

Nanosensors and their Pharmaceutical Applications: A Review

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ABSTRACT

Nanotechnology research is rolling worldwide, having an impact on multiple sectors and with a general belief that medical and biological applications will form the greatest area of expansion over the next decade. This field is mainly driven by an endeavour to bring radical solutions to areas of medical need, not fulfilled presently. This article discusses the basic concepts and developments in the field of nanosensors and their applications in pharmaceutical and medicine fields.

Various types of nanosensors including optical nanosensors, electrochemical nanosensors, chemical nanosensors, electrometers, biosensors, and deployable nanosensors are described. It describes the progression of this field of research from its birth up to the present, with emphasis on the techniques of sensor construction and their application to biomedical systems.

KEYWORDS: Nanosensors; Optical nanosensors; Electrochemical nanosensors; Chemical nanosensors; Electrometers, Biosensors.

Introduction

Nanotechnology stands to produce significant scientific and technological advances in diverse fields including medicine and physiology. It can be defined as the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale. A nanometer is one billionth of a meter or three orders of magnitude smaller than a micron (Palit and Datta, 2010).

For applications to medicine and physiology, these materials and devices can be designed to interact with cells and tissues at a molecular (i.e., subcellular) level with a high functional specificity, allowing a degree of integration between technology and biological systems which was not attained previously (Urban, 2009).

There are many advances in nanotechnology that if perfected could help make our world a safer and ultimately a better place to call home. The industry surrounding sensors is no exception. Nanosensors have been under research by many institutions for as long as ten years. A nanosensor is a sensor built on the atomic scale based in measurements of nanometres. There have been a number of advances in the research and development of nanosensors for a number of different applications. Some of the major applications are the medical field, national security, aerospace, integrated circuits, and many more. Along with many different applications for nanosensors, there are also many

different types of nanosensors, and a number of ways to manufacture them. There are a number of challenges currently with the production of these nanosensors, however, when they become perfected for regular use they will have a number of advantages over the sensors that are used in today's technology. A nanosensor's purpose is mainly to obtain data on the atomic scale and transfer it into data that can be easily analyzed. These sensors can also be defined as "A chemical or physical sensor constructed using nanoscale components, usually microscopic or submicroscopic in size." These sensors are very sensitive and can detect single virus particles or even very low concentrations of a substance that could be potentially harmful (Riu et al., 2006).

Structure and Mechanism of Action

A nanosensor generally consists of a biosensitive layer that can either contain biological recognition elements or be made of biological recognition elements covalently attached to the transducer. The interaction between the target analyte and the bioreceptor is designed to produce physicochemical changes that can be converted into a measurable effect such as an electrical signal (Colombo et al., 2009). Bioreceptors are important and provide specificity for biosensor technologies. They allow for binding of the specific analyte of interest to the sensor for the measurement with minimum interference from other components in sampling mixtures. Biological sensing elements can be either a biological molecular

species (eg, an antibody, an enzyme, a protein, or a nucleic acid) or a living biological system (eg, cells, tissue, or whole organisms) that uses a biochemical mechanism for recognition (Rouhanizadeh et al., 2006). Various readout techniques optical, electrochemical, or mass-sensitive can be used for detection in biosensors.

Two groups of receptor molecules form the majority of nanosensors: affinity-based and catalytic-based nanosensors. Affinity based nanosensors are used to bind molecular species of interest, irreversibly and non-catalytically. Examples include antibodies, nucleic acids, and hormone receptors. On the other hand, catalytic-based sensors such as enzymes and microbiological cells recognize and bind a molecule of interest, followed by a catalyzed chemical conversion of that molecule to a product that is then detected (Devreese, 2007).

Basically it operates by charge transfer which occurs between molecules and a sensitive material, resulting in an electrical and/or optical signal that is related to the molecules type and number (Yonzon et al., 2005).

Types of Nanosensors

Nanosensors can be classified depending upon structure and applications:

1. Classification based on Structure

Nanosensors are typically classified either by the bioreceptor employed for molecular recognition, or the type of transduction mechanism used for detection.

