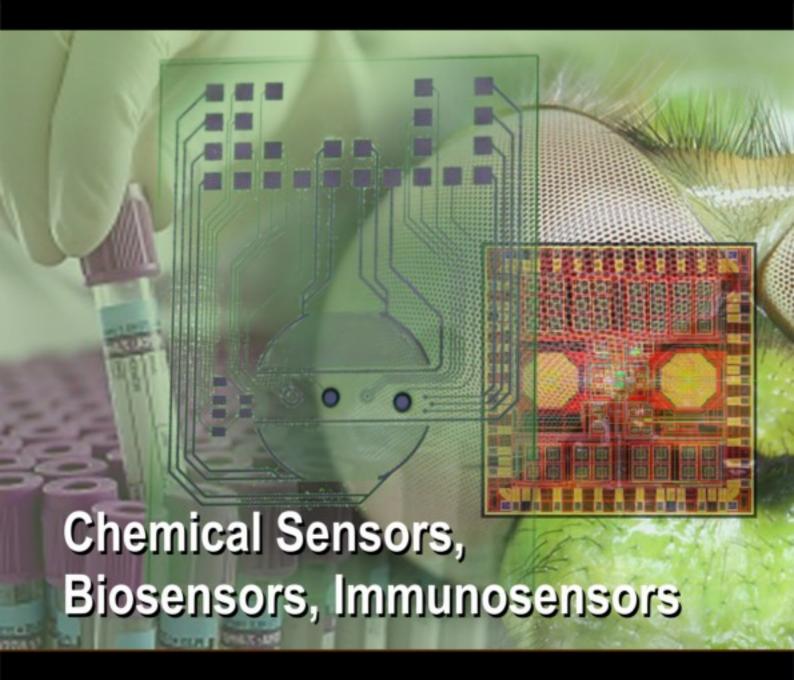
SENSORS 2/1 TRANSDUCERS







Sensors & Transducers

Volume 113, Issue 2, February 2010

www.sensorsportal.com

ISSN 1726-5479

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ISSN 1726-5479

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Sensors & Transducers

ISSN 1726-5479
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Biosensors and Biochips for Nanomedical Applications: a Review

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Received: 19 January 2010 /Accepted: 19 February 2010 /Published: 26 February 2010

Abstract: In the last few decades, a tremendous growth in the field of nanotechnology has been witnessed and its applications to various disciplines are being continuously explored. In this review article, we focus on a very important and emerging area of nano-bio sensors and biochips, which have prospects of numerous applications to nanomedicine. We will discuss various topics of biosensors and transducers based on quantum dots (QD), porous silicon (PS), and Si-nanoparticles. A short discussion on biochips, along with their classification and applications to microarrays and drug delivery systems, is also presented. Some powerful optical techniques like Fluorescence Resonance Energy Transfer (FRET), and Surface Enhanced Raman Spectroscopy (SERS) that are often deployed in conjunction with biosensors and biochips, as an interface mechanism, are also reviewed. *Copyright* © 2010 IFSA.

Keywords: Biochips, Biosensors, Porous silicon, Quantum dots, Nanoparticles, Nanomedicine

1. Introduction

Recent advances in nanoscience and technology have fueled a complete shift of paradigm in the physical, chemical, material, biological, healthcare, medical, and agricultural sciences, and also in mechanical, electrical, and computer engineering, as a result of attaining control of matter at the molecular or atomic level, i.e., at the single nanometer scale [1-56]. At the nanometer scale, matter is observed to exhibit numerous unique properties and phenomena, fundamentally different from their macroscopic counterparts. These properties can be harnessed to create new materials for novel applications such as biosensors or drug delivery systems, or to design new types of circuits and

computers [26] using quantum dots or single-electron transistors, which are 10,000 times smaller than the current ones. Colloidal gold, iron oxide crystal, and semiconductor quantum dots having sizes 1-20 *nm*, have showed unique diagnostic applications in biology, medicine, and agri-biotechnology [7].

The domain of nanotechnology encompasses a very important area called "nanomedicine", which is concerned with the development of minimally invasive and targeted delivery of diagnostic, pharmaceutical and therapeutic agents to various body organs, tissues, and cells in a controlled manner [7]. Research in nanomedicine includes three important aspects: (i) nanosensors, (ii) nanofluidics and (iii) lab-on-a-chip. Nanosensors consist of nanostructured particles, or nanoparticles, or nanodevices that respond to physical, chemical, or biological stimuli. Nanofluidics is concerned with navigation, mixing, and controlled delivery of nanoliter volume of fluids through microchannels on a chip [9]. Lab-on-a-chip integrates sensors, fluidics, optics and electronics on a silicon chip to be used as a biochip for drug delivery systems or for biochemical diagnostics or DNA detection [9, 42-45, 86-88, 119, 120].

