Ahmed et al. 2015). The investigations primarily involve further development of conventional biological detectors like antibodies, aptamers, peptides, etc. and several organic and inorganic materials like graphene, zinc oxide, nonporous membrane, 3D macroporous gold electrode, graphitized macroporous carbon, and nanoporous anodic alumina oxide (Reta et al. 2016; Harraz 2014; Betty 2009; Das et al. 2015; Munje et al. 2015; Shanmugam et al. 2015; Song et al. 2008; Lu et al. 2009; Santos et al. 2013). Considerable research is being conducted involving porous silicon (PS) as solid substrate because of conducive physiochemical properties like large surface area, biocompatibility, flexible structural properties, and controlling surface hydrophobicity by modulating formation parameters and its easy integration with silicon technology (Jarvis et al. 2012; Li et al. 2007; Jane et al. 2009; Kovacs and Mescheder 2012; Shahbazi et al. 2013; Mahmoudi et al. 2007; Alvarez et al. 2009).

Recent reports reveal the development of integrated optical biosensors and PS-based biosensors is being further investigated for their striking optical properties like optical signal transduction (Szili et al. 2011; Zhang et al. 2013; Cunin et al. 2002; Nguyen et al. 2013; Rea et al. 2010; Orabona et al. 2011; Hernandez-Montelongo et al. 2015). Optical properties of biosensors can be attuned to one's requirement by varying the refractive index in each direction.

There has also been remarkable research done in the field of label-free electrochemical biosensors based on PS as these simple detection systems are of high sensitivity with lower costs. The large internal surface area of PS which leads to

greater biomolecular immobilization as well as its exclusive structural capability of guiding the electric field lines through analyte-filled pores has set off research in this domain which has subsequently resulted in the development of highly specific and sensitive biosensors. At present there is an inadequacy of comprehensive review related to PS electrochemical biosensors, and the majority of the literature lacks critical analysis of its performance, reliability, and commercial aspects (Ensafi 2016; RoyChaudhuri 2015; Ciampi et al. 2008; Salis et al. 2011; Mora et al. 2013; Dhanekar and Jain 2013; Das and RoyChaudhuri 2015). For point-of-care diagnostics, its integration with microfluidics is also a vital step. Thus broader analysis on label-free electrochemical PS biosensors with specific discussion on performance reliability and electrical transduction mechanism with appropriate models is pertinent.

This chapter discusses the fabrication of stable electrical contacts and various surface derivatization protocols adopted for electrochemical biosensing. There is emphasis about the influence of PS in modulating the electrical attributes for different transduction schemes with the help of equivalent circuit models. The performance of the PS electrochemical biosensors has been compared with its counterparts. The reliability aspects and its integration with microfluidics platform have also been discussed. Finally the existing challenges and future direction of research for development of commercially viable PS electrochemical biosensors have been highlighted.

Fabrication Issues

This section discusses the formation of electrical contacts followed by immobilization of biomolecules of PS which have been adopted for electrochemical biosensors.

Fabrication of Metal Contacts

Most of the electrochemical biosensors are based on amperometry, resonant frequency, and impedance mode of measurements where one of the contacts is from the back silicon and the other from the electrolyte (Zimin et al. 1995; Fonthal et al. 2007; Lundstrom et al. 2007; Kanungo et al. 2009; RoyChaudhuri et al. 2008; Maji et al. 2010). This sandwich configuration bypasses the requirement of stable electrical contacts from PS. However, for the purpose of improved sensitivity especially for impedance biosensors, both the contacts need to be placed laterally on PS layer. Archer et al. (2004) first reported the placement of lateral contacts by aluminum evaporation or colloidal silver (Ag) paint on self-supporting PS layer. In a recent report, Harraz et al. (2015) created electrical contacts onto the front porous film by colloidal Ag paint for real-time measurements of conductance. For conductometric glucose sensors, the formation of metal contacts has been realized by silver and gold metal through ion beam scattering using mask (Melikjanyan and Martirosyan 2011, 2012) and by aluminum metal (Lopez-Garcia et al. 2007) by evaporation. Some

groups report the use of sputtered gold–nickel–chromium layers as metal contacts onto the nanoPS layers (Recio-Sanchez et al. 2010; Tembe et al. 2009). The typical thickness of the metal layer deposited by evaporation or sputtering is of the order of 0.5 μm, and they do not penetrate the pores. Thus the evaporated metal film is discontinuous in the porous regions. As the mechanism of conduction in conductometric biosensors is primarily a surface phenomenon, metal need not fill the pores. Similarly for impedance biosensors, the metal contacts need not fill the pores since the electric field lines should actually interact significantly with the electrolyte and not with the oxidized crystallites. However a continuous and thicker metal film would be desirable to reduce the sheet resistance of metal. In this direction, there are some reports on impedance biosensors (Das et al. 2009, 2011, 2012) where metal contacts have been fabricated on oxidized surfaces by aluminum paste with 3 mm by 1 mm dimension and spacing of 1 mm using screen printing technique. Deposition of metal paste by screen printing method helps to maintain continuity in the metal film through the pores of PS and also reduces the cost. For PS substrates which are oxidized, the metal–silicon–oxide–silicon junction is primarily capacitive in nature like the gate oxide capacitance in a MOSFET, and the apparent limitations of noisy metal contact junction in nanoPS are not present (Balagurov et al. 2001; Dhar and Chakraborti 1996; Saha and Pramanik 2006).

In miniaturization of PS sensors using reference electrodes, Schoning et al. (2000a) reported the fabrication of micro reference electrode structures by anodization process. A thick chromium and silver layer are deposited by physical vapor deposition in contact with silicon. Then the silver layer is anodized in KCl solution with a typical current density of 100 $\mu A/cm^2$ for 100 seconds to form a silver chloride layer. Finally, a KCl solution has been filled into the porous structure, and the electrolyte has been evaporated which is then covered by a Nafion membrane. A new technique of forming interdigitated metal electrodes on PS has been reported by Scheen et al. (2012). The electrodes have been patterned on the surface of silicon substrates using lift-off technique on gold. Metal has been first evaporated by dual e-beam evaporator and the patterned. Gold is HF resistant; hence after the patterning of gold, PS has been fabricated by electrochemical etching.

Ensafi et al. demonstrated the use of Pt/PS nanocomposite as a new electrocatalyst for simultaneous determination of morphine and codeine by means of carbon ionic liquid electrode (Ensafi et al. 2015). Combination of antifouling properties of ionic liquids, biocompatibility of PSi, and electrocatalytic effect of Pt nanoparticles lead to new sensing surface for voltammetric determination with good sensitivity.

Surface Derivatization

Immobilization of biomolecules by surface derivatization is an essential step for any biosensor from the point of view of selectivity (Das et al. 2010; Nagare and Mukherji 2009; Valentini and Polini 2011; Tao et al. 2008; Ressine et al. 2007; Li et al. 2009; Xu et al. 2012). The various types of biomolecules

functionalized range from enzymes and antibodies to DNA depending on the analyte to be sensed. 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 (APTES) (Gupta and Gooding 2016), 3-glycidoxypropyltrimethoxysilane (GOPS) (Wu et al. 2009), mercaptopropyltrimethoxysilane (MTS), 1-(3-(Dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC), and others. Glutaraldehyde and maleimidobutyric acid *N*-succinimidyl ester (GMBS) are the commonly used cross-linkers (Vemulachedu et al. 2009; Fernandez et al. 2009, 2008; Stolyarova et al. 2008; Reddy et al. 2003, 2001; Luth et al. 2000; Simonisa et al. 2003; Stolyarova and Nemirovsky 2011; Schoning et al. 1997; Ressine et al. 2010; Stolyarova et al. 2008; Lugo et al. 2007; Zhang and Alocilja 2008; Tao et al. 2008; Betty 2009; Betty et al. 2009, 2004; Das et al. 2009, 2011, 2012, Ghosh and RoyChaudhuri 2013, Das et al. 2010, Ciampi et al. 2012). For high density of antibody immobilization, it is required that a monolayer of silane is formed so that maximum thiol groups are available for attachment of cross-linker. In order to achieve the optimum silane film coating, all the parameters like the proportion of water, silane concentration, pH of silane solution, condensation time, and incubation temperature have been optimized. The succinimidyl group of GMBS reacts with the SH group of silane. For PS layers which have been coated with different conducting polymers (Reddy et al. 2001, Jin et al. 2009; Betty 2009; Betty et al. 2009; Jin et al. 2008, 2010), the biomolecules like DNA, antibody, and enzymes are electrostatically adsorbed by the doping process. Also DNA molecules have been electrostatically linked with the oxidized surface of PS (Archer et al. 2004). PS surface has also been modified by click chemistry method (Ciampi et al. 2007). Cu (I)-catalyzed alkyne–azide cycloaddition reactions have been employed to modify the internal pore surfaces through a two-step hydrosilylation/cycloaddition procedure. Currently, due to effective chemical transformations culminating in sufficient functionalization, hydrosilylation is gaining importance and popularity.