Optical nanosensors: Optical measurement includes Absorption/ Fluorescence/ Phosphorescence/ Raman/ Dispersion/ Refraction/ Interference spectroscopy etc. The properties measured include amplitude, energy, polarization, decay time and/or decay phase (Fehr et al., 2002; Kim et al., 2009);).

Electrochemical nanosensors: Electrochemical transduction mechanisms are amperometric, potentiometric, etc; whereas mass transduction includes surface acoustic wave, microbalance, microcantilever (Francla et al., 2009).

2. Classification based on Applications

Chemical nanosensors: The chemical sensor uses capacitive readout cantilevers and electronics in order to analyze the signal. This type of sensor is sensitive enough to detect a single chemical or biological molecule. Several different optical chemical nanosensors have been reported for the measurement of properties such as pH, various ion concentrations, and other entities (Gopel, 1996).

Previously opto-chemical nanosensors were used for measurements inside biological samples on rat embryos. In the experiment, pH Nanosensors were inserted into the extra-embryonic space of a rat conceptus, with minimal damage to the surrounding visceral yolk sac, and pH measurements were obtained. Values of the pH in rat conceptuses 10–12 days old were then compared. In addition to pH measurements, indirect measurements were also performed of nitrite and chloride levels in the yolk sac of rat conceptuses. Such minimally invasive

techniques seize great promise for biological measurements and could help to improve our understanding of the effect that environmental factors play in the growth of embryos (Modi et al., 2003). Opto-chemical nanosensors have been used for the measurement of Na⁺ concentrations in the cytoplasmic space inside a single mouse oocyte, one of the largest mammalian cells (~100 μm in diameter). The relative Na⁺ concentrations were measured while the ion channels were opened and closed by the external stimulant kainic acid. Measurement of Ca²⁺ concentrations have also been performed in single cells using optochemical nanosensors. Nanosensors were inserted into vascular smooth-muscle cells when the cells were stimulated. Ca²⁺ fluctuations were then measured and correlated with the cellular stimulation (Cusano et al., 2008).

Deployable nanosensors: A different type of sensor is referred to as a deployable nanosensor. These mostly refer to sensors that would be used in the military or other forms of national security. One sensor in particular is the Sniffer STAR, which is a nano-enabled chemical sensor that can be integrated into a micro unmanned aerial vehicle. This sensor is a lightweight, portable chemical detection system that combines a nanomaterial for sample collection and a concentration with a micro-electromechanical (MEM) based “chemical lab-on-a-chip” detector. An image of this sensor is shown to the left both in circuit form and in a field demonstration. This would likely be used in homeland security and during times of war in which it could easily detect chemicals in the air without risking human lives by sending it up in the air instead (Yonzon et al., 2005).

Electrometers: Another type of nanosensor is the electrometer, which is a nanometer-scale mechanical electrometer that consists of a torsional mechanical resonator, a detection electrode, and a gate electrode, which are used to couple charge to the mechanical element.

Biosensors: One of the most largely funded areas of research in nanosensors is biosensors. This is mostly due to the possibilities that this technology could lead to the early cancer detection and detection of other various diseases. The biosensors can also be used to detect specific types of DNA. The sensors are created from synthetic polymers called dendrimers and are created layer-by-layer into spheres with a diameter of less than five nanometers. It is because of their small size that the goal of these sensors is to be able to administer them transdermally, through the skin.

Nanofabrication

The experimental procedures involved in the fabrication of nanosensors using the “heat-and-pull” method are schematically shown in Figure 1. The fabrication of reproducible optical nanofibre sensors is critical for their successful development. The two common techniques for fabricating the nanofibre tips have been used (Zheng et al., 2004).

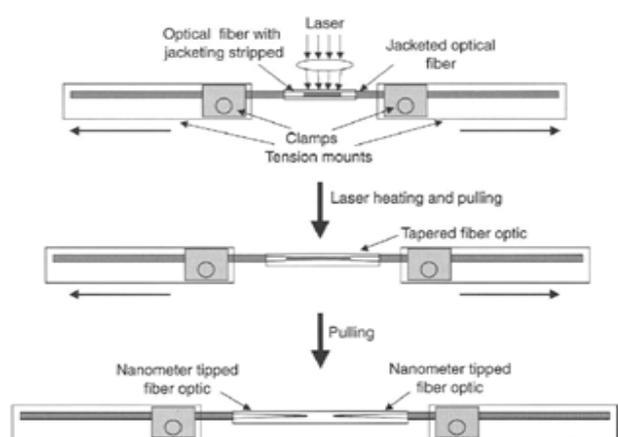


Fig. 1. Nanofabrication of nanosensors.