During the early eighties, powerful microscopes were invented, which could scan the surface of the specimen using physical probes to produce images of the surface at the nanometer region. Several simultaneous interactions can also be imaged with these microscopes. Some microscopes used electron beams for studying surface topography, compositions and also other properties of a sample. These powerful microscopes like Atomic Force Microscope (AFM), Scanning Electron Microscope (SEM), Tunneling Electron Microscope (TEM), etc., helped not only to understand and study materials at the nanoscale region, but also to pick them up and move them around to form basic nanostructures, allowing some materials to be built molecule by molecule.

For the past several years, various materials have been developed whose dimension lies in the nanoscale region, such as, inorganic nanocrystals or quantum dots (QD), nanoparticles, nanocomposites, different nanostructured materials and many other for sensor applications. In the nano region, materials nomenclatures, i.e. wire, dots, quantum well etc., are defined according to the size of the crystals and the way the atoms are arranged in it. Nanostructured materials show interesting optical, electronic and catalytic properties.

Among the nanostructured materials, porous silicon (PS) shows very amazing features like biocompatibility, biodegradable, electroluminescence (EL), and photoluminescence (PL) at room temperature. It also shows non-toxic behavior when applied to human body. These unique properties of PS make it particularly suitable for biosensor application and also as a drug delivery material for *in vivo* applications. Sailor and his group reported a review on PS where it has been used as a drug delivery material [84]. Nanostructured porous silicon (PS) is also used to create optical biosensors, DNA detection sensors and photodetectors. A review on the scope of PS in nanotechnology has been reported by C.A. Betty [81]. As PS is fabricated from Si wafers, its production cost is considerably lower and can be easily integrated with electronic equipments to produce a link between CMOS technology and photonic devices to create smart sensors and biochips.

From the inorganic nanocrystal category, the luminescent semiconductor nanocrystals or quantum dots (QDs) appears to be very promising material in the biosensor industry. QDs generated a huge interest in the biosensing industry due to their excellent fluorescent properties which may help in eliminating the problems faced during the use of conventional organic or protein based fluorophores.

Luminescent porous silicon nanoparticles were also synthesized and studied as a replacement of fluorescent dyes and for *in vivo* applications as a drug delivery system.

In this review, we will discuss how QDs, PS, and Si-nanoparticles, can be used as biosensors and transducers or as drug delivery materials in biomedical studies.

2. Nanobiosensors

A biosensor is a device that *detects*, *transmits* and *records* the information regarding an analyte that combines a biological component with a physiochemical detection system. A nanosensor is a biosensor which acts on the nano-scale region. There are different types of nanobiosensors – optical biosensors, electrical biosensors, electrochemical biosensors, nanowire biosensors, nanotube based biosensors, viral nano biosensors and nanoshell biosensors.

Fig. 1 [118] shows a basic biosensor assembly, which includes a bioreceptor, i.e., a biological recognition element, a transducer and a processor. The biological recognition elements used are living biological systems like, cells, tissues, or whole organism and biological molecular species such us antibody, enzyme, protein etc. The transducer essentially acts like a translator which recognizes the biological or chemical event from the biological component and transforms it into another signal for interpretation by the processor, which then converts it into a measurable output.

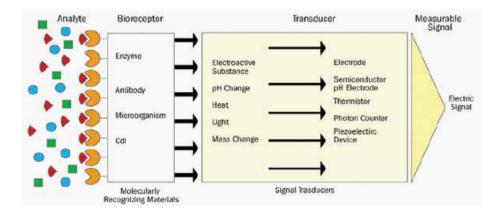


Fig. 1. Components of a biosensor [Source: Ref. 118].

The transducers may have different forms depending on the type of parameters being measured. These are (a) Amperometric transducers [76]; (b) Potentiometric transducers [77]; (c) Piezoelectric transducers [78]; (d) Thermal transducers [79]; (e) Optical transducers [80]. Transducers act as an interface, which measures the physical changes occurred at the bioreceptor [102], and transforms that energy into a readable output.

Nanobiosensors have wide applications in the field of biology and environment. Among the biological applications, there are (a) DNA sensors: genetic monitoring of diseases, (b) Immunosensors: HIV, hepatitis, other viral disease, drug testing, environmental monitoring, (c) Cell-based sensors: functional sensors, drug testing, (d) Point-of-care sensors: blood, urine, electrolytes, gases, steroids, drugs, hormones, proteins and others, (e) Bacteria (*E-coli*, streptococcus, other) sensors: food industry, medicine, environmental and others, (f) Enzyme sensors: diabetics, drug testing, and others are important. The environmental applications are (a) Detection of environmental pollution and toxicity, (b) Agricultural monitoring, (c) Ground water screening, (d) Ocean monitoring.

