Nanostructured and nanoscale devices, sensors and detectors

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Abstract

In reduced dimensions, materials display characteristics quite different from their bulk behavior. Interestingly, most viruses, bacteria, pathogens, particles in gas phase and other chemical–biological agents, which form the basis of this investigation, also have comparable dimensions. Our present and ongoing investigation is aimed towards the detection of chemical and biological agents using nanostructured materials. We have studied nanotubes, nanoparticles, nanowires, and nanoporous materials for biocompatibility and subsequent detection. In addition, based on our field emission studies on carbon nanotubes, we have suggested device designs to detect environmental emission. Detection strategies and preliminary data from electrochemical sensors, atomic force microscopy, and surface plasmon resonance are presented for the detection of chemical–biological agents immobilized on layers of nanoparticles. As a critical step in the development of sensors/detectors, strategies for surface functionalization and immobilization for carbon nanostructures, nanoparticles, and nanoporous materials are also presented. Such devices display unique characteristics, morphological flexibility, and biocompatibility. The eventual objective of our investigation is to develop a nanotechnology based sensor platform that will enable the direct electrical, optical, or electro-optical detection of biological and chemical agents in a label-free, highly multiplexed format over a broad dynamic range.

Keywords: Carbon nanotubes; Chem-biosensors; Field emission devices; Integrated sensor technology; Nano-biotechnology

1. Introduction

“At each new level of complexity, entirely new properties appear, and the understanding of these behaviors requires research which I think is as fundamental in its nature as any other”, quoted by P. W. Anderson, in ‘More is different’, in Science [1]. The reduced dimensionality of a system has a profound influence on its physical behavior more specifically for the nanostructured materials where the size is comparable to the size of the fundamental physical quantities. Recent technological advances have provided fabrication routes and strategies to reproducibly develop and study reduced-dimensional systems. A recent study of nanostructured materials has projected tremendous potential towards the development of new devices and sensor designs with unique capabilities [2]. The materials under investigation for the present study are biocompatible, chemically inert but capable of altering electronic properties in presence of some chemical species, dimensionally compatible with biomolecules, and have interesting electronic characteristics, thus rendering them as potential chem-bio sensors. More specifically, carbon-based nanostructures exhibit unique properties and morphological flexibility, which renders them inherently multifunctional and compatible with organic and inorganic systems. Carbon nanotubes (CNTs) have aspect ratios on the order of 1000, which coupled with high conductivity, makes them ideal candidates for low voltage field emitters, with applications in CNT based cold-cathode for X-ray generation [3]. Recent progress in nanostructured materials and its possible applications in chemical and biological sensors could have a significant impact on efficient data collection, processing, and recognition with minimum false positive count. The potential and risk for inadvertent or deliberate contamination of the environment, food and agricultural products has recently increased due to the global war on terrorism, rendering decentralized sensing as an important issue for several federal agencies. In clinical medicine, the current
trend is to decentralize laboratory facilities and conduct clinical trials employing direct reading, portable, lab-on-chip systems. A nanotechnology based sensor platform will enable the direct electrical detection of biological and chemical agents in a label-free, highly multiplexed format over a broad dynamic range. This platform utilizes functionalized nanotubes and nanowires to detect molecular binding with high sensitivity and selectivity. The platform is capable of detecting broad range of molecules, viz., DNA, RNA, proteins, ions, small molecules, cells and even the pH values. Detection is possible in both liquid and gas phase and is highly multiplexable, allowing for the parallel detection of multiple agents. Our present and ongoing investigation is aimed towards evaluating the applications of nanostructures of carbon and other materials in unique devices and chem–bio sensor platforms.