A widely used technique, the heat-and-pull method, consists of pulling nanotips from a larger diameter (600 μm) silica optical fibre using a special fibre-pulling device. This procedure involves local heating of a glass fibre using a CO_2 laser or a heat filament and subsequently pulling the fibre apart. The resulting tip shapes depend mainly on experimental parameters, such as the temperature and the timing of the procedure. One end of a 600- μm silica/silica fibre is polished to a 0.3- μm finish using an Ultratec fibre polisher to produce an even, flat surface to couple optical fibre delivering laser excitation light. The fibre is then fixed into the fibre-pulling device and the laser heating source is focused onto the median point of the fibre. The optical fibre is then pulled to the breaking point, thus producing two fibres with nanosized tip diameters (Jianrong *et al.*, 2004).

The second fabrication method is based on chemical etching of glass fibres. A variation of the standard etching scheme has been introduced; in this variation the taper is formed inside the polymer cladding of the glass fibres. The next step in the nanosensor fabrication process involves coating the tapered sidewalls of the optical fibre with a thin layer of silver, aluminium, or gold (100–200 nm) using a Cooke Vacuum Evaporator system using a thermal source at 10–6 torr to avoid leakage of the excitation light from the tapered sides of the nanofibre. The coating procedure leaves the distal end of the fibre free from silver for subsequent derivatization to allow covalent immobilization of biological sensing elements to the exposed silica nanotip. The nanofibres are secured after production onto a rotating stage inside the thermal evaporation chamber to ensure a uniform silver coating. The fibre axis and the evaporation direction form an angle of approximately 45° to the normal. While the nanofibre is rotated, the metal is heated and allowed to evaporate onto the tapered sides of the nanofibre tips, forming a thin, uniform, highly reflective coating. Because the fibre tip is pointed away from the metal source, it does not become coated with metal. With the metal coating, the final tip diameter size is adjusted approximately to 150 to 250 nm.

Applications of Nanosensors

Gas Leak Detection

Mass spectrometry is a proven technology that can be used to monitor all necessary gas constituents at the required limits of detection (ppm). However, the size, weight, and power requirements of the mass spectrometers is such that all the monitoring cannot always be done locally. Rather, the monitoring is done remotely by means of gas sample lines. One of the drawbacks of remotely sensing for gases is that gas samples have to be transported to the mass spectrometer through long transport lines (sometimes hundreds of meters long). This means the samples being analyzed could potentially be tens of seconds old by the time they reach the mass spectrometer (Ren *et al.*, 2007). The use of small sensors, with sensitivity and selectivity similar to that achieved by the mass spectrometer, results in a more timely analysis of the gas samples, especially when the sensors are physically placed in the area of interest (Riu *et al.*, 2006).

The use of small and sensitive nanosensors can allow for the distributed placement of devices over a large area, thus allowing for a more precise and timely determination of a gas leak. Nanosensors are being developed for the detection of various hazardous gases, including but not limited to: H_2 , NH_3 , N_2O_4 , hydrazine, and others (Medelius, 2009).

The small size inherent to nanosensors allows for multiple devices, each responsive in a different way to different gases, to be placed on a single substrate. One of the side advantages of embedding multiple devices on a single substrate results in the increased reliability achieved as a result of enabling the use of redundant sensors within the same sensing environment.

Glucose Monitoring

One of the main reasons for developing *in vivo* glucose sensors is the detection of hypoglycemia in people with insulin dependent (type 1) diabetes. It is possible to engineer fluorescent micro/nanoscale devices for glucose sensing. Exploitation of micro/nanoparticles in the dermis may allow transdermal monitoring of glucose changes in interstitial fluid. Nanotechnology of the coated colloids and microcapsules allows precision control over optical, mechanical, and catalytic properties to achieve sensitive response. Non-invasive glucose sensing will maximize patient's acceptance and overcome biocompatibility problems of implants (Kong *et al.*, 2000).