Nanosensors can be used to measure biotargets in a living cell without affecting its viability in a major way [44]. The effect of nanotechnology is enormous on the sensor industry because most chemical, biological and even physical sensors depend on the interactions occurring at the nanoscale level. The

improvements required in sensor designing is low cost, small size, lower weight, greater sensitivity and better specificity all of which may be achieved through nanotechnology.

3. Types of Biosensors

3.1. Quantum Dots Biosensors

Quantum dots (QD) appears to be the most promising functional and reproducible nanostructures available in the nanotechnology research circle. Quantum dots are very small and the smallest objects that can be synthesized on the nanoscale. Its structure is like a small dot which suggests its name. Different kinds of quantum dots can be synthesized in the laboratory through different techniques and the shapes normally appear to be pyramids, cylinders, lens shapes, and spheres. It confines electrons in three dimensions. The total diameter of a quantum dot varies between 3-60 nm depending on its application. OD exhibits quantum confinement properties in all three dimensions, i.e., the electrons are not allowed to move freely around in any direction. As its behavior is similar to atoms, it is also called an 'artificial atom'. This has a lot of important consequences for researchers. First of all, they exhibit quantized energy levels like an atom. For a particular incident radiation, for instance, a quantum dot will only emit certain specific spectra of light. The quantum theory also predicts that with the decreasing diameters of quantum dots, there will be a corresponding increase in energy of emitted light. From the solution of Schrödinger equation for an electron confined in an 1-dimentional box of length L, the energy difference between two successive levels E_n and E_{n+1} can be given as, $\Delta E = (2n+1) \{h^2/8mL^2\}$. This equation shows that if the length of the box decreases the energy difference between the levels increases and for $L \to \infty$ (ΔE will be 0, i.e., the electrons are delocalized and there is no quantization). This particular emission property of QDs has huge applications in diagnostics. Quantum dots are already in use as markers that are inserted into patients' body. These markers can be seen under medical scanners helping detection of biological processes as they occur.

Quantum dots can be fabricated with either top-down or bottom-up techniques. Top down techniques are very effective for generating a uniform distribution of diameters. This is important if it is desirable to create a large array of dots that will emit the same wavelength of light. The top down approaches like lithography are diffraction limited and cannot create dense networks of quantum dots. This approach inherently implies material damage and many quantum dots produced with these techniques have defects that reduce their effectiveness.

The commonly used methods for producing quantum dots are bottom-up approaches. This can be done either with chemical vapor deposition or molecular beam epitaxy on a highly mismatched substrate. By layering a desired material that does not fit properly with the lattice of the substrate, high strain occurs at the interface and that layer will start nucleating into small quantum dots. Bottom-up approaches are acceptable ways to create quantum dots in dense arrays that will self-assemble in an orderly manner. However, the uniformity of their size distribution is not as precise as that produced through top-down approach mainly because it's impossible to control their formation as strictly.

From early eighties, quantum dots are being deployed in nano-scale computing applications, where light is used to process information. However, this technology is now being used in medicine. The QD crystals are one ten-millionth of an inch in size and can be dissolved in water, which when illuminated, act as molecule-sized LEDs, and can be used as probes to track antibodies, viruses, proteins, or DNA within the human body. Biomolecules labeled with luminescent colloidal semiconductor quantum dots (QDs) have various applications to fluoro-immunoassays and biological imaging. Because of their small size, quantum dots can be used to visualize, measure, and track individual molecular events using fluorescence techniques, as they have the ability to visualize and track dynamic molecular

processes over long time scales, which is a unique property. A review on QD biosensors was reported earlier by Sapsford et al. [93].

It has been found that single QD incorporated nanosensors for DNA detection can reduce significantly or even eliminate the complication of background fluorescence encountered by conventional molecular fluorescence resonance energy transfer (FRET) technique [35]. Zhang, et al. [35] reported the extraordinary performance characteristics of a QD-FRET nanosensor for DNA detection with ultrahigh sensitivity, discrimination capacity and great simplicity. Fig. 2 shows how FRET induced QD DNA nanosensor works. Tran, et al. [38] described in their paper how CdSe-ZnS QDs can be designed and used as nanosensors for detection both in water soluble and in solid phase conditions using Förster energy transfer method.