2. Biosensors: function, specificity and sensitivity

Biosensors are simple, inexpensive measurement systems that use biological molecules, usually enzyme, antibody, or nucleic acid to recognize sample molecules of interest via hydrogen bonding, charge–charge interactions, and other biochemical interactions to provide molecular information. The integration of biological systems and nanostructured materials requires information to be induced across the interface in a consistent and reproducible format. Recent advances in the field of nanotechnology and processing have resulted in solid-state biosensors offering unprecedented compatibility of inorganic materials with the chemical/biological agents, thus enabling stable, direct, and reproducible screening and detection. As compared to earlier catalyst systems based biosensors, the next generation affinity biosensors deliver real-time information about the antibodies to antigens, cell receptors to their glands, and DNA and RNA to nucleic acid with a complimentary sequence. Food safety has become one of the most critical issues in sensing due to the zero-tolerance mandate for sequence. Food safety has become one of the most critical issues in sensing due to the zero-tolerance mandate for sequence. Food safety has become one of the most critical issues in sensing due to the zero-tolerance mandate for sequence. Food safety has become one of the most critical issues in sensing due to the zero-tolerance mandate for sequence. Food safety has become one of the most critical issues in sensing due to the zero-tolerance mandate for sequence. Food safety has become one of the most critical issues in sensing due to the zero-tolerance mandate for sequence. Food safety has become one of the most critical issues in sensing due to the zero-tolerance mandate for sequence.

CNTs can be used as biosensors for detecting individual bio-molecules and other biological agents. The CNTs are conducting, can act as electrodes, can generate electrochemiluminescence (ECL) in aqueous solutions, can be derivatized with functional group that allows immobilization of biomolecules, have high surface-to-volume ratio for adsorption, and surface-to-weight ratio (~300 m² g⁻¹), which is accessible to both electrochemistry and immobilization of biomolecules. The functionalization of the CNTs is a key step in the formation of biosensors, as modified nanotubes tips offer possibility of probing systems at the nanometer scale. CNTs can be functionalized in several different configurations, viz.-covalent and non-covalent functionalization, defect, sidewall, and endohedral functionalization, and π-stacking and biosensor applications often require uniform functionalization. Oxidation of the nanotubes with HNO₃–H₂SO₄ leads to high concentrations of carboxylic, carbonyl, and hydroxyl groups on the surface and removal of the tip to expose the tube interior. Carboxyl groups can be readily derivatized by a variety of reactions allowing linking of biomolecules, such as proteins, enzymes, DNA, or even metal nanoparticles. The covalent modification of nanotubes facilitates the creation of well-defined probes that are sensitive to specific intermolecular interactions of many chemical and biological systems. Integration of transducer and probe enables quick, accurate, and reversible measurement of target analytes without using reagents. The possibility of covalent modification of SWNTs offers mapping of functional groups at a true molecular resolution. Furthermore, chemical processes to link catalysts, such as transition-metal complexes to the ends of CNT, will be useful in creating or modifying the structures at a molecular scale, creating interconnections for electronic devices, and even develop new classes of materials. Covalent functionalization of the sidewalls for the SWNTs provides stability and best accessibility, but at the expense of damaging the sidewalls, thereby diminishing the mechanical and electronic properties of the SWNT. Due to these issues, non-covalent routes to
nanotube functionalization offer ease of synthesis and minimum disruption of the tubular structure. As an example, non-covalent functionalization of the SWNT by careful selection and application of a polymer wrapping around the SWNT can be obtained. The non-covalent association of polymers with nanotubes provides an electrically non-interactive baseline association with the nanotubes; while the reactive groups of the polymers allow for interaction with specific molecules. When specific molecules interact with the polymers coating on the SWNT, the electrical properties of the nanotubes are altered, enabling detection of the molecules. Preliminary results of CNT based sensors have shown change in conductance by several orders of magnitude. By using sequence specific-attachment, CNT electronic devices with specific molecular-recognition feature of DNA have been reported [4]. Furthermore, a possible interfacing of CNTs with electro active proteins, by establishing molecular links, is likely to become a convenient vehicle for studying cell organization. Recently obtained readings from oocyte cells using single CNT, albeit preliminary, strongly suggest a bio-probing feasibility through such a nano-approach.

2.2. Nanoparticles

Electrochemical biosensors use gold or silver nanoparticles or magnetic particles, for detecting the oxidation signal of electroactive DNA bases in the presence of DNA hybridization [7]. For electrochemical sensors, the hybridization detection of samples was transduced by means of different electrochemical techniques performed using a potentiostat such as the Autolab PGSTAT 30 electrochemical analysis system. The oxidation or reduction signal of the electro active DNA bases or metal indicators was measured and used as an analytical signal. The detection limit for the different PCR amplicons was found to be between 1 and 100 femtomoles of DNA [8].