Electrical Transduction Mechanisms

Porous Silicon as Amperometric Biosensor

Amperometric biosensors function by the production of a current when a potential is applied between two electrodes. The working electrode of the amperometric biosensor is usually either a noble metal or a screen-printed layer covered by the biorecognition component where redox reaction takes place. Usually those molecules/analytes that can exchange electrons due to a few redox reactions like DNA and urea are sensed by way of this procedure. Antibody–antigen binding also can be detected by using this approach using a redox mediator like ferrocyanide (Prabhakar et al. 2012). For specific gene detection, ruthenium has been used which also acts as a redox couple (Lugo et al. 2007). PS working electrodes show greater electrical behavior over planar silicon for its higher electrochemically active surface area.

There are some reports on PS amperometric sensors for urea, cholesterol, bilirubin, alanine aminotransferase, and aspartate aminotransferase detection (Yun et al. 2012; Song et al. 2007, 2009; Kumari et al. 2012). In all of these, PS substrate not only improves the binding efficiency of the analyte due to large surface-area-to-volume ratio but also shows a possible catalytic effect in the diffusion of the analyte molecules within the nanopores which is not possible with planar silicon since the oxide layer for immobilization would restrict the faradaic charge transfer at the working electrode. Hence amperometric biosensors on silicon would not have been practically feasible without the formation of PS.

Some group uses conducting polymer as a working electrode for facile immobilization of DNA or protein. For this purpose, PS surface has been electropolymerized by polypyrrole or poly(3-methylthiophene) (P3MT) whereby the probe DNA (Jin et al. 2008, 2010) or urease molecules have been used as dopants into the polymer matrix (Jin et al. 2009). The enhanced surface roughness of the PS substrates compared to bare silicon promotes formation of strong adsorption bond with the polymer film through the improved surface energy. Miniaturization of polymer PS sensor has been reported by lithographic patterning of electrodes (Jin et al. 2009). By doping with P3MT during the electropolymerization process with urease enzyme, this miniaturized sensor has been applied for urea sensing. The PS substrate with sensing electrodes showed increased effective area by 1.6 times compared with the planar silicon-based sensing electrodes. Recently, copper–PS nanocomposite has been reported for sensing glucose using nonenzymatic process (Ensafi et al. 2014). This sensor reduces response time of less than 4 s, long-time stability, and good signal reproducibility with a detection limit of 0.2 mmol dm^{-3} glucose.

Porous Silicon as Conductance Biosensor

Conductance biosensor sensing mechanism is based on change in dc conductance caused by the field, upon attachment of biomolecules. This sensing mechanism has been deployed for the detection and quantification of glucose, catechol, and bacteria where two lateral contacts have been fabricated on nanostructured PS layer. Before immobilization, the baseline current–voltage (IV) characteristics have been observed to be rectifying in all the cases which has been attributed to the nonohmic nature of the metal–PS junction as the Fermi level of the silicon nanocrystallites does not usually align with that of the metal owing to the presence of a large number of surface states. Usually the conduction current is given by the thermionic emission model (Jang 2008), and for highly disordered nanostructures of PS, the connectivity between the crystallites becomes poor, and the carrier conduction is thought to be assisted by variable range hopping (Islam et al. 2009).

Another application of conductance-based biosensor is glucose sensing (Melikjanyan and Martirosyan 2011, 2012; Lopez-Garcia et al. 2007), where varying concentration of dissolved glucose has been immobilized onto the surface of PS. The observed conduction in such sensors is of thermionic emission type given by Eq. 1. It has been observed that with increasing concentration, current decreases at a certain

voltage. This has been attributed to the surface charge distribution of the nanocrystallites which can lead to the depletion in the nano-silicon regions, resulting in the decrease of concentration of free carriers available for conduction current transport at the surface (Ben-Chorin et al. 1994; Moeller et al. 1995). A nonlinear current change has been observed with glucose concentration.

Bacteria fragments have also been detected by this conductance principle using antibody immobilization (Archer et al. 2004; Moeller et al. 1995). It has been observed that after antibody immobilization conductivity has decreased which may be owing to the presence of a large surface density of amino groups on the surface of nanoPS. But after introduction of *E. coli* fragments, the overall conductivity increased. This may be due to the fact that some fraction of the amino charges get neutralized resulting in increase of carrier density of nanocrystallites. For all these reports on conductivity sensing using PS, the sensitivity is modulated via interaction of the carriers at the interface between the nanocrystallites and the biomolecule, not only by the surface-area-to-volume ratio factor.

Recently, graphene-coated nanoporous silicon has been demonstrated to be capable of enhanced conductance sensing with detection limit down to attomolar range (Basu and RoyChaudhuri 2016). This has been made possible due to two reasons. Firstly, in the nanopores, the biomolecules usually reside within a distance shorter than the original pore radius which statistically raises the charge transfer probability between the biomolecules and the surface. As a consequence, the heterogeneous charge transfer gets facilitated, and the electrode potential reaches equilibrium faster for a given electrochemical system. Secondly, the nanoporous silicon template makes the graphene nanostructure smooth which suffers from less edge defects.

Porous Silicon as Impedance Biosensor

Impedance biosensors measure the electrical impedance at an interface in steady-state conditions. Impedance biosensors on PS are usually of two classes: one with lateral contacts and the other with sandwich configuration of metal contacts leading to an electrolyte–insulator–semiconductor (EIS) structure. The lateral contact configuration of PS is much like that of interdigitated electrode-based impedance biosensor. One of the most common patterns for impedance-based biosensors is interdigitated microelectrode array (IDE) (RoyChaudhuri and Das 2010; Radke and Alocilja 2004; Laczka et al. 2008). But they have some limitation toward sensitivity and specificity. PS has been used to replace the conventional substrates to enhance the performance. For lateral contact configuration, the metal contacts need to be fabricated on PS oxide rather than only PS, and hence the apparent limitations of noisy metal contact junctions are not present.

There are some reports on impedance biosensor using macroPS (Das et al. 2009, 2011, 2012) where metal contacts have been fabricated on oxidized surfaces for bacteria detection. The expected advantages of impedance detection by macroPS trapping array are (a) natural trapping of bacteria in the self-assembled optimum size

pores near the electrodes without using dielectrophoresis, (b) localization of the electric field lines through the pores adjacent to the electrodes filled up with bacterial analyte solution permitting much wider spacing of electrodes, and (c) sensitivity at a significantly lower processing cost compared to the IDE structures. The oxidized macroPS substrate offers a high-resistance hydrophilic platform which is essential for achieving high-Q capacitance required for reliable impedance measurement. The distribution of current lines has been verified by finite element analysis using ANSYS as well as analytically by transmission line method. The results of ANSYS shown in Fig. 1a indicate that the solution enters through the short pores and comes in contact with the bottom silicon since it provides a relatively low impedance parallel path compared to the lateral current path through the solution.

