Chapter 4
Advancing Nanostructured Porous Si-Based Optical Transducers for Label Free Bacteria Detection

Naama Massad-Ivanir, Giorgi Shtenberg, and Ester Segal

Abstract Optical label-free porous Si-based biosensors for rapid bacteria detection are introduced. The biosensors are designed to directly capture the target bacteria cells onto their surface with no prior sample processing (such as cell lysis). Two types of nanostructured optical transducers based on oxidized porous Si ($PSiO_2$) Fabry-Pérot thin films are synthesized and used to construct the biosensors. In the first system, we graft specific monoclonal antibodies (immunoglobulin G's) onto a neat electro-chemically-machined $PSiO_2$ surface, based on well-established silanization chemistry. The second biosensor class consists of a $PSiO_2$/hydrogel hybrid. The hydrogel, polyacrylamide, is synthesized *in situ* within the nanostructured $PSiO_2$ host and conjugated with specific monoclonal antibodies to provide the active component of the biosensor. Exposure of these modified-surfaces to the target bacteria results in "direct-cell-capture" onto the biosensor surface. These specific binding events induce predictable changes in the thin-film optical interference spectrum of the biosensor. Our studies demonstrate the applicability of these biosensors for the detection of low bacterial concentrations, in the range of 10^3–10^5 cell/ml, within minutes. The sensing performance of the two different platforms, in terms of their stability in aqueous media and sensitivity, are compared and discussed. This preliminary study suggests that biosensors based on $PSiO_2$/hydrogel hybrid outperform the neat $PSiO_2$ system.

Keywords Porous Si • Biosensor • Bacteria detection • Optical transducer • Hydrogel • Hybrid

4.1 Introduction

Porous Si (PSi) or SiO_2 matrices have emerged as an attractive and versatile material for the construction of complex functional nanostructures (Li et al. 2003, 2005; Yoon et al. 2003). PSi is typically synthesized by anodic electrochemical etching of a single-crystal Si wafer in a hydrofluoric acid (HF)

N. Massad-Ivanir
Department of Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Haifa 32000, Israel

G. Shtenberg
The Interdepartmental Program of Biotechnology, Technion – Israel Institute of Technology, Haifa 32000, Israel

E. Segal (✉)
Department of Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Haifa 32000, Israel

Russell Berrie Nanotechnology Institute, Technion – Israel Institute of Technology, Haifa 32000, Israel
e-mail: esegal@tx.technion.ac.il

based electrolyte solution. At the end of this simple and cost-effective process, a nanostructure with complex properties is obtained (Sailor and Link 2005). One of the favorable properties of this nanomaterial is its large surface area (up to 500 m^2/cm^3), which enables large amounts and a variety of biomolecular interactions, including enzymes (DeLouise et al. 2005b), DNA fragments (Zhang and Alocilja 2008) and antibodies (Bonanno and DeLouise 2007), occurring over a small working area. The resulting activated surface can be used for several biosensing applications, including the detection of DNA (Zhang and Alocilja 2008), proteins (Dancil et al. 1999; Pacholski et al. 2005, 2006; Schwartz et al. 2007), enzyme activity (DeLouise et al. 2005b) and bacteria (Alvarez et al. 2007; Chan et al. 2001; Massad-Ivanir et al. 2010; Massad-Ivanir et al. 2011; Radke and Alocilja 2005). PSi optical sensors are based on changes of photoluminescence (Chan et al. 2001; de Leon et al. 2004) or reflectivity (Archer et al. 2004; Stewart and Buriak 2000) upon exposure to the target analyte (D'Auria et al. 2006), which replace the media in the pores. A change in the refractive index of the film is observed as a modulation in the photoluminescence spectrum or as a wavelength shift in the reflectivity spectrum, respectively.