A new type of optical nanosensor, based on single walled carbon nanotube can be used as glucose sensor (Deuschle *et al.*, 2006). It modulates emission in response to the adsorption of specific biomolecules. It works on two distinct mechanisms of signal transduction-fluorescence quenching and charge transfer. It is coated with glucose oxidase, an enzyme that breaks down glucose molecules. Then ferricyanide, an electron-deficient molecule, is sprinkled, onto the nanotube surfaces. Ferricyanide moves electrons from the nanotubes, quenching their capacity to glow when excited by infrared light. When

glucose is present, it reacts with the oxidase, producing hydrogen peroxide. In turn, the hydrogen peroxide reacts with ferricyanide in a way that satisfies the molecule's hunger for electrons. The higher the glucose level, the greater is the infrared fluorescence revealed by the nanotube (Kong et al., 2000). To test the feasibility of implanting the sensors in the body, oxidase and ferricyanide coated nanotubes were placed inside a sealed glass tube a centimeter long and 200 Å thick. The tube is riddled large enough to let glucose enter but small enough to keep the nanotubes inside. The tube is then implanted in a sample of human skin and the sensor can be excited with infrared light to detect fluorescence.

A technique has been reported for detection of biologically relevant glucose concentrations by immobilization of glucose oxidase onto a microcantilever surface. The enzyme-functionalized microcantilever undergoes bending due to a change in surface stress induced by the reaction between glucose in solution and the glucose oxidase immobilized on the surface (Smith and Nagel, 2003).

Asthma Detection

A nano-biosensor that can be used to detect asthma attacks up to three weeks before they happen just by using a handheld device to test the nitric oxide level in the patients breath. Testing regularly, as a diabetic patient would test their blood sugar level, could save lives. By knowing the nitric oxide level in their breath they could be alerted if the level were too high, or were increasing. This would indicate the risk of an asthma attack in the patient. The diagram below shows the most crucial pieces of the nanosensor that would accomplish this. The base of the sensor is a polymer coated Nano Tube Field Effect Transistor (NTFET) containing a random network of single-walled carbon nanotubes between source and drain gold electrodes on a silicon oxide substrate (Tallury et al., 2010).

Astronaut's Diagnosis

Astronauts working in a space vehicle or eventually living in an extra planetary environment, as well as ground-based personnel working within or nearby spacecrafts can potentially be exposed to lethal amounts of hazardous gases. Space vehicles often use hydrazine and similar gases as fuel for some small engines. These gases can be extremely dangerous even in low as tens of parts per billion concentrations. It is therefore important to be able to detect, identify, and quantify the presence of a gas, especially when its existence could result in serious injury or death. The nanosensors are being optimized to detect and quantify the presence of a variety of gases in real time. Another biosensor can pass through membranes and into the white blood cells called lymphocytes, in order to detect early radiation damage or infection in astronauts by sensing signs of biochemical changes. When on space missions, because of the amount of radiation, astronauts are at a higher risk of developing cancer due to cell damage. The sensors are created from synthetic polymers called dendrimers and are created

layer-by-layer into spheres with a diameter of less than five nanometers. It is because of their small size that the goal of these sensors is to be able to administer them transdermally, through the skin. Being able to accomplish this and administer them every few weeks would avoid the need for injections or IVs during space missions. The development of these sensors would also eliminate the need to draw and test blood samples (Flinn, 2005).

Detection of Organophosphorus Compounds

The techniques used can be divided into three main types based on the sensing mechanism: the inhibition of cholinesterases, immunoassays and Organophosphorus hydrolase (OPH). The enzyme inhibition mechanism is that the pesticide, as an inhibitor, can be determined by means of measuring the kinetic performance of the initial velocity of the reaction catalyzed by this enzyme.