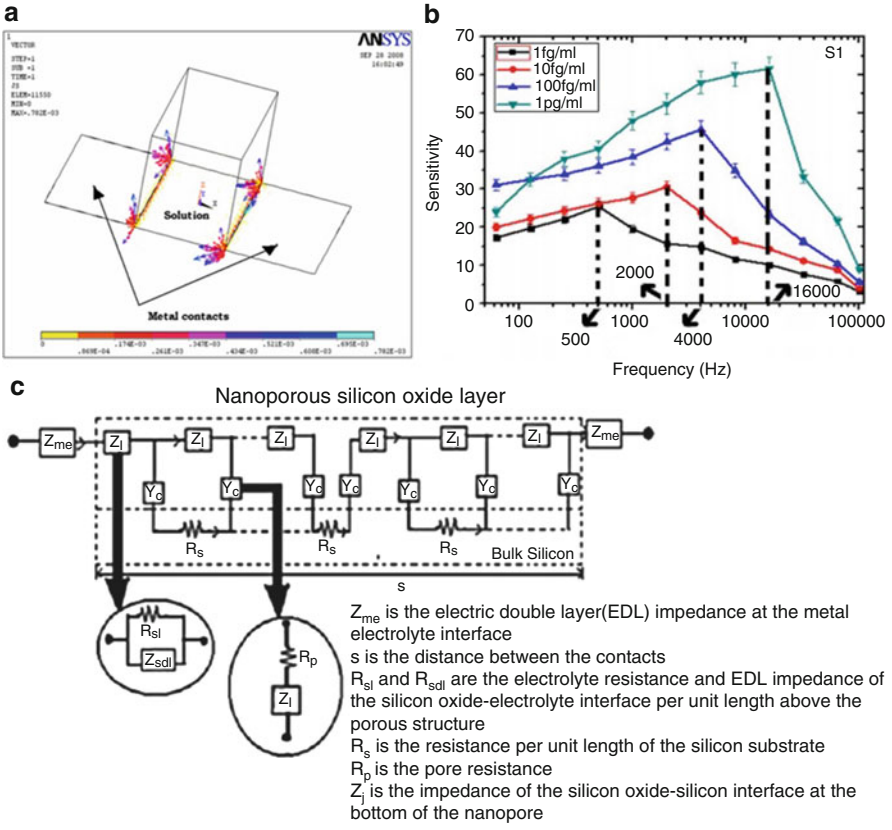

Fig. 1 (**a**) Current line distribution on application of an AC electric field in an oxidized PS substrate in the presence of solution with lateral contacts (Reproduced from Das et al. 2009). (**b**) Equivalent circuit of PS with lateral contacts (Reproduced from Das and Chaudhuri 2015). (**c**) Sensitivity for different hepatitis B virus concentration in nanoPS impedance biosensor (Reproduced from Das and Chaudhuri 2015)

This causes the field lines to scan only a small portion of the solution near the electrodes irrespective of the horizontal and vertical spread of the solution above the surface, leading to an efficient confinement of field lines through the bacterial cells even with widely spaced electrodes. This is the primary advantage of macroPS over planar substrates. The transmission line model of the same is shown in Fig. 1b.

Similarly for different electrode patterns (Dengll et al. 2002; Das et al. 2012), the sensitivity increases over planar surface but not monotonically with surface-area-to-volume ratio of PS. This was attributed to the optimum area utilization factor (AUF) of the electrode which is calculated as the ratio of the effective area occupied by captured bacterial cells (A_{beff}) to the effective exposed area available for capture between the electrodes. A detection limit of 100 CFU/ml has been obtained with the optimized macroPS silicon sensor which is better than most of the label-free impedance sensors.

NanoPS has been recently reported to detect down to 1 fg/ml hepatitis B virus and food toxins by a novel mechanism of concentration-dependent shift in peak frequency as shown in Fig. 1c (Das and RoyChaudhuri 2015; Ghosh and RoyChaudhuri 2013). This happens due to two reasons: opposite change of the double-layer impedance at the pore walls and the bottom of the nanopore after antigen capture and the electrolyte resistance in nanopore being greater than or equal to the double-layer impedance at the pore walls. Also it has been observed coupling noise spectroscopy analysis can lower the detection limit by one order of magnitude using PS (Ghosh and RoyChaudhuri 2015; Samanta and RoyChaudhuri 2015).

Archer et al. (2004) and Vamvakaki and Chaniotakis (2008) have also reported the use of macro- and nanoPS for impedance-based DNA hybridization detection where both self-supporting layers and silicon-supported PS layer have been used for experimentation. It has been observed that the sensitivity is affected not only by the surface area but also by the charged backbone of DNA. In fact there is a trade-off between the surface area and the number of binding sites that can be accommodated without affecting the hybridization. For self-supporting layers, the capacitance changes due to the change in the dielectric constant inside the pores. The difference in the range of values of the impedance and phase angle between the heterostructures and the self-supporting membranes originates from the variation in electric field propagation. Despite the fact that the self-supporting membranes produced larger signal, the fabrication and handling process is difficult.

Bergveld (1970, 1972) and Siu et al. (Siu and Cobbold 1979) developed a theory on electrolyte–insulator–semiconductor-based capacitive (EISCAP) structure. The working principle of EISCAP is the flat band voltage shift in response to the pH of the electrolyte and is explained in great detail with different dielectrics. Incorporating PS on EISCAP sensors has been widely used for detection of antigen–antibody binding, triglycerides, penicillin, urea, and viruses of plants (Vemulachedu et al. 2009; Betty 2009). These structures use two contacts, one from silicon and the other from the electrolyte; thus there is no direct contact from the oxidized PS. The resulting equivalent structure is primarily a capacitor in series with resistors between the two contacts. The capacitor is a

parallel combination of oxidized crystallites (C_{ox}) with the double-layer capacitance (C_{dl}) within the pores. The resistors comprise of the silicon bulk resistance (R_{bulk}) and the electrolyte ($R_{electrolyte}$) and are expected to be small than the capacitive impedance. It has been observed that due to the much larger surface area of the porous silicon sample, there is a 34.5 times increase in accumulation capacitance over the single crystalline silicon sample. The responsivity as well as the dissipation factor of PS is better at lower frequencies. However for miniaturized sensors, even though PS binds more enzyme, the abundance of enzyme alone does not necessarily correspond to a higher triglyceride sensitivity as the triglyceride concentration is the limiting factor for the miniaturized EISCAPs. In addition, reproducibility of PS EISCAPs was found to be a problem. By deploying macroPS for enzyme-based detection, the longevity could be improved.

Using macroPS, antigen–antibody binding event also has been detected by capacitive mode (Betty et al. 2004, Betty 2009). Though the increase in sensitivity observed with higher frequency for the squat irregular column structure is of the same order as that of the increase in surface area, there are constraints on the length and size of the columns in order to have maximum capacitance change at the measurement frequencies. These are: the columns should not get fully depleted at the measurement voltage and the length should be optimum enough to allow complete penetration of the electrical signal in the electrolyte in the pore. PS–polyaniline composite structure has also been reported to detect antigen–antibody binding by capacitance measurement (Betty 2009). Capacitance increase observed after PANI deposition has been attributed due to either the dielectric formed on the surface causes a change in the surface charge of the semiconductor altering the space charge layer thickness or due to the high dielectric constant of the dielectric formed. The high sensitivity observed in such structure has not been attributed to the increased surface-area-to-volume ratio but to the combined effect of depletion or accumulation of charge carriers and the change in dielectric layer thickness produced by specific binding of a biological molecule on the surface of the nanochannels.