A key challenge in PSi biosensors is to effectively stabilize the nanostructure during experiments in biological solutions, as PSi oxidation and dissolution in aqueous environments lead to significant signal baseline drifts, signal loss, and ultimately to structural collapse of the PSi thin film (Jane et al. 2009; Janshoff et al. 1998; Kilian et al. 2009). Another difficulty presented by PSi transducers is the susceptibility of proteins to undergo undesired conformation changes during deposition and patterning onto the Si (Burnham et al. 2006). Thus, it was demonstrated that the use of PSi as template or as a host matrix may eliminate these issues, while providing the means for construction of complex optical structures from flexible materials, such as polymers (Bonanno and DeLouise 2009a, b; DeLouise et al. 2005a; Li et al. 2003; Massad-Ivanir et al. 2010; Segal et al. 2007; Yoon et al. 2003). Specifically, the incorporation of hydrogels offers significant advantages due to their high optical transparency, good mechanical properties, ability to store and immobilize reactive functional groups and biological compatibility (Bonanno and DeLouise 2009a, b; Massad-Ivanir et al. 2010). Recent work on (oxidized) PSi/hydrogel hybrids (Bonanno and DeLouise 2009a, b; Massad-Ivanir et al. 2010; Perelman et al. 2010; Sciacca et al. 2011; Segal et al. 2007; Wu and Sailor 2009) demonstrated the potential of these nanomaterials for application in drug delivery, sensing and biosensing.

In the present work we describe the basic considerations in designing PSi-based optical transducers for label-free detection of microorganisms e.g., bacteria. We compare two strategies for the preparation of the biosensor surface. In the first approach, neat oxidized PSi ($PSiO_2$) Fabry–Pérot thin films are used as the transducer and biofunctionalized with a monoclonal antibody (as the capture probe) using well established coupling chemistry. In the second approach, an antibody-modified $PSiO_2$/hydrogel hybrid is designed and synthesized. The hydrogel is synthesized *in situ* within the $PSiO_2$ host and conjugated with a monoclonal antibody through a biotin–streptavidin (SA) system. We demonstrate rapid detection of *E. coli* K-12 bacteria (as a model microorganism) via a "direct cell capture" approach onto these two types of PSi-based biosensors.

4.2 Materials and Methods

4.2.1 Materials

Highly-doped p-type Si wafers (0.0009 Ω-cm resistivity, <100>oriented, boron-doped) were purchased from Siltronix Corp. Aqueous HF (48%) and ethanol absolute were supplied by Merck. Acrylamide, N,N′-methylenebis(acrylamide) (BIS), bis(acryloyl)cystamine (BIS-CA), 2,2-dimethoxy-2-phenyl-acetophenone (DMAP), tris(2-carboxyethyl) phosphine (TCEP), Bis(N-succinimidyl)

carbonate (SC), (3-aminopropyl)triethoxysilane (APTES), diisopropylethylamine (DIEA) and PEO-iodoacetyl biotin were obtained from Sigma Aldrich Chemicals. Streptavidin (SA), *E. coli* antibody and biotinylated *E. coli* antibody were purchased from Jackson ImmunoResearch Labs Inc. *E. coli* (K-12) was generously supplied by Prof. Sima Yaron (Technion).

4.2.2 *Preparation of PSiO$_2$ Substrates*

PSi Fabry-Pérot thin films are prepared by anodic etch of highly doped p-type single-crystal Si wafers in a solution of 3:1 v/v 48% aqueous hydrofluoric acid:ethanol. A constant current density of 385 mA/cm^2 is applied for 30 s. The resulting freshly-etched samples are thermally oxidized at 800°C, to create a SiO$_2$ matrix.

4.2.3 *Preparation of PSiO$_2$/Hydrogel Hybrids*

The detailed synthesis scheme of these hybrids was previously described (Massad-Ivanir et al. 2010). Briefly, an aqueous pre-gel solution contains acrylamide monomers, cross-linking agents (BIS and BIS-CA) and photoinitiator (DMAP). The pre-gel solution is cast onto the PSiO$_2$ film and allowed to infiltrate into the nanostructure. Photo-polymerization is initiated by UV irradiation (254 nm, 10 min).

4.2.4 *Immobilization of Recognition Motives*

4.2.4.1 Biofunctionalization of Neat PSiO$_2$ Films

Preparation of APTES-modified surfaces: A PSiO$_2$ sample is incubated with an aqueous solution of 42 mM APTES and 56 mM DIEA for 30 min. After the solution is removed, the surface is rinsed with purified water and ethanol for 10 min each and dried under a nitrogen stream.