AChE-based biosensors have a major drawback: since organophosphorus and carbamic pesticides, heavy metals, and detergents exert strong specific inhibition of AChE. AChE inhibition test is restricted to only a limited number of analytes. Immunosensors based on anti-pesticide antibodies offer selective, sensitive and low-cost tools for pesticide analysis direct immunosensors based on quartz crystal microbalance (QCM), surface plasma resonance (SPR) and impedimetric devices have been reported to detect direct binding of the analyte with the antibody (Liu et al., 2008).

pH Sensing

The first example demonstrating the ability of nanowire field effect devices to detect species in liquid solutions was demonstrated in 2001 for the case of hydrogen ion concentration or pH sensing. A basic p-type Si nanowire device can be used by converting it into such a sensor. It is modified with 3-aminopropyltriethoxysilane instead of the silicon oxide surface, which yields amino groups at the nanowire surface along with the naturally occurring silanol (Si-OH) groups of the oxide, as shown in Fig 2A.

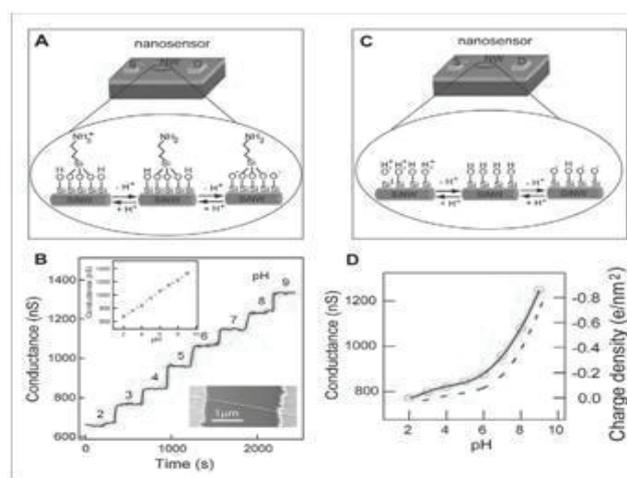


Fig. 2. Nanosensors in pH sensing Adapted and printed from Petolsky and Charles 2005.

The amino and silanol moieties function as receptors for hydrogen ions, which undergo protonation/deprotonation reactions, thereby changing the net nanowire surface charge. Significantly, as illustrated in Fig. B., p-type Si nanowire devices modified exhibits stepwise increases in conductance as the pH of the solution, which is increased stepwise from 2 to 9. The nearly linear increase in conductance with pH results from the presence of two distinct receptor groups that undergo protonation/deprotonation over different pH ranges. From a mechanistic standpoint, the increase in conductance with increasing pH is consistent with either a decrease of the surface positive or increase of the surface negative charge, which 'turns on' the p-type FET via the accumulation of carriers (Petolsky and Charles, 2005).

Surface receptor plays a key role in defining the response of the nanowire sensors which was further tested by probing the pH response without modifying the silicon oxide surface layer.

As illustrated in Fig. 2C, only the silanol group can function as a receptor for hydrogen ions in this case. Measurements of the conductance as a function of pH shown in Fig. 2D exhibit two different responses, unlike nanowire surfaces containing both amino and silanol receptors, where the conductance change is small at low pH (2 to 6) but larger and comparable to Fig. 2B for the high pH range (6 to 9). The pH-dependent changes in conductance are in excellent agreement with previous measurements of the pH-dependent surface charge density derived from silica. This comparison clearly demonstrated that the sensing mechanism was indeed the result of a field effect (Petolsky and Charles, 2005).

Protein and DNA Detection

Biological macromolecules, such as proteins and nucleic acids, are typically charged in aqueous solution and, as such, can be detected readily by nanosensors when appropriate receptors are linked (Fritz *et al.*, 2002). By using p-type Si nanosensor devices in which the molecule biotin, which binds selectively to the protein streptavidin, was linked to the oxide surface of the nanosensor, as illustrated schematically in Fig.3A. When solutions of streptavidin protein are delivered to nanosensor devices modified with biotin receptors, the conductance increases rapidly to a constant value, and that this conductance value is maintained after the addition of pure buffer solution, as shown in Fig. 3B. These results are consistent with the net negative charge on streptavidin at the pH of these experiments (i.e. causing accumulation of carriers in p-type material) and the very small dissociation rate of the streptavidin-biotin system, respectively. The biotin surface receptor plays a key role in the specific detection of streptavidin. For example, addition of a streptavidin solution to an unmodified Si nanosensor does not produce a change in conductance, as shown in Fig. 3C. Blocking the streptavidin binding sites also leads to an absence of response. In addition, this work showed that real-time electrical detection could be carried out up to

concentrations of 10 pM, below the detection level required for a number of disease marker proteins. These experiments show that the binding interaction is highly specific, and that nanosensor devices are ultrasensitive detectors, and thus provided clear sign that this approach could lead to the development of sensor devices of real value (Petolsky and Charles, 2005).