From Table 1, the trend of development of PS electrochemical biosensors may be analyzed. Most of biomolecules have been detected by impedance and amperometric biosensor category. For all the cases, there is a steady improvement in the lower detection limit and a decrease in the response time over the years. As conductometric biosensors are driven by the interface phenomena, the metal contacts with PS have a tendency to fluctuate, thus dampening the overall signal-to-noise ratio. Impedance biosensors have the greatest potential for commercialization owing to their label-free operation, larger signal magnitude, and hence low cost of detection instruments. To summarize, the state-of-the-art detectivity of PS electrochemical biosensors for different biomolecules and analytes has reached the clinically significant values. Also the detection time varies from a few seconds to around 30 minutes which is commercially viable and comparable to many reports on optical PS biosensors (Samanta et al. 2013; Maedler et al. 2013; Stefano et al. 2013; Rea et al. 2011). Despite this achievement, majority of research has been

Table 1 Comparison of the performance of the PS electrochemical biosensors. This list does not include those references which reported only pH sensing of a standard solution (nd: not determined in the references)

Detection mechanism	Analyte	Detection range	Response time	References
Amperometric	DNA	0.167–0.9 μM	Around 2.5 hrs	Jin et al. (2008)
	Alpha fetoprotein	0.01 ng /ml	nd	Wu et al. (2009)
	DNA	0.05–0.909 μM	Around 3 hrs	Jin et al. (2010)
	DNA hybridization	50 pM–500 nM	Around 30 min	Lugo et al. (2007)
	DNA of *Salmonella enteritidis*	0.1–0.0001 μg/ml	nd	Zhang and Alocilja (2008)
	Urea	0.1–125 mM	Around 100 secs	Jin et al. (2009)
	Glucose	1.0 μmol dm^{-3} to 2.3 mmol dm^{-3}	4 sec	Ensafi et al. (2014)
	Urea	0.3–4.5 mM	nd	Yun et al. (2012)
	Antigen	10 ng/ml	nd	Prabhakar et al. (2012)
	Alanine aminotransferase Aspartate aminotransferase Cholesterol Bilirubin	1.3–250 U/l 1.3–250 U/l 1–50 mM 0.002–0.02 mM	Around 20 sec Around 20 sec Around 20 sec Around 20 sec	Song et al. (2007)
	Alanine aminotransferase Aspartate aminotransferase	1.3–250 U/l 1.3–250 U/l	Around 20 sec Around 20 sec	Song et al. (2009)
	MS2 bacteriophage	6 pfu/ml	nd	Reta et al. (2016)
Conductance	Glucose	3–7 gm/l	nd	Melikjanyan and Martirosyan (2011)
	Glucose	1–1000 μg/ml	nd	Lopez-Garcia et al. (2007)
	Glucose and *Escherichia coli*	Glucose: 1–1000 μg/ml and *E.coli* fragments: 10–100 μg/ml	30 min	Recio-Sanchez et al. (2010)
	Catechol	50–100 μM	2 min	Tembe et al. (2009)
	Aflatoxin B1	1 fg/ml–1 pg/ml	15 min	Das et al. (2015)
	Hepatitis B	50 aM−10 pM	15 min	Basu and RoyChaudhuri (2016)

(*continued*)

Table 1 (continued)

Detection mechanism	Analyte	Detection range	Response time	References
Impedance	Triglyceride	5 mM	3 min 30 sec	Vemulachedu et al. (2009)
	Mouse IgG	0.6–480 ng/ml	33 min approx.	Betty (2009)
	DNA hybridization	0.1–25 μM	Around 5 min	Archer et al. (2004)
	Triglyceride	1–40 mM	15 min	Reddy et al. (2001)
	Goat anti-mouse IgG	100 μg/ml	nd	Betty et al. (2004)
	penicillin	0.01–1 M	Around 5 min	Schoning et al. (2000a)
	Penicillin G	0.1–10 mM	nd	Schoning et al. (1997)
	S. typhimurium	10^3–10^7 CFU/ml	Around 20 min	Das et al. (2009)
	E.coli O157	10^3–10^6 CFU/ml	Around 20 min	Das et al. (2011)
	E.coli O157	10^2–10^6 CFU/ml	Around 20 min	Das et al. (2012)
	Aflatoxin B1	1 fg/ml–1 pg.ml	Around 15 min	Ghosh and RoyChaudhuri (2013)
	Hepatits B virus	1 fg/ml–1 pg/ml	Around 15 min	Das and RoyChaudhuri (2015)
	Cortisol	1 pg/ml	nd	Munje et al. (2015)
	Troponin-T	0.01 pg/ml	Around 15 min	Shanmugam et al. (2015)
	Goat anti-mouse IgG	260 μg/ml	nd	Betty (2016)
	Aflatoxin B1	0.1 fg/ml–1 pg.ml	Around 15 min	Ghosh and RoyChaudhuri (2015); Samanta and RoyChaudhuri (2015)

targeted toward optical PS biosensors. One of the driving reasons is that PS was a well-known optical material since its discovery for its conveniently achievable room temperature photoluminescence but was observed to be a difficult electronic material in terms of stable metal contacts (Kanungo and Basu 2014). In the last 20 years, there has been enormous research targeted toward the development of reproducible and low-noise electrical contacts on PS. In a report on PS amperometry biosensor (Schoning et al. 2000b), metal contacts had been deposited conformally within the pores, but the metal–PS junction was not an active component of such sensors. It was only in Das et al. (2009), the first report on PS impedance biosensor with lateral metal contacts claimed sensitive, stable and reproducible measurements.

Reliability Aspects

Another aspect of porous silicon-based electrical biosensors is reliability. One of the major concerns of reliability in any biosensor is the stability and degradation problems of biorecognition elements. The degradation problems of the biorecognition elements like antibodies, enzymes, and others have been addressed by improved biochemical techniques of immobilization so that they retain their activity for a long time. However, for electrochemical biosensors, the other reliability issues can be attributed to the variability in sensor processing and time-related drift in the sensor characteristics. A critical factor that has been recently studied in impedance biosensors (Das and RoyChaudhuri 2015) is the time-related device integrity with respect to the interfacial behavior of the oxidized PS under prolonged exposure to buffer solution at a low temperature. In the presence of electrolyte, the interfacial properties of a particular material are expected to depend largely on surface roughness. The enhanced selectivity of these sensors is dependent on the pore morphology and surface roughness of the nanoporous silicon oxide (Das and RoyChaudhuri 2015). Surface roughness can change the contact angle which can affect the degree of contamination upon long-term exposure to buffer solution. Three different surface morphologies have been studied with respect to the repeatability in sensitivity and selectivity as shown in Fig. 2. The magnitude of the sensitivity is less for sensors with higher surface roughness due to the fact that the antibody-binding density is less for higher surface roughness (Das and RoyChaudhuri 2015). On the other hand, the deviation in the sensitivity characteristics during detection in blood serum is more for lower surface roughness. This may be correlated with the fact that the larger-sized nonspecific ions/molecules of the blood serum are expected to face more hindrances from the antibody molecules and may not be able to reach the valleys of the asperities with increasing surface roughness which will effectively reduce the nonspecific adsorption. Figure 3a shows the sensitivity variation with frequency for three consecutive cycles for all the sensor samples corresponding to 1 fg/ml hepatitis B virus after two different storage times. It is observed from Fig. 3b that increasing the surface roughness by decreasing the pitch reduces the sharpness of the peak frequency, i.e., selectivity (S_p), but enhances the longevity of the sensors. Hence the design of sensor surface may be decided by a figure of merit combining all the sensing parameters. The figure of merit (*FOM*) has been evaluated by the product of maximum sensitivity(S), selectivity (S_p), and repeatability(R) given by Eq. 1, and the results are shown in Fig. 3c. It has been observed that till 15 days of storage, *FOM* has a negative correlation with surface roughness for 1 fg/ml, but the trend gradually reverses after 3 months. This may be attributed to the fact that for low concentration of virus, standard deviation(σ_s) of sensitivity is significantly lowered for sensors with higher surface roughness compared to the deterioration in their S_p. But for 1 pg/ml, initially there is a positive correlation of *FOM* with surface roughness, but the trend reverses after a particular roughness at any instant of time. This may be due to the fact that after a certain surface roughness, the lowering of S and S_p dominates over the decrease in σ_s. Thus it may be concluded that for different range of virus concentration, tuning the surface roughness may provide