Preparation of NHS-modified surfaces: The APTES-modified surface is immersed in a 10 mM SC solution in acetonitrile for 7 min. After the solution is removed, the surface is washed extensively with acetonitrile three times for 10 min each and dried under a nitrogen stream.

Preparation of IgG-modified surfaces: The NHS-modified surface is incubated in 100 μg/mL *E. coli* antibody solution at room temperature for 60 min. After the solution is removed, the surface is rinsed with PBS, 1 M NaCl, and PBS for 10 min each.

4.2.4.2 Biofunctionalization of PSiO$_2$/Polyacrylamide Hybrids

The hybrids are exposed to a reducing agent, TCEP (10 mM), to generate reactive thiol groups throughout the hydrogel. The activated hydrogel is then treated with PEO-iodoacetyl biotin (100 μM), a thiol-reactive biotin linker molecule, and reacted with SA (100 μg/mL). The resulting SA-modified hybrids are incubated with biotinylated *E. coli* antibody (100 μg/mL).

4.2.5 Measurement of Interferometric Reflectance Spectra

Interferometric reflectance spectra of the samples are collected using an Ocean Optics charge-coupled device (CCD) USB 4000 spectrometer fitted with a microscope objective lens coupled to a bifurcated fiber-optic cable. A tungsten light source is focused onto the center of the sample surface with a spot size of approximately 1–2 mm^2. Reflectivity data are recorded in the wavelength range of 400–1,000 nm, with a spectral acquisition time of 100 ms. Both illumination of the surface and detection of the reflected light are performed along an axis coincident with the surface normal. All the optical experiments are conducted in a fixed cell to ensure that the sample reflectivity is measured at the same spot during all the measurements. Spectra are collected using a CCD spectrometer and analyzed by applying fast Fourier transform (FFT).

4.2.6 Bacteria Culture

E. coli K-12 is cultivated in a 10 mL tube with LB medium (5 mL) (medium composition in deionized water (1 L): NaCl (5 g), yeast extract (5 g), and tryptone (10 g)). The bacteria are incubated overnight at 37°C with shaking.

4.2.7 Bacteria Sensing

IgG-modified $PSiO_2$, IgG-modified hybrid, neat $PSiO_2$ and unmodified hybrid (as controls) samples are incubated with *E. coli* K-12 suspensions (at concentrations ranging from 10^3 to 10^5 cell/mL) for 30 min in a fixed cell. After the bacteria suspension is removed, the cell is flushed for 30 min with a buffer solution. Optical measurements are recorded throughout the experiment. The FFT intensity changes are expressed as percentages and are calculated using the following equation:

$$\text{Intensity change } (\%) = \frac{A_1 - A_2}{A_2} \times 100 \qquad (4.1)$$

where A_1 is the intensity before modification and A_2 is the intensity after modification with the bacteria suspensions.

4.3 Results and Discussion

4.3.1 Preparation and Characterization of PSiO$_2$ Films

The $PSiO_2$ film is prepared from a highly doped p-type single-crystal Si wafer, polished on the <100>face using an anodic electrochemical etch, at a constant current density of 385 mA/cm^2 for 30 s. The resulting freshly etched PSi template is then thermally oxidized at 800°C to create a hydrophilic $PSiO_2$ matrix. The resulting porous layer is approximately 7,880 nm thick with interconnecting cylindrical pores ranging in diameter from 60 to 100 nm and the calculated porosity is approximately 80%. The structural properties, i.e., thickness and porosity, of the $PSiO_2$ layer are thoroughly

characterized by scanning electron microscopy, gravimetry (for porosity), and a spectroscopic liquid infiltration method, as previously described by Massad-Ivanir et al. (2010).

4.3.2 Biofunctionalization of Neat PSiO$_2$ and PSiO$_2$/Hydrogel Hybrids with Antibodies

4.3.2.1 Neat PSiO$_2$

The synthetic approach for grafting the monoclonal antibodies (IgG) onto neat PSiO$_2$ surfaces is based on a well established silanization technology (Xia et al. 2006), as previously described (Massad-Ivanir et al. 2011).