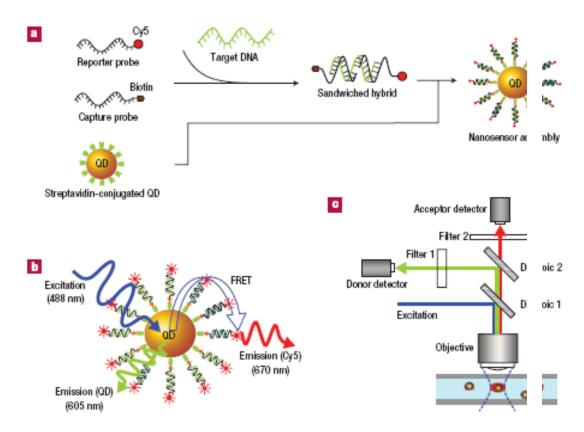


Fig. 2. Schematic of single QD-based DNA nanosensors: a) Conceptual scheme showing the formation of a nanosensor assembly in the presence of targets; b) Fluorescence emission from Cy5 on illumination on QD caused by FRET between Cy5 acceptors and QD donor in a nanosensor assembly; c) Experimental setup [Source: Ref. 35].

Quantum dot technology presents a promising tool in neuroscience research [104]. Several researchers are trying hard to produce new quantum-dot-based tools for applications to neurobiology. Triller, et al. [98] used antibody functionalized quantum dots to study diffusion of glycine receptors in cultures of primary spinal cord neurons. Vu et al. [99] tagged nerve growth factor to quantum dots and used them to promote neuronal-like differentiation in cultured pheochromocytoma 12 (PC12) cells. This method could be used to visualize and track functional responses in neurons. A technique of producing biocompatible water-soluble quantum dot micelles that retain the optical properties of individual quantum dots, was developed by Brinker, et al. [100]. Ting, et al. [101] developed a modified quantum dot labeling approach that presented the relatively large size of antibody—quantum-dot conjugates and the instability of some quantum-dot—ligand interactions. The problems with semiconductor QDs are its toxic effects, which prevent it from being used in-vivo applications.

3.2. Porous Silicon Biosensors

Porous silicon (PS) and Si-nanocrystal have amazing properties that are particularly suitable for applications [57, 59] in the biosensor industry. Both PS and Si-nanocrystals have potential applications to optical biosensors, DNA detection sensors, or photodetectors [32, 46-51]. Sensors based on PS offer enhanced sensitivity, reduced power demands and low cost. A review article explaining various applications of PS as a transducer material has been reported recently by Andrew Jane, et al. [95]. The interesting features about PS are its high surface area and reactive surface chemistry. Si-nanocrystals can also be obtained from PS [54-55] in aqueous form.

Porous Silicon is an electrochemically derived nanostructured material consisting of nanometer-sized silicon regions surrounded by empty space, and can be prepared as quantum wires or quantum dots. The quantum confinement of Si atoms in PS leads to interesting optical, chemical, and electronic properties. The visible room temperature photoluminescence (PL) and the electroluminescence properties of PS, along with the simplicity of its fabrication process, make it extremely convenient and useful material for several opto-electronic and sensor applications. The wavelength of the photoluminescent light can be changed by simply increasing or decreasing the porosity of the material. For example, a highly porous sample (70-80% porosity) will emit green/blue light while a less porous sample (40%) will emit red light. The most acceptable theory about this photoluminescence (PL) property is the quantum confinement effect where by confining the matter in the nanoscale dimension, the interaction between matter and light can be limited in nano dimension (as described in Section 3.1).

PS can be divided into three main categories based on their pore size: 1) for microporous porous silicon the pore width is less than 2 nm, 2) for mesoporous the pore width is in between 2nm to 50 nm, 3) for macroporous the pore width is greater than 50 nm. With appropriate modification of the electrochemical process, PS can also be fabricated to behave as 1-D photonic crystals [58]. The intensity and wavelength of the reflected light is determined by the nanostructure, and these optical properties can be deployed in sensing of chemical and biological agents like viruses and bacteria [34]. Because of their non-invasive and non-radioactive nature, they promise versatile applications to medical diagnostics, pathogen detection, gene identification, and DNA sequencing [12-13, 39].

The standard procedure for fabrication of nanostructured porous silicon is the electrochemical etching method in hydrofluoric (HF) acid solution. The etching resulted in a system of disordered pores with nanocrystals remaining in the inter-pore region. The pores propagate primarily in the <100> direction of the crystal. Almost all properties of PS, such as porosity, porous layer thickness, pore size and shape, as well as microstructure, strongly depend on the fabrication conditions. In the case of anodization, these conditions include HF concentration, chemical composition of electrolyte, current density (and potential), wafer type and resistivity, crystallographic orientation, temperature, time, electrolyte stirring, illumination intensity, and wavelength, etc. Thus, a complete control of the fabrication is complicated and all possible parameters should be taken into account. Some of these parameters also depend on each other.