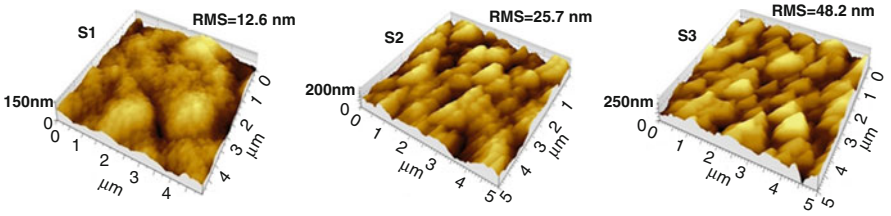

Fig. 2 Surface roughness of electrical biosensors (Reproduced from Das and Chaudhuri 2015)

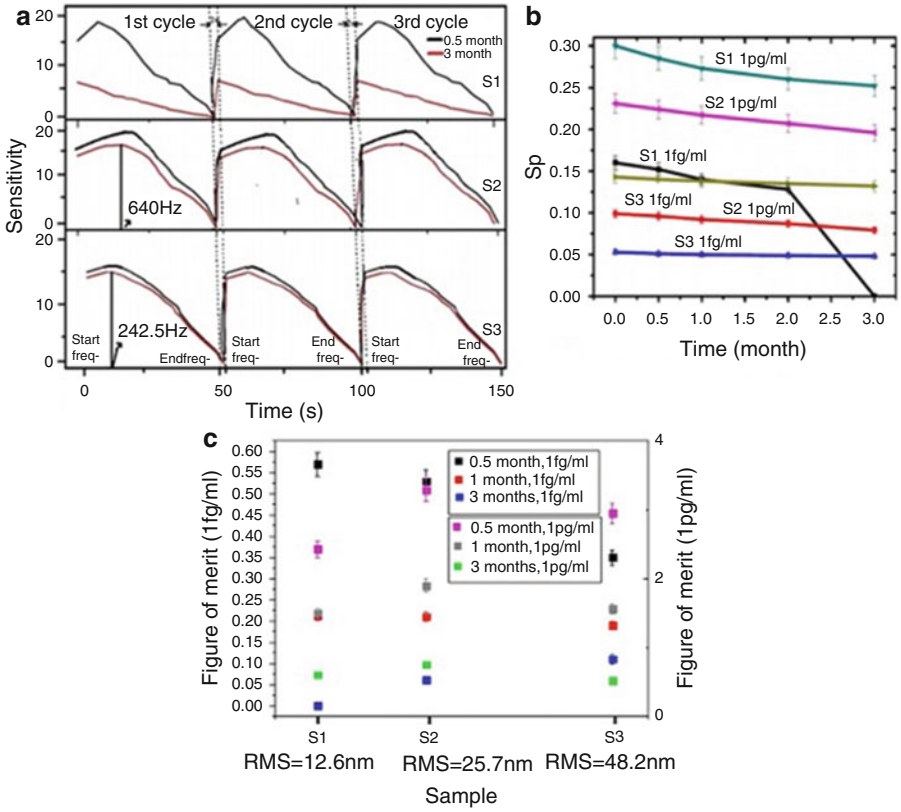

Fig. 3 (**a**) Sensitivity measured for three consecutive frequency scans for 1 fg/ml. (**b**) *Sp* (selectivity) with time for different sensors and virus concentration. (**c**) Correlation plot of figure of merit with different surface roughness (Reproduced from Das and Chaudhuri 2015)

optimum response in terms of sensitivity, selectivity, and repeatability. This study highlights the necessity for exploring the reliability aspects of such biosensors and also presents a guideline for selecting the optimum fabrication parameters to maximize the figure of merit.

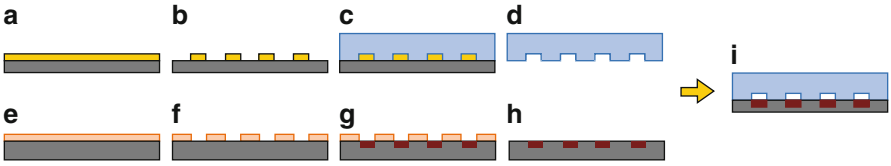

Fig. 4 Technological steps of the PS microarray fabrication process and its integration with the microfluidic circuit: (**a**–**d**) microfluidic circuit fabrication; (**e**–**h**) porous silicon transducers realization. (**a**) SU8 spin coating, (**b**) photolithography and development, (**c**) soft imprinting, (**d**) microchannels, (**e**) photoresist spin coating, (**f**) same as (**b**), (**g**) electrochemical etch, (**h**) porous silicon microarray, and (**i**) assembly of the whole device (Reproduced from Stefano et al. 2013)

$$FOM = SxS_pxR \tag{1}$$

Integration of PS Platform with Microfluidics

Microfluidic platform possesses remarkable features for simple, low-cost, and rapid disease diagnosis, such as low volumes of reagent consumption, fast analysis, and high portability along with integrated processing and analysis of complex biological fluids with high sensitivity for biomedical applications. Stefano et al. (2013) report a PS-based microarray integrated with microfluidic circuit in PDMS for the study of DNA–cDNA interactions as a proof of concept device as shown in Fig. 4. The PS elements constituting the array have been functionalized by directly injecting the DNA probe molecules into the microfluidic system. Smaller sample amounts and functionalization time significantly shorter than those required for the not integrated device have been used. The integrated microarray using a label-free detection method has revealed great potentiality, and it can also be of interest for various bioanalytical applications (Rea et al. 2011). In this aspect Barillaro et al. demonstrate the actual compatibility of the post-processing procedure for porous silicon fabrication with commercially available microelectronic processes (Barillaro et al. 2010).

Challenges and Future Scope

One of the well-known challenges of PS is their chemical stability. However for biosensor applications, the problem of instability, in general, is much less compared to other chemical sensors as the oxidation followed by surface derivatization reduces the surface activity of PS structure. Repeatability issues from stable electrical contacts in capacitive biosensors on porous silicon are better than their resistive counterparts, probably due to the reduced effect of flicker noise originating from the interface states at the junction of PS and metal contacts (Parkhutik and Timashev 2000). Other reliability aspects like the integrity of the bio-functionalized PS surface upon prolonged exposure to buffer solution need to be maximized by optimum selection of pore morphology, oxidation parameters, and immobilization procedures.

However, the problem posed by reproducibility or time-related drifts may be partially addressed by suitable electronic interface design (Samanta et al. 2013). The detection and quantification of the target biomolecule has to be estimated by measuring the sensitivity which is the relative change between control and test reading. Further, the peak frequency-based impedance detection is encouraging from the commercial aspects since it can be directly applied to blood serum without pre-concentration or centrifugation. However, for practical deployment of such sensors, integration of microfluidics platform with PS biosensors along with their metal contacts has to be standardized and tested rigorously using real-life samples like blood, urine, and others.