4.3.2.2 PSiO$_2$/Hydrogel Hybrids

Biofunctionalization of the hybrids is achieved by exposure of the PSiO$_2$/di-sulfide linked hydrogel to a reducing (TCEP) to generate reactive thiol groups throughout the hydrogel phase. Next, the activated hydrogel is incubated with a SH-reactive biotin linker molecule, followed by attachment of SA to the hydrogel via the SH-linked biotin. Functionalization is accomplished through the bioconjugation of biotinylated *E. coli* monoclonal antibodies to the SA linker molecule.

4.3.3 Stability of PSiO$_2$–Based Transducers in Aqueous Media

The optical readout stability of the two different transducers (neat PSiO$_2$ and hybrid) under flow conditions is characterized by long buffer (PBS) flow experiments of several hours. The samples are placed in a plexiglas custom-made flow cell, in which a buffer solution is delivered at a constant buffer flow of 0.5 mL/min for 5 h and the reflectivity spectra is collected every 15 s.

The reflectivity spectrum of the thin PSi layer consists of a series of interference fringes that result from a Fabry–Pérot interference. This fringe pattern arises from reflections at the top and at the bottom of the film, so that the measurement is made over the entire volume of the system. The maxima of these fringes are governed by the following relationship (Fabry–Pérot equation) (Sailor and Link 2005):

$$m\lambda = 2nL \qquad (4.2)$$

The effective optical thickness (EOT) of the sample refers to the 2 nL term in the Fabry–Pérot formula (where m is an integer, n is the average refractive index, L is the thickness of the film, and λ is the wavelength of the incident light). A change in the average refractive index (n) leads to a shift in the observed reflectivity spectrum that correlates with EOT changes.

Figure 4.1 shows the results of the buffer flow experiments for both silanized-PSiO$_2$ and hybrid transducers. Changes in the EOT values and the reflected light intensity with time are depicted in Fig. 4.1a and b, respectively. This study demonstrates that the optical readout of the hybrid transducer is highly stable throughout the entire experiment. Changes of less than 0.5% in the intensity and 10 nm in the EOT values are recorded. Thus, a robust optical readout with no evident baseline drift is obtained. On the contrary, the silanized-PSiO$_2$ transducer exhibits significant baseline drifts of both
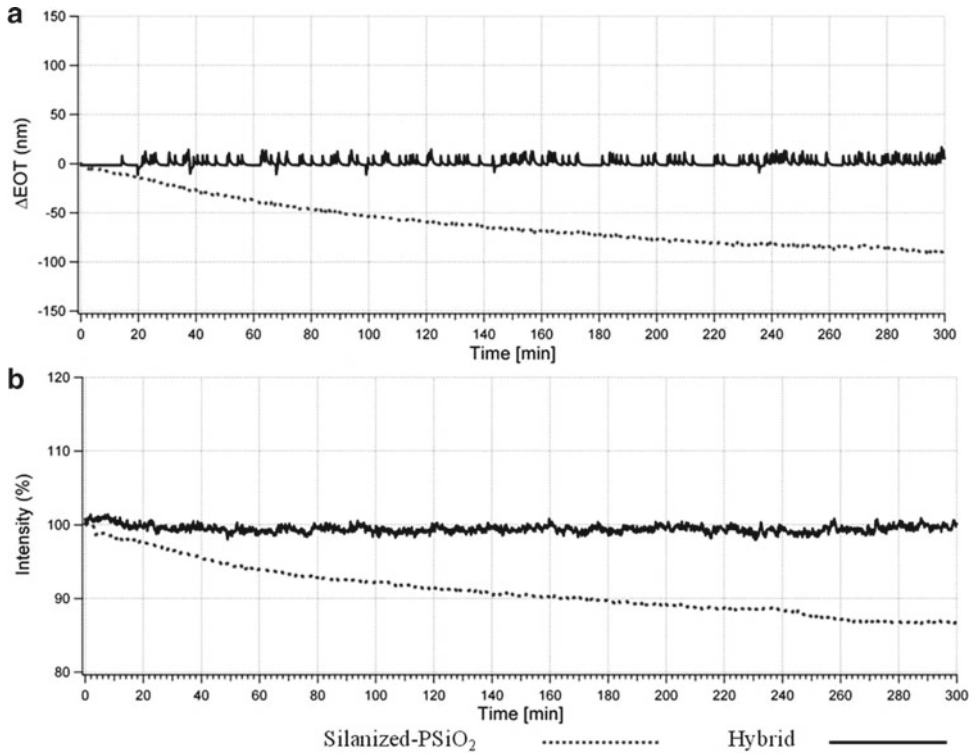
Fig. 4.1 Buffer flow experiments onto silanized-PSiO$_2$ and PSiO$_2$/polyacrylamide hydrogel hybrid transducers. (**a**) EOT changes with time and (**b**) Intensity changes with time. The reflectivity spectra of the samples is collected every 15 s under constant PBS buffer flow of 0.5 mL/min for 5 h