The average diameter of the pores can be tuned from a few nanometers to several micrometers. Tuning the pore diameters and chemically modifying the surface allow developers to control the size and type of molecules adsorbed [60, 111]. The large surface area enables bio-organic molecules to adhere to the surface of the PS [81, 85]. Aqueous HF is suitable for the etching process because the silicon surface is hydrophobic. The porous layer can be made more structurally uniform if an ethanoic solution is used – this increases the wettability of the silicon and allows more surface penetration by the acid. Fig. 3 shows scanning electron microscope (SEM) images of varying n-type doped PS with different etching current densities [106, 107].

Although the electrochemical anodization is commonly used in the fabrication of PS, several other fabrication methods have been introduced. Stain etching method is one of them. The stain films are produced by immersion of Si substrate in HF solutions without any electrical bias [89-91]. This method is even simpler than the previously presented anodization. However, the control of the porosity, layer thickness and pore size of PS is quite limited. In addition, in the stain etching of Si microparticles, it is quite difficult to control the porosification of particles. Incomplete porosification of Si particles might cause problems in drug delivery applications. Porous silicon fabricated by stain etching method shows low photoluminescence efficiency than the electrochemically etched one.

Nanoporous silicon consists of a complicated network of silicon threads of 2-5 nm thickness with an internal surface area-to-volume ratio of around $500 \ m^2/m^3$. Thus PS can absorb large amounts of foreign molecules onto its surface eventually changing the effective refractive index of the semiconductor porous material. Due to the quantum confinement effect strong luminescence at room temperature is observed from the tiny pores. This photoluminescence (PL) intensity changes when PS is exposed to various chemicals and biological samples and the final photoluminescence efficiency depends on the dipole moment of the molecules attached to the pores. Similarly the effective dielectric constant and the conductivity of PS layer changes when the pores are filled with some other molecule. This property helps in developing electrical and optical PS biosensors adsorbing foreign materials on its surface.

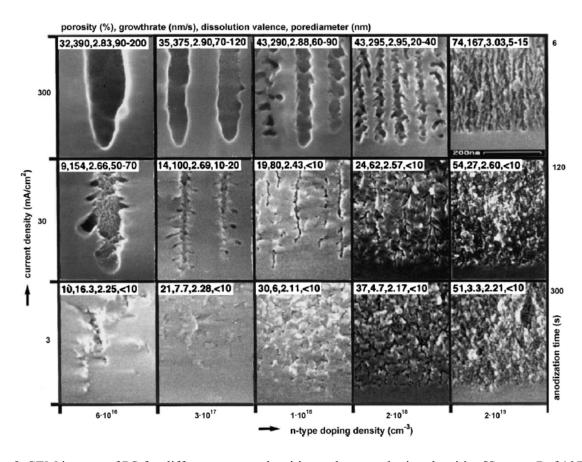


Fig. 3. SEM images of PS for different current densities and *n*-type doping densities [Source: Ref 107].

It has been shown that porous silicon can be used as a base material for passive or active optical devices like Fabry-Perot interferometers, Bragg filters and optical microcavities [82], because of its lower effective refractive index than that of bulk silicon. It can also be used as an antireflection coating for silicon solar cells. A wide range of refractive index varying from 1.25 to 3, allows this material for

many optical application. The PS Fabry-Perot film with two planer and parallel interfaces can produce high contrast optical fringes. Shift in the fringe occur when an analyte binds to the surfaces in the pores, providing a sensitive transduction modality [114]. Multilayer devices, like Bragg filters, can be prepared by periodically varying the current density during the etching process. Such multiplayer structures act as 1-D photonic crystals with reflectivity maxima that depends on the refractive index gradient and the periodicity of the superlattice. Porous silicon 1-D photonic crystals used as a label free optical sensor for detection of bacteria has been reported earlier [103].

The freshly etched PS surface is hydrogen terminated and hydrophobic in nature. Impurities like, carbon and fluorine are also found attached to the surface. The PS surface in unmodified form is unstable for sensor application and also very much fragile. For biosensor applications the PS surface needs to be stabilized. Different surface treatments have been reported to achieve a stable and hydrophilic surface [60, 96-97]. Mild oxidation removes the Si-H bonds which stabilizes and protects the surface. For biological molecules to be attached to the PS surface silanization or hydrosilanization treatment is to be done [94, 96-97].

The enormous medical application of silicon was recognized very recently. Researchers investigate PS material as a transducer in sensing systems [67, 68] because of its physical and structural properties. High sensitivity results have been obtained using PS by monitoring changes in optical properties, such as photoluminescence [69-70] and ellipsometry [71]. The special features of PS material which led to its applications in the sensor industry are large surface area within a small volume, controllable pore sizes, convenient surface chemistry and compatibility with conventional silicon microfabrication technologies [72]. Scientists used these properties to develop PS sensors to detect toxic gases, volatile organic compounds, explosives, DNA and proteins. Porous Silicon is a well known material for sensing layers in different gas and humidity sensors. It shows great effectiveness when combined with titanium, ceramics, composites, polymers and other materials, which are mainly used for biological implants.