References

Ahmed MU, Hossain MM, Safavieh M, Wong YL, Rahman IA, Zourob M, Tamiya E (2015) Toward the development of smart and low cost point-of-care biosensor based on screen printed electrodes. Crit Rev Biotechnol. https://doi.org/10.3109/07388551.2014.992387

Alvarez SD, Derfus AM, Schwartz MP, Bhatia SN, Sailor MJ (2009) The compatibility of hepatocytes with chemically modified porous silicon with reference to in vitro biosensors. Biomaterials 30:26–34

Archer M, Christophersen M, Fauchet PM (2004) Macroporous silicon electrical sensor for DNA hybridization detection. Biomed Microdevices 6:203–211

Balagurov LA, Bayliss SC, Kasatochkin VS, Petrova EA, Unal B, Yarkin DG (2001) Transport of carriers in metal/porous silicon/c-Si device structures based on oxidized porous silicon. J Appl Phys 90:4543–4548

Barillaro G, Bruschi P, Lazzerini GM, Strambini LM (2010) Validation of the compatibility between a porous silicon-based gas sensor technology and standard microelectronic process. IEEE Sens 10:893–899

Basu J, RoyChaudhuri C (2016) Attomolar sensitivity of FET biosensor based on smooth and reliable graphene nanogrids. IEEE Elec Dev Lett 37:493–495

Ben-Chorin M, Kux A, Schechter I (1994) Adsorbate effects on photoluminescence and electrical conductivity of porous silicon. Appl Phys Lett 64:481–483

Bergveld P (1970) Development of an ion-sensitive solid-state device for neurophysiological measurements. IEEE Trans Biomed Eng 17:70–71

Bergveld P (1972) Development, operation, and application of the ion-sensitive field-effect transistor as a tool for electrophysiology. IEEE Trans Biomed Eng 19:342–351

Betty CA (2009) Highly sensitive capacitive immunosensor based on porous silicon–polyaniline structure: bias dependence on specificity. Biosens Bioelectron 25:338–343

Betty CA (2016) Nanostructured semiconductor based biochemical sensors. Mater Sci Technol 32:375–379

Betty CA, Lal R, Sharma DK, Yakhmi JV, Mittal JP (2004) Macroporous silicon based capacitive affinity sensor-fabrication and electrochemical studies. Sensors Actuators B Chem 97:334–343

Betty CA, Lal R, Yakhmi JV (2009) Impedance model of electrolyte–insulator–semiconductor structure with porous silicon semiconductor. Electrochim Acta 54:3781–3787

Cai X, Gao X, Wang L, Wu Q, Lin X (2013) A layer by layer assembled and carbon nanotube/gold nanoparticles based bienzyme biosensors for cholesterol detection. Sensors Actuators B Chem 181:575–583

Ciampi S, Bocking T, Kilian KA, James M, Harper JB, Gooding JJ (2007) Functionalization of acetylene-terminated monolayers on Si(100) surfaces: a click chemistry approach. Langmuir 23:9320–9329

Ciampi S, Bocking T, Kilian KA, Harper JB, Gooding JJ (2008) Click chemistry in mesoporous materials: functionalization of porous silicon rugate filters. Langmuir 24:5888–5892

Ciampi S, Guan B, Darwish NA, Zhu Y, Reece PJ, Gooding JJ (2012) A multimodal optical and electrochemical device for monitoring surface reactions: redox active surfaces in porous silicon Rugate filters. Phys Chem Phys 14:16433–16439

Cunin F, Schmedake TA, Link JR, Li YY, Koh J, Bhatia SN, Sailor MJ (2002) Biomolecular screening with encoded porous silicon photonic crystals. Nat Mater 1:39–41

Das N, RoyChaudhuri C (2015) Reliability study of nanoporous silicon oxide impedance biosensor for virus detection: influence of surface roughness. IEEE Trans Device Mater Reliab 15: 402–209

Das RD, RoyChaudhuri C, Maji S, Das S, Saha H (2009) Macroporous silicon based simple and efficient trapping platform for electrical detection of salmonella typhimurium pathogens. Biosens Bioelectron 24:3215–3222

Das RD, Maji S, Das S, RoyChaudhuri C (2010) Optimization of covalent antibody immobilization on macroporous silicon solid supports. Appl Surf Sci 256:5867–5975

Das RD, Dey A, Das S, RoyChaudhuri C (2011) Interdigitated electrode-less high performance macroporous silicon structure as impedance biosensor for bacteria detection. IEEE Sens 11:1242–1252

Das RD, Mondal N, RoyChaudhuri C (2012) Optimized electrode geometry for an improved impedance based macroporous silicon bacteria detector. IEEE Sens 12:1868–1877

Das N, Basu J, RoyChaudhuri C (2015) Graphene coated nanoporous silicon immunosensor for food toxin detection. Int J Adv Eng Sci Appl Math 7:204–209

Dengll LH, Karagiannoglou SH, Sakkas WI, Barbenel JC (2002) Effects of geometrical factors of coplanar electrodes on biomedical measurements as exemplified by the electrical impedance measurement of saline and blood. In: Proceedings of the second joint EMBS/BMES conference IEEE, pp 1667–1668

Dhanekar S, Jain S (2013) Porous silicon biosensor: current status. Biosens Bioelectron 41:54–64

Dhar S, Chakraborti S (1996) Electroless nickel plated contacts on porous silicon. Appl Phys Lett 68:1392

Ensafi AA (2016) Porous silicon electrochemical biosensors: basic principles and detection strategies. In: Handbook on porous silicon. Springer, Cham

Ensafi AA, Abarghoui MM, Rezaei B (2014) A new non-enzymatic glucose sensor based on copper/porous silicon nanocomposite. Electrochim Acta 123:19–226

Ensafi AA, Abarghoui MM, Rezaei B (2015) Simultaneous determination of morphine and codeine using Pt nanoparticles supported on porous silicon flour modified ionic liquid carbon paste electrode. Sensors Actuators B Chem 219:1–9

Esteves A, Patarata L, Saraiva C, Martins C (2008) Assessment of the microbiological characteristics of industrially produced Alheira, with particular reference to foodborne pathogens. J Food Saf 28:88–102

Fernandez RE, Bhattacharya E, Chadha A (2008) Covalent immobilization of Pseudomonas cepacia lipase on semiconducting materials. Appl Surf Sci 254:4512–4519

Fernandez RE, Stolyarova S, Chadha A, Bhattacharya E, Nemirovsky Y (2009) MEMS composite porous silicon/polysilicon cantilever sensor for enhanced triglycerides biosensing. IEEE Sensors J 9:1660–1666

Fonthal F, Trifonov T, Rodriguez A, Goyes C, Vilanova X, Pallares J (2007) Fabrication and Characterization of Porous Silicon on crystalline Silicon based devices. In: IEEE conference on fourth congress of electronics, robotics and automotive mechanics, pp 170–174

Ghosh H, RoyChaudhuri C (2013) Ultrasensitive food toxin biosensor using frequency based signals of silicon oxide nanoporous structure. Appl Phys Lett 102:243701–243704

Ghosh H, RoyChaudhuri C (2015) Noise spectroscopy as an efficient tool for impedance based sub-femtomolar toxin detection in complex mixture using nanoporous silicon oxide. Biosens Bioelectron 67:757–762

Gupta B, Gooding JJ (2016) Biomolecule attachment to porous silicon. In: Handbook on porous silicon. Springer, Cham

Harraz FA (2014) Porous silicon chemical sensors and biosensors: a review. Sensors Actuators B Chem 202:897–912

Harraz FA, Ismail AA, Bouzid H, Al-Sayari SA, Al-Hajry A, Al-Assiri MS (2015) Electrical porous silicon sensor for detection of various organic molecules in liquid phase. Phys Status Solidi A. https://doi.org/10.1002/pssa.201431780

Hernandez-Montelongo J, Munoz-Noval A, García-Ruíz JP, Torres-Costa V, Martín-Palma R, Manso-Silvan M (2015) Nanostructured porous silicon: the winding road from photonics to cell scaffolds – a review. https://doi.org/10.3389/fbioe.2015.00060

Islam MN, Ram SK, Kumar S (2009) Mott and Efros-Shklovskii hopping conductions in porous silicon nanostructures. Phys E 41:1025–1028

Jan SD, Patel KR (2013) Enzyme immunoassay and enzyme linked immunosorbent assay. J Invest Dermatol 13:e12(1-3)