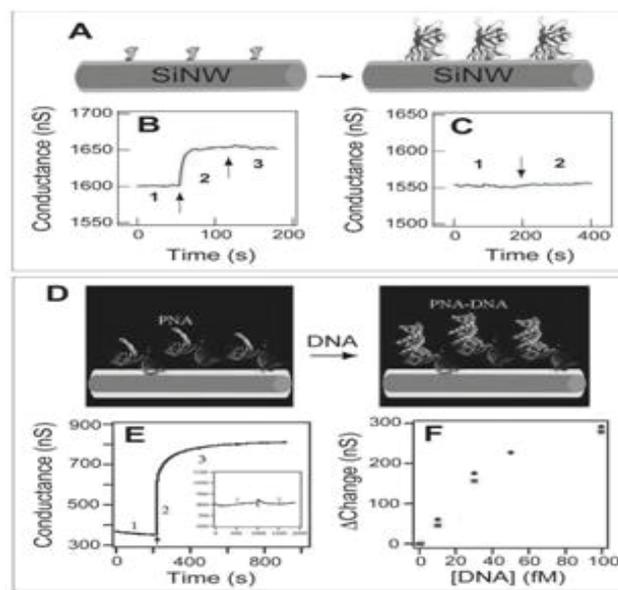


Fig. 3. Protein and DNA detection by nanosensors Adapted and printed from Petolsky and Charles 2005.

More recently, Si nanowire field-effect devices investigated as sensors for the detection of single-stranded DNA. This negatively charged macromolecule binds to p-type nanowire surfaces leading to an increase in conductance. Recognition of the DNA target molecules is carried out with a complementary sequence of single-stranded material, i.e. peptide nucleic acids (PNAs), as illustrated in Fig.3D. PNA was used as a receptor for DNA detection since the uncharged PNA molecule has a greater affinity and stability than corresponding DNA detection sequences. Studies of p-type Si nanosensor devices modified with a PNA receptor designed to recognize wild type versus the DF508 mutation site in the cystic fibrosis transmembrane receptor gene. It showed that the conductance increases following the addition of a 60 fM wild-type DNA sample solution, as shown in Fig. 3E. The increase in conductance for the p-type Si nanowire device is consistent with an increase in the negative surface charge density associated with the binding of negatively charged DNA at the surface. The binding response was specific to the wild-type sequence and the sequence with the DF508 mutation site does not show this stable change in conductance (Fig. E, inset). This sequence specificity is a critical step toward the development of the nanowire devices for genetic-based disease detection. There are several other features of the nanowire DNA sensors that deserve mention (Perez and Weissleder, 2002). First, the studies of the conductance change versus target sequence concentration

demonstrate that direct electrical detection is possible down to at least the 10 fM level, as shown in Fig. F. Second, the DNA detection data obtained from independent Si nanowire devices exhibited very similar changes in conductance with increasing DNA concentration as illustrated in Fig. 3F.

Drug Discovery

Organic molecules that bind specifically to proteins are fundamental to the discovery and development of pharmaceuticals, and thus represent an important target for sensors. A representative example of this area is the identification of molecular inhibitors to tyrosine kinases (Nowak-Lovato and Rector, 2009). Tyrosine Kinases are proteins that mediate signal transduction in mammalian cells through phosphorylation of a tyrosine residue from a substrate protein using adenosine triphosphate (ATP).