The PS optical biosensors normally measure the change in the average refractive index of the device when a bioconjugation event takes place [67], because the immobilization of the probe and the target biological sample changes the effective refractive index of the PS surface, thus modifying the interference pattern on the output. In the case of label free optical biosensors, the biological probe is attached with a signaling material, which automatically transduces the hybridization effect into an optical signal. The label free optical detection of single strand of DNA (ssDNA) and its complementary (cDNA) conjugation is carried out on the PS chip by comparing the signals taken after the surface modification, then after probe immobilization on the chip surface and finally after its hybridization with the cDNA. In each step of the chip preparation, the optical path length changes which is recorded in the reflectivity spectrum [68-69]. Vicky Vamvakaki, et al. [113] developed PS DNA sensors, which can be used for label-free detection of oligonucleotides in DNA microarrays and microfabricated PS field effect sensors. Francia et al. [109] reported photoluminescence measurements for label-free optical porous silicon DNA sensors.

Singh, et al. [112] in their work, showed how PS films with good mechanical and optical properties can be effectively used for the biofunctionalization purpose for its possible application in immunosensors.

Measurement techniques of molecular binding interactions have been patented by Rauh-Adelmann and his coworkers [117], where ligands are immobilized within pores of a PS interaction region produced in a Si subtrate, after which analytes suspended in a fluid are flowed over the PS region. A large surface area with easily modified chemistry makes porous silicon an effective transducer for optical and electrical biosensing. Porous Silicon optical biosensors sensitivity and performance depend strongly on their nanomorphology and calculated as a function of the pore size [66].

Due to its biocompatibility and biodegradability properties, PS can be injected inside the body which over time releases it without any harm. Current research targets on how to find out the possible applications of PS as a biodegradable material in the field of medicine, for slow release of drugs or essential trace elements for *in vivo* applications [84]. PS can be used to treat everything from broken bones to cancer. Label –free optical biosensor using PS for detection of immunoglobulin G (IgG) in serum and whole blood sample were also reported [105]. Salonel and his co-workers [116] studied the effect of size reduction of PS particles from micro to nanosize, which affects in vitro cytotoxicity and biochemical mechanism of toxicity when these PS particles are applied to human cells. According to their findings, this cytotoxicity depends on the particle size and also on the surface chemistry of the PS particles.

Porous silicon has potential of several nanomedical applications, particularly, as a biomaterial in cancer detection because of its property of reflectivity and its resistance to stomach acid. Reflectivity of PS increases in the presence of cancer related chemicals in the blood, which indicate possible growth of tumors in the body. A silicon capsule containing the required drug can thus be directly administered orally to reach colon through the stomach without biodegradation therein [110].

3.3. Silicon Nanoparticle Sensors

To prepare Si nanoparticles, first PS is obtained by electrochemical etching of single-crystal silicon wafers in ethanolic HF solution. This PS layer was then lifted off and ultrasonicated to get silicon nanocrystals. A silicon oxide layer then grows on these nano crystals. These crystals, in aqueous solution, generate visible luminescence at room temperature due to quantum confinement effect.

In the case of medical or biological imaging, dyes are used as markers, which are not photostable. The dyes can break down under photoexcitation or visible light or at higher temperatures. The amazing property of visible room temperature luminescence of PS created an interest among the scientists for synthesizing and characterizing silicon nanoparticles. In addition to its luminescence property, PS is biocompatible and stable against photobleaching. These properties are ideal for replacing fluorescent dyes with silicon nanoparticles. Silicon nanoparticles can even replace highly toxic cadmium quantum dots for *in vivo* applications [92]. For biomedical applications, it is essential that they have high stability, a substantial photoluminescence quantum yield in the visible region, and solubility in aqueous media. Nanomaterials that can circulate inside the body, have great advantage for disease diagnosis and treatment. These nanomaterials ought to be harmlessly eliminated from the body shortly after they carry out their diagnostic or therapeutic functions.

Nanoparticle-based sensors and drug delivery systems have considerable potential for various types of medical treatment. The important technological advantages of nanoparticles used as drug carriers are high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration, including oral application and inhalation. Nanoparticles can also be designed to allow controlled (sustained) drug release [92] from the matrix. These properties of nanoparticles enable improvement of drug bioavailability and reduction of the dosing frequency, and may resolve the problem of nonadherence to prescribed therapy.

Despite efforts to improve their targeting efficiency, significant quantities of systematically administered nanomaterials are cleared by the mononuclear phagocytic system before finding their targets, increasing the likelihood of unintended acute or chronic toxicity. However, there has been little effort to engineer for self-destruction of errant nanoparticles into non-toxic, systematically eliminated products. M. J. Sailor and his group [92] showed that luminescent porous silicon nanoparticles (LPSiNPs) producing near infrared luminescence can be used as drug payload for *in vivo* monitoring.