Jane A, Dronov R, Hodges A, Voelcker NH (2009) Porous silicon biosensors on the advance. Trends Biotechnol 27:230–239

Jang J (2008) Organic light-emitting diode with polyaniline-poly (styrene sulfonate) as a hole injection layer. Thin Solid Films 516:3152–3156

Jarvis KL, Barnes TJ, Prestidge CA (2012) Surface chemistry of porous silicon and implications for drug encapsulation and delivery applications. Adv Colloid Interf Sci 175:25–38

Jin J, Zhang D, Alocilja EC (2008) Label-free DNA sensor on nanoporous silicon-polypyrrole chip for monitoring salmonella species. IEEE Sensors J 8:891–895

Jin J, Hong S, Min NK (2009) Integrated urea sensor module based on poly(3-methylthiophene)-modified p-type porous silicon substrate. J Porous Mater 16:379–386

Jin J, Alocilja EC, Grooms DL (2010) Fabrication and electroanalytical characterization of label-free DNA sensor based on direct electropolymerization of pyrrole on p-type porous silicon substrates. J Porous Mater 17:169–176

Kanungo J, Basu S (2014) Ohmic and rectifying contacts to porous silicon. In: Handbook on porous silicon. Springer, Cham

Kanungo J, Maji S, Saha H, Basu S (2009) Stable aluminium ohmic contact to surface modified porous silicon. Solid State Electron 53:663–668

Kovacs A, Mescheder U (2012) Transport mechanisms in nanostructured porous silicon layers for sensor and filter applications. Sensors Actuators B Chem 175:179–185

Kumari V, Mishra P, Islam SS (2012) Development and standardization of porous silicon for application as a working electrode in electrochemical immunosensor. In: 16th international workshop on physics of semiconductor devices, p 85491I

Laczka O, Baldrich E, Munoz FX, Campo FJD (2008) Detection of escherichia coli and salmonella typhimurium using interdigitated microelectrode capacitive immunosensors: the importance of transducer geometry. Anal Chem 80:7239–7247

Li XJ, Chen SJ, Feng CY (2007) Characterization of silicon nanoporous pillar array as room-temperature capacitive ethanol gas sensor. Sensors Actuators B Chem 123:461–465

Li HL, Zhu Y, Xu D, Wan Y, Xia L, Zhao X (2009) Vapor-phase silanization of oxidized porous silicon for stabilizing composition and photoluminescence. J Appl Phys 105:114307

Li S, Cui H, Yuan Q, Wu J, Wadhwa A, Eda S, Jiang H (2014) AC electrokinetics enhanced capacitive immunosensor for point of care serodiagnosis of infectious diseases. Biosens Bioelectron 51:437–443

Lopez-Garcia J, Martin-Palma RJ, Manso M, Martinez-Duart JM (2007) Porous silicon based structures for the electrical biosensing of glucose. Sensors Actuators B Chem 126:82–85

Lu X, Xiao Y, Lei Z, Chen J (2009) Graphitized macroporous carbon microarray with hierarchical mesopores as host for the fabrication of electrochemical biosensor. Biosens Bioelectron 25:244–247

Lugo J, Ocampo M, Kirk AG, Plant DV, Fauchet PM (2007) Electrochemical sensing of DNA with porous silicon layers. J New Mater Electrochem Syst 10:113–116

Lundstrom I, Sundgren H, Winquist F, Eriksson M, Rulcker CK, Spetz AL (2007) Twenty-five years of field effect gas sensor research in linkoping. Sensors Actuators B Chem 121:247–262

Luo X, Davis J (2013) Electrical biosensors and the label free detection of protein disease biomarkers. Chem Soc Rev 42:5944–5962

Luth H, Thust M, Steffen A, Kordos P, Schoning MJ (2000) Biochemical sensors with structured and porous silicon capacitors. Mater Sci Eng B69–70:104–108
Maedler C, Erramilli S, LJ H, Hong MK, Mohanty P (2013) Tunable nanowire Wheatstone bridge for improved sensitivity in molecular recognition. Appl Phys Lett 102:043112(1-4)
Mahmoudi B, Gabouze N, Haddadi M, Mahmoudi BR, Cheraga H, Jilali KB, D. Dahmane (2007) The effect of annealing on the sensing properties of porous silicon gas sensor: use of screen-printed contacts. Sensors Actuators B Chem 123:680–784
Maji S, Das RD, Jana M, RoyChaudhuri C, Mondal N, Dutta SK, Bandopadhyay NR, Saha H (2010) Formation of ohmic contact by pre-annealing of shallow nanopores in macroporous silicon and its characterization. Solid State Electron 54:568–574
Melikjanyan GA, Martirosyan KS (2011) Possibility of application of porous silicon as glucose biosensor. Am J Physiol 4:225–227
Melikjanyan GA, Martirosyan KS (2012) Influence of anodization parameters of porous silicon on its glucose sensitivity. J Contemp Phys 47:294–297
Moeller F, Ban-Chorin M, Koch F (1995) Post-treatment effects on electrical conduction in porous silicon. Thin Solid Films 255:16
Mora MB, Ocampo M, Doti R, Lugo JE, Faubert J (2013) Chapter 6 porous silicon biosensors. In: State of the art in biosensors-general aspects. Intech Publishers
Munje RD, Muthukumar S, Selvam AP, Prasad S (2015) Flexible nanoporous tunable electrical double layer biosensors for sweat diagnostics. Sci Rep 5:14586
Nagare GD, Mukherji S (2009) Characterization of silanization and antibody immobilization on spin-on glass (SOG) surface. Appl Surf Sci 255:3696–3700
Nguyen MH, Tsai HJ, Wu JK, Wu YS, Lee M, Tseng FG (2013) Cascaded nano-porous silicon for high sensitive biosensing and functional group distinguishing by Mid-IR spectra. Biosens Bioelectron 47:80–85
Orabona E, Rendina I, Stefano LD, Rea I (2011) Chapter 12 Porous silicon integrated photonic devices for biochemical optical sensing. In: Crystalline silicon-properties and uses. Intech Publishers, Rijeka
Parkhutik V, Timashev S (2000) Kinetics of porous silicon growth studied using flicker-noise spectroscopy. J Appl Phys 87:7558–7566
Prabhakar D, Kumari V, Islam SS (2012) Nanoporous silicon based electrochemical immunosensor. Sci Adv Mater 4:121–125
Radke SM, Alocilja EC (2004) Design and fabrication of a microimpedance biosensor for bacterial detection. IEEE Sensors J 4:434–440
Rea I, Lamberti A, Rendina I, Coppola G, Gioffrè M, Iodice M, Casalino M, Tommasi ED, Stefano LD (2010) Fabrication and characterization of a porous silicon based microarray for label-free optical monitoring of biomolecular interactions. J Appl Phys 107:014513(1-5)
Rea I, Orabona E, Lamberti A, Rendina I, Stefano LD (2011) A microfluidics assisted porous silicon array for optical label-free biochemical sensing. Biomicrofluidics 5:0341201–03412010
Recio-Sanchez G, Torres-Costa V, Manso M, Gallach D, Lopez-Garcia J, Martin-Palma RJ (2010) Towards the development of electrical biosensors based on nanostructured porous silicon. Materials 3:755–763
Reddy RRK, Chadha A, Bhattacharya E (2001) Porous silicon based potentiometric triglyceride biosensor. Biosens Bioelectron 16:313–317
Ressine A, Marko-Varga G, Laurell T (2007) Porous silicon protein microarray technology and ultra−/superhydrophobic states for improved bioanalytical readout. Biotechnol Annu Rev 13: 149–200
Ressine A, VazDomínguez C, Fernandez VM, Lacey ALD, Laurell T, Ruzgas T, Shleev S (2010) Bioelectrochemical studies of azurin and laccase confined in three-dimensional chips based on gold-modified nano/microstructured silicon. Biosens Bioelectron 25:1001–1007
Reta N, Michelmore A, Saint C, Prieto-Simón B, Voelcker NH (2016) Porous silicon membrane-modified electrodes for label-free voltammetric detection of MS2 bacteriophage. Biosens Bioelectron 80:47–53