the EOT and the intensity signals with time. The EOT value of the film decrease by approximately 90 nm within 5 h and the reflected intensity drops by 13%. These significant changes in the optical readout are ascribed to time-dependent degradation of the porous scaffold (Janshoff et al. 1998; Massad-Ivanir et al. 2010; Pacholski et al. 2006). Thus, these experiments clearly show that the presence of the hydrogel within and delicate and highly porous SiO$_2$ scaffold stabilize the nanostructure under aqueous conditions. Similar results were recently reported by Cunin et al. (Sciacca et al. 2011) for a PSi/chitosan hybrid used as an optical transducer for the detection of carboxylic acid-containing drugs in water.

4.3.4   Optical Detection of E. coli Bacteria

Our biosensing approach is based on monitoring changes in the light reflected from the biofunctionalized PSiO$_2$ or hybrid nanostructures. Changes in the amplitude (intensity) of the FFT peak of the biosensor are correlated to specific immobilization of bacteria cells, "direct cell capture" onto the transducer surface via antibody-antigen interactions. Since *E. coli* bacteria are large biological species, with typical dimensions of 0.8–2 μm (Sundararaj et al. 2004), binding of bacteria will occur only on the biosensor surface and not inside the porous nanostructure. Moreover, we expect that their presence on the surface will scatter the light, resulting in a significant decrease in the reflected light intensity (Alvarez et al. 2007; Massad-Ivanir et al. 2010, 2011). An experimental

Fig. 4.2 *E. coli* K-12 biosensing experiments. IgG-modified $PSiO_2$ and hybrid are incubated with different concentrations of *E. coli* suspensions and the maximal intensity decrease value is presented. Control experiments, incubation of unmodified $PSiO_2$ and hybrid surfaces with bacteria suspensions
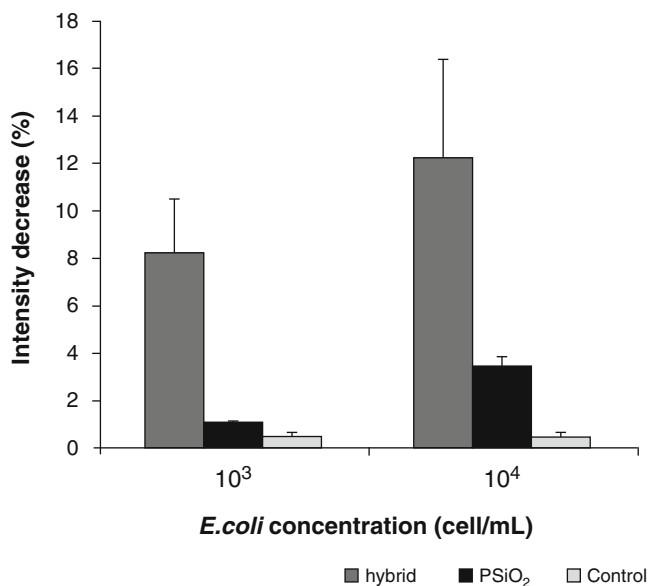
setup for continuous monitoring of the reflectivity spectrum of the hybrids upon incubation with bacteria suspensions is designed and constructed. The biosensors are exposed to *E. coli* K-12 suspensions with different concentrations, ranging from 10^3 to 10^5 cell/mL. The incubation time was set to 30 min, after which the samples were washed with a buffer solution for 30 min. Figure 4.2 displays the optical response of the different biosensors upon introduction to different concentrations of *E. coli* suspensions. Indeed, both biosensors exhibit a decrease in intensity upon exposure to *E. coli*, while insignificant changes in the FFT spectrum are recorded for the unmodified surfaces. In order to confirm that this intensity change results from bacteria capture onto IgG-modified surfaces, the biosensors are studied under a light microscope immediately after the biosensing experiment. Indeed, immobilized bacteria cells are observed onto the biosensor surface, while no cells are observed onto the unmodified surfaces (data not shown). Moreover, the intensity signals of the two biosensors are proportional to the bacteria suspension concentration (see Fig. 4.2). As expected, exposure of the biosensors to a lower bacteria concentration (10^3 cells/mL) results in a signal decrease; i.e., a smaller change in intensity is observed. When comparing the response of the two biosensors ($PSiO_2$ vs. hybrid) to *E. coli* exposure, the hybrid-based biosensors exhibit significantly higher intensity changes (~four fold higher). These results indicate that the hybrid system outperforms the $PSiO_2$ biosensors in terms of their sensitivity. It is well established that the immobilization step is crucial in maintaining the conformation and the immunoactivity of the IgG molecules (Somasundaran 2006). We assume that the presence of the hydrogel (a soft organic component) maintains the proteins desired conformation during the immobilization and patterning onto the biosensor surface.