The most interesting property manifested by these particles is, when tested on mouse, they self-destruct and are cleared from the body within a short period of time without producing any toxic effect. Their work presents a new type of multifunctional nanostructure for *in vivo* applications with low toxicity. Other uses of silicon nanoporous particles as effective carriers for *in vivo* simultaneous application for different nanotherapeutics have been reported recently [116].

4. Biochips or Lab-on-a-Chips

A biochip or a lab-on-a chip can be broadly classified as three categories: (a) microarray-based chips for genomic analysis, (b) microfluidic-based chips for executing biochemical assays, and (c) *in vitro* chips as drug reservoirs and/or monitoring purposes. A microarray contains large number of miniaturized test sites, which can perform thousands of biochemical reactions instantaneously, such as decoding genes, DNA/protein analysis, etc., in a few seconds. The microfluidic-based biochips are widely used for on-chip implementation of several biochemical laboratory assays, for sample preparation, dilution and mixing [119, 120]. These chips use only nanoliter volumes of fluids and thus offer the advantages of low sample and reagent consumption, high throughput and sensitivity, and minimal intervention. The fluidic operations can be performed on-chip either in a continuous fashion (continuous-flow microfluidic chips), or in a discrete fashion (digital microfluidic biochips). Their applications include clinical diagnostics, enzymatic analysis, e.g., glucose and lactate assays, DNA analysis, immunoasays, and environmental toxicity monitoring. The third type of biochips are those, which can be implanted inside the human body or administered orally, for drug release or for controlling/monitoring some biological functioning, *in vivo*. All these biochips need several types of sophisticated optical and electronic sensors as interface.

In microarray type of chips, the term "gene expression" is used to describe the transcription of the information contained within the DNA, into messenger RNA (mRNA) molecules that are then translated into the proteins that perform most of the critical functions of the cells. In our body, all genes are not "expressed" in the same cell, though almost all cells contain the same gene. Many genes represent unique features to a particular type of cell. For example, liver cells express genes for enzymes that detoxify poisons, while pancreas cells express genes for making insulin. Scientists are working in these areas to identify which genes are expressed by each type of cells.

In a microarray, mRNA molecules bind specifically to a complementary DNA, to hybridize and to form a double helix structure. By using an array containing many DNA samples, scientists can determine, in a single experiment, the expression levels of hundreds or thousands of genes within a cell by measuring the amount of mRNA bound to each site on the microarray. The amount of mRNA bound to the spots on the microarray is precisely measured by a microprocessor attached to it, generating a profile of gene expression in the cell.

In a microarray, nucleic acid sensing is done by immobilizing single stranded oligonucleotide (5 to 50 nucleotides long) probe onto transducer surface forming a recognition layer that binds its complementary (target) DNA sequence to form a hybrid for the purpose of expression profiling, monitoring expression levels for thousands of genes simultaneously or for comparative genomic hybridization. The hybridization reaction means coupling of any four different nucleotides, adenine (A), thymine (T), guanine (G), and cytosine (C) with its complementary one e.g. the complementary sequence of G-T-C-C-T-A is C-A-G-G-A-T. Fig. 4 [121] shows how a hybridization reaction takes place. This process of hybridization helps in identifying diseases, where fluorescently labeled nucleic acid molecules are used as mobile probes to identify the complementary molecular sequences that are able to base-pair with one another.

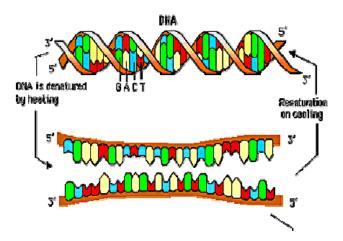


Fig. 4. Schematic diagram of hybridization reaction [Source: Ref. 121].

A review article on protein based lab-on-a-chip sensors reported by Borini et al. [65], describes different approaches for fabrication of biochips with PS and their future perspectives. Hu Lingang et al. [73] in their paper, reported a new technique for fabrication of a biochip on porous silicon and its application for detection of small molecule—protein interactions with desorption/ionization on PS (DIOS) [74]. Other applications of PS for building DNA sensors have also been reported earlier [108].

One of the major challenges in nanobiosensor industry is to design efficient sensors and integrating them on a biochip. The information captured by the sensors must be converted into a readable form [32]. Several optical, electrical, chemical, and biological data from the nanosensors are to be transformed into signals for processing, analysis, and for deciding actions. Thus, integration of sensing and controlling probes with the fluidic and the electronic world remains the main challenge while designing a multi-function biochip.