Deregulation of the phosphorylation process causes a number of diseases including cancer. To organize nanosensor devices for screening small-molecule inhibitors to tyrosine kinases, the kinase Abl adsorbed on the surface of Si nanosensors and investigated the binding of ATP as well as competitive inhibition of ATP binding with organic molecules, such as the drug Gleevec. Binding or inhibition to the binding of the negatively charged ATP to Abl was detected simply as an increase or decrease in the conductance of the p-type nanosensor device. Time-dependent data recorded from Abl-modified p-type Si nanowire devices exhibited reversible, concentration dependent increase in conductance upon introducing solution containing ATP. The increase in conductance was consistent with the binding of negatively charged ATP to Abl. Plots of the normalized conductance recorded from Abl-modified p-type Si nanosensors devices exhibit reversible decreases in conductance because of competitive inhibition of ATP (Petolsky and Charles, 2005). Notably, the conductance decreases at constant small molecule concentration, which demonstrates that the degree of inhibition depends strongly on molecular structure (Schellenberger, 2010; Akyildiz and Jornet, 2010).

Microorganism Detection

1. Detection of bacteria: The rapid and sensitive detection of pathogenic bacteria is extremely important in medical diagnosis and measures against bioterrorism. Limitations of most of the conventional diagnostic methods are a lack of ultra-sensitivity or delay in getting results. Several nanotechnology based methods have already been described including ferrofluid magnetic nanoparticles and ceramic nanospheres. A bioconjugated nanoparticle based bioassay for *in situ* pathogen quantification can detect a single bacterium within 20 min. The nanoparticle can be easily used in a biorecognition of molecule such as an antibody due to their high fluorescence. One limitation of quantum dot technology is that it gives qualitative information but not provide quantitative information. The nanoparticle-based colorimetric assay, in comparison to a previously reported absorbance-based method, increases detection

sensitivity by over four orders of magnitude. Detection of a small number of *Salmonella* enteric bacteria is achieved due to a change in the surface stress on the silicon nitride cantilever surface *in situ* upon binding of bacteria. Scanning electron micrographs indicate that adsorption of less than 25 organisms may suffice for detection. A nanotechnology-based technique, Sensing of Phage-Triggered Ion Cascade (SEPTIC), uses a nanowell device with two antenna-like electrodes to detect the electric-field fluctuations and then identifies the bacterium (Kaittanis et al., 2010).

2. Detection of viruses: Rapid, selective, and sensitive detection of viruses is crucial for implementing an effective response to viral infection. Established methods for viral analysis include plaque assays, immunological assays, transmission electron microscopy, and PCR-based testing of viral nucleic acids. These methods have not achieved rapid detection at a single virus level and often require a relatively high level of sample manipulation that is inconvenient for infectious materials. Single virus particles, real-time electrical detection has been achieved by the use of nanowire field effect transistors (Jain, 2005). Measurements made with nanowire arrays modified with antibodies for influenza A showed discrete conductance changes characteristic of binding. Simultaneous electrical and optical measurements using fluorescently labelled influenza A were used to demonstrate conclusively that the conductance changes correspond to binding/unbinding of single viruses at the surface of nanowire devices.

The pH dependent studies further showed that nanowire devices can be used to rapidly determine isoelectric points and variations in receptor-virus binding kinetics for different conditions. It has been clear that when a virus particle binds to an antibody receptor on a nanowire device, the conductance of that device changed from the baseline value, and when the virus unbinds again, the conductance returned to the baseline value. Significantly, delivery of highly dilute influenza A virus solutions (10^{-18} M or 50 viruses/ μ l) to p-type Si nanowire produces well-defined, discrete conductance changes that are characteristic of binding and unbinding of single negatively charged influenza viruses. These devices are modified with monoclonal antibody for influenza A. The discrete conductance changes are observed in these studies due to detection of single virus binding/unbinding. The optical and electrical data showed that, the conductance remained at the baseline value as a virus diffused near a nanowire device and the conductance dropped in a quantized manner similar to that observed with unlabeled viruses only after binding at the nanowire surface. When that virus diffused from the nanowire surface, the conductance returned rapidly to the baseline value. These measurements showed that a virus must be in contact with the nanowire device to yield an electrical response. It suggested that it will be possible to develop ultradense nanowire device arrays without unwanted signals in future, where the minimum size scale is set by that of the virus (Vo-Dinh et al., 2006).