In terms of the sensitivity of the different biosensors, these preliminary experiments show relatively low detection limits of 10^3 and 10^4 cell/mL for the modified hybrids and $PSiO_2$ biosensing platforms, respectively. For comparison, the detection limit of current state-of-the-art surface plasmon resonance (SPR) biosensors is in the range of 10^2–10^6 cells/mL (Dudak and Boyaci 2007, 2009; Skottrup et al. 2008; Taylor et al. 2008). Moreover, the response time of these biosensors to bacteria exposure is comparable to that of SPR techniques.

4.4 Conclusions

Two classes of label-free optical biosensors for bacteria detection are synthesized and characterized. The first platform is based on a $PSiO_2$ nanostructure (Fabry–Pérot thin film) and the second consists of a $PSiO_2$/polyacrylamide hydrogel hybrid. The different nanostructures' surfaces are biofunctionalized with IgG, as a capture probe, and their potential applicability as a biosensor for bacteria detection is demonstrated. The hybrids show improved optical readout stability under aqueous conditions. Moreover, the presence of the hydrogel also enables desired conformation of the antibodies onto the biosensor surface during deposition and patterning, resulting in a higher sensitivity. This proof-of-concept work demonstrates a simple and sensitive detection scheme of bacteria via a "direct cell capture" approach. Our preliminary biosensing experiments demonstrate a detection limit of 10^3–10^4 cells/mL for *E. coli* and a response time of several minutes. We are currently exploring several approaches to enhance the sensitivity of these biosensors, including improving optimization of the antibody concentration and orientation, enhancement of the coupling chemistry and use antibody fragments.

References

Alvarez, S. D., Schwartz, M. P., Migliori, B., Rang, C. U., Chao, L., & Sailor, M. J. (2007). Using a porous silicon photonic crystal for bacterial cell-based biosensing. *Physica Status Solidi a–Applications and Materials Science, 204*, 1439–1443.

Archer, M., Christophersen, M., Fauchet, P. M., Persaud, D., & Hirschman, K. D. (2004). Electrical porous silicon microarray for DNA hybridization detection. *Micro- and Nanosystems, 782*, 385–391.

Bonanno, L. M., & Delouise, L. A. (2007). Steric crowding effects on target detection in an affinity biosensor. *Langmuir, 23*, 5817–5823.

Bonanno, L. M., & Delouise, L. A. (2009a) Design of a hybrid amine functionalized polyacrylamide hydrogel-porous silicon optical sensor. *Proceedings of SPIE, 7167*, 71670F1-11.

Bonanno, L. M., & Delouise, L. A. (2009b). Optical detection of polyacrylamide swelling behavior in a porous silicon sensor. *Materials Research Society Symposium. Proceeding., 1133*, 1133-AA01-05.