5. Optical Detection Techniques

For functioning of biochips, one of the most important criteria to be fulfilled is to attach a powerful transduction or signal processing unit to the system, which can directly and accurately detect the biological event and convert it in to a human readable output. The biological events like, antibody/ DNA binding, oxidation/reduction, etc., need to be transduced into a format understandable by a computer (voltage, light, intensity, mass, etc.), for analyzing and processing the signal to produce the final output. Several optical techniques are commonly used for detecting and quantifying biomolecules [62-63] as one does not require electrical contacts with the system for capturing data with optical devices. Instead, one can use fluorescent tags either with the probe or with the analyte to detect any change in the system. The major advantages of the optical transduction methods are that the devices are small, lightweight and portable due to the integrability of all optical components.

Several optical transduction methods such as FRET (fluorescence resonance energy transfer), SERS (surface enhanced Raman spectroscopy), and fluorescence spectroscopy are used for detection of biological samples. Spectroscopic techniques are used, for detecting biological samples or events occurring in it because cells or tissues can absorb or emit light, thereby producing a signal or spectrum, which is a characteristic of that particular event. From this fingerprint spectrum, one can directly identify or quantify the sample or the event.

Fluorescence Resonance Energy Transfer (FRET) is a nonradiative energy transfer process from excited state donor molecule to an acceptor molecule, when appreciable overlap exists between the emission spectrum of the donor and the absorption spectrum of the acceptor. This radiation-less transfer of energy, when the excited state fluorophore and the second chromophore lie within a range of approximately 10 nm, provides vivid structural information about the donor-acceptor pair. This is a quantum mechanical process that does not require a collision and does not involve production of heat. When energy transfer occurs, the acceptor molecule quenches the donor molecule fluorescence, and if the acceptor is itself a fluorochrome, increased or sensitized fluorescence emission is observed [35, 36, 38]. Fig. 5 shows the underlying principle of FRET. The information obtained by this method is unique because the surrounding solvent shell of a fluorophore does not affect the FRET measurements.

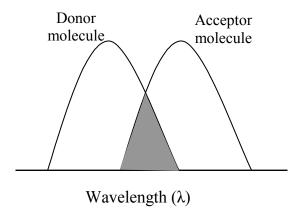


Fig. 5. Fluorescence resonance energy transfer.

The Surface Enhanced Raman Spectroscopy or Surface Enhanced Raman Scattering (SERS) is a surface sensitive technique that results in the enhancement of Raman scattering by molecules adsorbed on rough metal surfaces. The vibrational modes of the adsorbates on the roughened surface are sometimes observed to have about one million times the intensity that would be predicted by comparison with their Raman spectra in the gaseous phase [39].

Fluorescent measurement techniques are commonly used for the detection of biomolecules. In fluorescence spectroscopy, fixed or living cells or single stranded DNA probes are often labeled with fluorescent tags or fluorophores, each specific to a particular intercellular component, which absorps light at one wavelength (excitation), followed by a subsequent emission of secondary fluorescence at a longer wavelength. The excitation and emission wavelengths are usually separated from each other by tens to hundreds of nanometers.

Cellular components are labeled with specific fluorophores to identify their localization within fixed and living parameters. In microarray systems, the target molecules are labeled, which is a process of covalently binding a molecule or particle to the target DNA strand, for generating transducer signal. This approach takes care of the issue related to safety and disposal associated with radioactive markers and allows the researchers to study several experimental parameters simultaneously with multiplex samples. In the case of multiple probes, different dyes are attached to different probes which can be simultaneously detected at different wavelengths using optical filters. After hybridization, the fluorescent signals from a DNA chip are studied using specific instruments. However, this method of labeling with fluorophores is not possible everywhere [64] because the optical labels are costly and unreliable and also the optical scanners are expensive and the procedure of extracting information from the data is complicated [70]. Researchers are trying to work it out with label free techniques or reagent-less optical biosensors where the target sample can be detected in a heterogeneous solution without adding anything but the sample [96].

6. Conclusion

In this review article, attempts have been made to highlight how nanomaterials or nanostructured materials like PS, Si nanoparticles, and QDs are being used as highly efficient biosensors for several biomedical applications. Inorganic nanocrystals or QDs have been used in nano scale computing for the past several years. These crystals dissolve in water and fluoresce when exposed to light radiation, and are usable as markers or trackers in biological recognition events. However, these QDs have toxic effects and therefore, are unsuitable for *in vivo* applications. In contrast, PS and Si nanoparticles are non-toxic and are easily removed from the body without leaving any trace of harmful effect. They are very effective as a biosensor/transducer material and because of their luminescence properties and strong biocompatibility, they have wide applications in drug delivery systems and in the design of biomedical implants. Other research endeavors aiming at producing low cost sensor material are currently being explored.

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