Cancer Diagnosis

Recent advances have led to QD bioconjugates that are highly luminescent and stable. These bioconjugates raise new possibilities for studying genes, proteins and drug targets in single cells, tissue specimens and even in living animals and enable visualization of cancer cells in living animals. A method for detecting protein analytes has been developed that relies on magnetic microparticle probes with antibodies that specifically bind a target of interest such as prostate-specific antigen (PSA) in case of prostate cancer. QDs, coated with a polyacrylate cap and covalently linked to antibodies or to streptavidin, have been used for immunofluorescent labelling of breast cancer marker Her2. Labelling is highly specific, and is brighter and more stable than that of with other fluorescent markers. QDs can be combined with fluorescence microscopy to follow cells at high resolution in living animals, offering considerable advantages over organic fluorophores for this purpose (Smith and Nagel, 2003).

Cell Monitoring

The most commonly used optical method is to monitor intracellular species with fluorescent labels. In a new approach, fibreoptic as well as cell-implantable nanosensors have been developed and tested for pH, calcium, sodium, potassium, nitric oxide, oxygen and glucose detection for their use in early rat embryos and single mammalian cells. The problems of fragility, photobleaching, leaching and invasiveness have been largely overcome. The selectivity and signal: noise ratio is adequate for intracellular work (Cullum and Vo-Dinh, 2003).

Optical imaging fibres may be implemented in the direct chemical analysis of single cells (Kneipp et al., 2010). These fibres comprise thousands of individual fibres that have been melted and drawn together in a coherent manner such that each fibre in the bundle carries its own isolated optical signal from one end of the fibre to the other. Through a wet-etch process that takes advantage of the differences in etch rate (the layer thickness that is removed in a certain time, which depends on the material) between core and cladding materials, individual femtolitre-sized wells are formed at the distal tip of an optical imaging fibre. The wells are fabricated so that the well diameter and depth allow for the accommodation of one cell per well. Fluorescently-labelled NIH 3T3 mouse fibroblast cells are dispersed into the well array by allowing a suspension of cells held above the collection to settle into the wells and adhere to the well bottom. The pattern of the cells populating the wells is determined by exciting the fluorescent cell membrane label at the appropriate wavelength (Okumoto et al., 2005). Once the location of the cells in the array has been determined, fluorescence measurements (of analyte) may then be made at other wavelengths. The chemical environment of each cell is directly monitored by correlating the release or consumption of a specific analyte with a change in fluorescent intensity (Cullum and Vo-Dinh, 2000).

Future Prospects and Conclusions

Advances in nanotechnology are providing nanofabricated devices that are small, sensitive and inexpensive enough to facilitate direct observation, manipulation and analysis of a single biological molecule from single cell. It seems quite likely that there will be numerous applications of inorganic nanostructures in as biomarkers. Given the inherent nanoscale of receptors, pores, and other functional components of living cells, the detailed monitoring and analysis of these components will be made possible by the development of a new class of nanoscale probes. Biological tests measuring the presence or activity of selected substances become quicker, more sensitive and more flexible when certain nanoscale particles are put to work as tags or labels.

Future trends in diagnostics will continue in miniaturization of biochip technology to nano-range. There is also a trend to build the diagnostic devices from bottom up starting with the smallest building blocks. Whether interest and application of nanomechanical detection will hold in the long range remains to be seen. Another trend is to move away from fluorescent labelling as miniaturization reduces the signal intensity but there have been some improvements making fluorescence viable with nanoparticles.

The nanosensor devices have a number of key features, including direct, label-free, and real-time electrical signal transduction, ultrahigh sensitivity, exquisite selectivity, and potential for integration of addressable arrays on a massive scale, which sets them apart from other sensor technologies available today. The examples described in this review illustrate unique capabilities in the detection of proteins, viruses, and DNA to the analysis of small organic molecule binding to proteins, which has the potential to significantly impact on disease diagnosis, genetic screening, and drug discovery, as well as serve as powerful new tools for research in many areas of biology. In the near future, we argue that these advances could and should be developed at the commercial level in simple nanosensor devices that would represent a clear application of nanotechnology and, more importantly, a substantial benefit to humankind. Looking to the longer term, we believe the future is exciting from both scientific and technological perspectives. For example, we believe that advances in capabilities of assembling larger and more complex nanosensor arrays and integrating them with first conventional and later nanoscale electronics for processing will lead to exquisitely powerful sensor systems that help to enable the dream of personalized medicine in the future.

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