RoyChaudhuri C (2015) A review on porous silicon based electrochemical biosensors: beyond surface area enhancement factor. Sensors Actuators B Chem 210:310–323
RoyChaudhuri C, Das RD (2010) A biomolecule compatible electrical model of microimpedance affinity biosensor for sensitivity improvement in cell detection. Sens Actuators A 157:280–289
RoyChaudhuri C, Kanungo J, Das RD, Dutta SK, RoyChaudhuri S, Majhi S, Saha H (2008) Electrical sensing of biochemicals using macroporous silicon. Lecture notes in electrical engineering, vol 20. Springer Berlin Heidelberg, pp 101–116
Saha H, Pramanik C (2006) Porous Silicon Based Sensors: prospects and Challenges. Mater Manuf Process 21:239–246
Salis A, Setzu S, Monduzzi M, Mula G (2011) Chapter 17 porous silicon-based electrochemical biosensors. In: Biosensors-emerging materials and applications. Intech Publishers, Rijeka
Samanta N, RoyChaudhuri C (2015) Nanocrystalline silicon oxide impedance immunosensors for sub-femtomolar mycotoxin estimation in corn samples by incremental fuzzy approach. IEEE Sens 16:1069–1078
Samanta N, Kundu O, RoyChaudhuri C (2013) A simple low power electronic readout for rapid bacteria detection with impedance biosensor. IEEE Sens 13:4716–4724
Santos A, Kumeria T, Losic D (2013) Nanoporous anodic aluminum oxide for chemical sensing and biosensors. Trends Anal Chem 44:25–38
Scheen G, Bassu M, Francis LA (2012) Metal electrode integration on macroporous silicon: pore distribution and morphology. Nanoscale Res Lett 7:395 (1-5)
Schoning MJ, Ronkel F, Crott M, Thust M, Schultze JW, Kordos P, Luth H (1997) Miniaturization of potentiometric using porous silicon microtechnology. Electrochim Acta 42:3185–3193
Schoning MJ, Kurowski A, Thust M, Kordos P, Schultze JW, Luth H (2000a) Capacitive micro-sensors for biochemical sensing based on porous silicon technology. Sens Actuators B 64:59–64
Schoning MJ, Malkoc U, Thust M, Steffen A, Kordos P, Luth H (2000b) Novel electrochemical sensors with structured and porous semiconductor/insulator capacitor. Sensors Actuators B Chem 65:288–290
Shahbazi MA, Hamidi M, Makila EM, Zhang H, Almeida PV, Kaasalainen M, Salonen JJ, Hirvonan JJ, Santos HA (2013) The mechanisms of surface chemistry effects of mesoporous silicon nanoparticles on immunotoxicity and biocompatibility. Biomaterials 34:7776–7789
Shanmugam NR, Selvam AP, Barrett TW, Kazmierczak SC, Rana MN, Prasad S (2015) Portable nanoporous electrical biosensor for ultrasensitive detection of Troponin-T. Future Sci OA 1: FSO24
Simonisa A, Rugeb C, Muller-Veggian M, Luth H, Schoning MJ (2003) A long-term stable macroporous-type EIS structure for electrochemical sensor applications. Sensors Actuators B Chem 91:21–25
Singh R, Mukherjee M, Sumana G, Gupta RK, Sood S, Malhotra BD (2014) Biosensors for pathogen detection: a smart approach towards clinical diagnosis. Sensors Actuators B Chem 197:385–404
Siu WM, Cobbold RSC (1979) Basic properties of the electrolyte-Si02-Si system: physical and theoretical aspects. IEEE Trans Electron Devices 26:1805–1815
Song M, Yun D, Min N, Hong S (2007) Electrochemical biosensor array for liver diagnosis using silanization technique on nanoporous silicon electrode. J Biosci Bioeng 1:32–37
Song YY, Li Y, Yang C, Xia XH (2008) Surface electric field manipulation of the adsorption kinetics and biocatalytic properties of cytochrome c on a 3D macroporous Au electrode. Anal Bioanal Chem 390:333–341
Song MJ, Yun DH, Hong SI (2009) An electrochemical biosensor array for rapid detection of alanine aminotransferase and aspartate aminotransferase. Biosci Biotechnol Biochem 73: 474–478
Stefano LD, Orabona E, Lamberti A, Rea I, Rendina I (2013) Microfluidics assisted biosensors for label-free optical monitoring of molecular interactions. Sensors Actuators B Chem 179:157–162
Stolyarova S, Nemirovsky Y (2011) Porous silicon as a nanostructured template for enhanced immobilization and crystallization of inorganic and biomaterials. ECS Trans 33:137–145

Stolyarova S, Cherian S, Raiteri R, Zeravik J, Skladal P, Nemirovsky Y (2008) Composite porous silicon-crystalline silicon cantilevers for enhanced biosensing. Sensors Actuators B Chem 131: 509–515

Szili EJ, Jane A, Low SP, Sweetman M, Macardle P, Kumar S, Smart RSC, Voelcker NH (2011) Interferometric porous silicon transducers using an enzymatically amplified optical signal. Sensors Actuators B Chem 160:341–348

Tang J, Wang Y, Li J, Da P, Geng J, Zheng G (2014) Sensitive enzymatic glucose detection by TiO_2 nanowire photoelectrochemical biosensors. J Mater Chem A 2:6153–6157

Tao X, Xiao-yi L, Zhen-hong J, Shi-bin H, Fu-chun Z (2008) Research on immunosensor based on porous silicon. Optoelectron Lett 4:0328–0330

Tembe S, Chaudhari PS, Bhoraskar SV, D'Souza SF, Karve MS (2009) Conductivity-based catechol sensor using tyrosinase immobilized in porous silicon. IEEE. Sensors 8:1593–1597

Valentini V, Polini R (2011) Confined functionalization of mesoporous silicon layers. J Electrochem Soc 158:K35–K41

Vamvakaki V, Chaniotakis NA (2008) DNA stabilization and hybridization detection on porous silicon surface by EIS and total reflection FT-IR spectroscopy. Electroanalysis 20:1845–1850

Veeramani MS, Shyam KP, Ratchagar NP, Chadha A, Bhattacharya E (2014) Miniaturised silicon biosensors for the detection of triglyceride in blood serum. Anal Methods 6:1728–1735

Vemulachedu H, Fernandez RE, Bhattacharya E, Chadha A (2009) Miniaturization of EISCAP sensor for triglyceride detection. J Mater Sci Mater Med 20:S229–S234

Wu Y, Chen C, Liu S (2009) Enzyme-functionalized silica nanoparticles as sensitive labels in biosensing. Anal Chem 81:1600–1607

Xu W, Riikonen J, Nissinen T, Suvanto M, Rilla K, Li B, Wang Q, Deng F, Lehto VP (2012) Amine surface modifications and fluorescent labeling of thermally stabilized mesoporous silicon nanoparticles. J Phys Chem C 116:22307–22314

Yun D, Song M, Hwang S, Hong S (2012) Fabrication and electrochemical characterization of nanoporous silicon electrode for amperometric urea biosensor. Jpn J Appl Phys 51:06FG02

Zhang D, Alocilja EC (2008) Characterization of nanoporous silicon-based DNA biosensor for the detection of salmonella enteritidis. IEEE Sensors J 8:775–780

Zhang H, Jia Z, Lv X, Zhou J, Chen L, Liu R, Ma J (2013) Porous silicon optical microcavity biosensor on silicon-on-insulator wafer for sensitive DNA detection. Biosens Bioelectron 44: 89–94

Zimin SP, Kuznetsov VS, Prokaznikov AV (1995) Electrical characteristics of aluminum contacts to porous silicon. Appl Surf Sci 91:355–358