Burnham, M. R., Turner, J. N., Szarowski, D., & Martin, D. L. (2006). Biological functionalization and surface micro-patterning of polyacrylamide hydrogels. *Biomaterials, 27*, 5883–5891.

Chan, S., Horner, S. R., Fauchet, P. M., & Miller, B. L. (2001). Identification of gram negative bacteria using nanoscale silicon microcavities. *Journal of the American Chemical Society, 123*(47), 11797–11798.

Dancil, K.-P. S., Greiner, D. P., & Sailor, M. J. (1999). A porous silicon optical biosensor: Detection of reversible binding of IgG to a protein A-modified surface. *Journal of the American Chemical Society, 121*, 7925–7930.

D'Auria, S., de Champdore, M., Aurilia, V., Parracino, A., Staiano, M., Vitale, A., Rossi, M., Rea, I., Rotiroti, L., Rossi, A. M., Borini, S., Rendina, I., & de Stefano, L. (2006). Nanostructured silicon-based biosensors for the selective identification of analytes of social interest. *Journal of Physics. Condensed Matter, 18*, S2019–S2028.

de Leon, S. B., Sa'Ar, A., Oren, R., Spira, M. E., & Yitzchaik, S. (2004). Neurons culturing and biophotonic sensing using porous silicon. *Applied Physics Letters, 84*, 4361–4363.

Delouise, L. A., Fauchet, P. M., Miller, B. L., & Pentland, A. A. (2005a). Hydrogel-supported optical-microcavity sensors. *Advanced Materials, 17*, 2199–2203.

Delouise, L. A., Kou, P. M., & Miller, B. L. (2005b). Cross-correlation of optical microcavity biosensor response with immobilized enzyme activity. Insights into biosensor sensitivity. *Analytical Chemistry, 77*, 3222–3230.

Dudak, F. C., & Boyaci, I. H. (2007). Development of an immunosensor based on surface plasmon resonance for enumeration of *Escherichia coli* in water samples. *Food Research International, 40*, 803–807.

Dudak, F. C., & Boyaci, I. H. (2009). Rapid and label-free bacteria detection by surface plasmon resonance (SPR) biosensors. *Biotechnology Journal, 4*, 1003–1011.

Jane, A., Dronov, R., Hodges, A., & Voelcker, N. H. (2009). Porous silicon biosensors on the advance. *Trends in Biotechnology, 27*, 230–239.

Janshoff, A., Dancil, K. P. S., Steinem, C., Greiner, D. P., LIN, V. S. Y., Gurtner, C., Motesharei, K., Sailor, M. J., & Ghadiri, M. R. (1998). Macroporous p-type silicon Fabry-Perot layers. Fabrication, characterization, and applications in biosensing. *Journal of the American Chemical Society, 120*, 12108–12116.

Kilian, K. A., Boecking, T., & Gooding, J. J. (2009). The importance of surface chemistry in mesoporous materials: Lessons from porous silicon biosensors. *Chemical Communications*, 630–640.

Li, Y. Y., Cunin, F., Link, J. R., Gao, T., Betts, R. E., Reiver, S. H., Chin, V., Bhatia, S. N., & Sailor, M. J. (2003). Polymer replicas of photonic porous silicon for sensing and drug delivery applications. *Science, 299*, 2045–2047.

Li, Y. Y., Kollengode, V. S., & Sailor, M. J. (2005). Porous silicon/polymer nanocomposite photonic crystals by microdroplet patterning. *Advanced Materials, 17*, 1249–1251.

Massad-Ivanir, N., Shtenberg, G., Zeidman, T., & Segal, E. (2010). Construction and characterization of porous SiO_2/hydrogel hybrids as optical biosensors for rapid detection of bacteria. *Advanced Functional Materials, 20*, 2269–2277.

Massad-Ivanir, N., Shtenberg, G., Tzur, A., Krepker, A. M., & Segal, E. (2011). Engineering nanostructured porous SiO_2 surfaces for bacteria detection via "direct cell capture". *Analytical Chemistry, 83*, 3282–3289.

Pacholski, C., Sartor, M., Sailor, M. J., Cunin, F., & Miskelly, G. M. (2005). Biosensing using porous silicon double-layer interferometers: Reflective interferometric Fourier transform spectroscopy. *Journal of the American Chemical Society, 127*, 11636–11645.

Pacholski, C., Yu, C., Miskelly, G. M., Godin, D., & Sailor, M. J. (2006). Reflective interferometric Fourier transform spectroscopy: A self-compensating label-free immunosensor using double-layers of porous SiO_2. *Journal of the American Chemical Society, 128*, 4250–4252.

Perelman, L. A., Moore, T., Singelyn, J., Sailor, M. J., & Segal, E. (2010). Preparation and characterization of a pH- and thermally responsive poly(N-isopropylacrylamide-co-acrylic acid)/porous SiO_2 hybrid. *Advanced Functional Materials, 20*, 826–833.

Radke, S. M., & Alocilja, E. C. (2005). A microfabricated biosensor for detecting foodborne bioterrorism agents. *IEEE Sensors Journal, 5*, 744–750.

Sailor, M. J., & Link, J. R. (2005). "Smart dust": Nanostructured devices in a grain of sand. *Chemical Communications*, 1375–1383.

Schwartz, M. P., Alvarez, S. D., & Sailor, M. J. (2007). Porous SiO_2 interferometric biosensor for quantitative determination of protein interactions: Binding of protein a to immunoglobulins derived from different species. *Analytical Chemistry, 79*, 327–334.

Sciacca, B., Secret, E., Pace, S., Gonzalez, P., Geobaldo, F., Quignard, F., & Cunin, F. (2011). Chitosan-functionalized porous silicon optical transducer for the detection of carboxylic acid-containing drugs in water. *Journal of Materials Chemistry, 21*, 2294–2302.

Segal, E., Perelman, L. A., Cunin, F., di Renzo, F., Devoisselle, J. M., Li, Y. Y., & Sailor, M. J. (2007). Confinement of thermoresponsive hydrogels in nanostructured porous silicon dioxide templates. *Advanced Functional Materials, 17*, 1153–1162.

Skottrup, P. D., Nicolaisen, M., & Justesen, A. F. (2008). Towards on-site pathogen detection using antibody-based sensors. *Biosensors and Bioelectronics, 24*, 339–348.

Somasundaran, P. (2006). *Encyclopedia of surface and colloid science*. Boca Raton: CRC Press.

Stewart, M. P., & Buriak, J. M. (2000). Chemical and biological applications of porous silicon technology. *Advanced Materials, 12*, 859–869.

Sundararaj, S., Guo, A., Habibi-Nazhad, B., Rouani, M., Stothard, P., Ellison, M., & Wishart, D. S. (2004). The CyberCell Database (CCDB): A comprehensive, self-updating, relational database to coordinate and facilitate in silico modeling of Escherichia coli. *Nucleic Acids Research, 32*, D293–D295.

Taylor, A. D., Ladd, J., Homola, J., & Jiang, S. (2008). Surface plasmon resonance (SPR) sensors for the detection of bacterial pathogens. In Z. Mohammed, E. Souna, & T. Anthony (Eds.), *Principles of bacterial detection: Biosensors, recognition receptors and microsystems*. New York: Springer.

Wu, J., & Sailor, M. (2009). Chitosan hydrogel-capped porous SiO_2 as a pH responsive nano-valve for triggered release of insulin. *Advanced Functional Materials, 19*, 733–741.

Xia, B., Xiao, S. J., Guo, D. J., Wang, J., Chao, M., Liu, H. B., Pei, J., Chen, Y. Q., Tang, Y. C., & Liu, J. N. (2006). Biofunctionalisation of porous silicon (PS) surfaces by using homobifunctional cross-linkers. *Journal of Materials Chemistry, 16*, 570–578.

Yoon, M. S., Ahn, K. H., Cheung, R. W., Sohn, H., Link, J. R., Cunin, F., & Sailor, M. J. (2003). Covalent crosslinking of 1-D photonic crystals of microporous Si by hydrosilylation and ring-opening metathesis polymerization. *Chemical Communications*, 680–681.

Zhang, D., & Alocilja, E. C. (2008). Characterization of nanoporous silicon-based DNA biosensor for the detection of salmonella enteritidis. *IEEE Sensors Journal, 8*, 775–780.