The second type of biosensor is developed by using SPR to detect \textit{E. coli} O157:H7 bacteria by immobilizing antibodies by a coupling matrix on the surface of a thin film of precious metal such as gold deposited on the reflecting surface of an optically transparent wave-guide. When visible or near-infrared radiation is made to totally internally reflect at the interface of the metal and the reflecting surface by a prism, SPR occurs. When the antigens interact with antibodies, the refractive index of the medium surrounding the sensor changes, which in turn causes a shift in the angle of resonance. The change in resonance angle is proportional to the change in the concentration of antigens bound to the surface. In order to investigate the capability of a self assembled monolayers (SAM) based SPR biosensor for pathogen detection, first an initial baseline was established and various experiments were conducted. Immobilization using \textit{E. coli} O157:H7 polyclonal antibodies suspended in NaOAc (pH 5.5) produced a pixel change of 12, while \textit{E. coli} O157:H7 (pH 7.4) polyclonal antibodies suspended in PBST produced a pixel change of 7. Increasing the concentration of antigen in direct assay resulted in an increased response. Upon passing the antigen sample at a concentration of $4 \times 10^8$ CFU/ml for 10 min, a change in the pixel value of 0.1 was noted, while a concentration of $7 \times 10^9$ CFU/ml produced an average change in the pixel value of 0.1667. One pixel change is equivalent to a 0.006° change in angle. For the same concentrations of antigens and different
concentrations of secondary antibodies in the sandwich assay, the response obtained was almost 30 times higher than for the direct assay. Fig. 2(b) shows a schematic of SPR and a typical response curve. Fig. 2(c) shows an AFM image of a rod shaped *E. coli*, bound to its corresponding antibody on a SAM based gold chip. Preliminary studies clearly showed that the SAM based LEICA SR 7000 SPR biosensor can be used to monitor biomolecular interactions, and to rapidly detect pathogens. With improvements in the detection protocol and sensor chips, the sensitivity and specificity can be enhanced considerably. As an extension to the detection, and to increase the sensitivity and specificity of the binding assays, particularly in the case of antibody binding, surface plasmon fluorescence spectroscopy (SPFS) will be applied simultaneously with SPS. As the reflectivity monitored at different incident angles reaches a minimum corresponding to the excitation of the surface plasmons, the surface field intensity is maximized, yielding a mirror image of the plasmon reflectivity curve. This intensity enhancement can be employed to increase the fluorescence emission of surface bound dye molecules excited by the amplified electromagnetic field.

### 2.3. Nanoporous materials

In addition to the nanotubes and nanoparticles, novel materials such as porous silicon (PS) [9] and porous carbon [10] with pore size compatible with the dimension of the chem-bio agents have been used for biosensor applications [11,12]. The mesoporous carbon matrix is used for stable immobilization of the biological molecule and C$_{60}$ serves as electron mediator. Both C$_{60}$ and nanotubes have shown to be a good electron mediator when used with mesoporous carbon matrix or modified metal electrodes. Carbon nanotubes based transducers, however, show significant advantage over materials due to the well defined, defect free structures and also that the nanotubes promote homogenous electron transfer reactions.

PS offers a controllable surface topography at a nanometer resolution in three dimensions, and allows
have values close to those of living tissue: medicine have shown that only carbon, gold, and platinum chemical potential of a number of materials used in with respect to the living body. Studies of the electro-normal electrochemical potential and the surface energy parameters. It is determined by two main properties: the can be improved by a suitable change of the surface PS surface is hydrophobic, and functionalization of the etching conditions, PS has a very complex, anisotropic, dissolution in hydrofluoric acid solutions. Depending upon using bulk silicon crystal, by partial electrochemical as it can be used as a sensitive biosensor for proteins, on-demand release of vaccine is currently been investigated, biodegradable material for in-vivo diagnostics and slow or DNA-assisted charge transport

detection

detection

detection

Table 1
Different types of sensors, sensitivity, and merits

| Type of sensor                                      | Range         | Merits                                             |
|----------------------------------------------------|---------------|----------------------------------------------------|
| Direct DNA electrochemistry                         | $10^{-15}$ m of target | Highly sensitive; requires no labeling step; amenable to a range of electrodes |
| Indirect DNA electrochemistry                       | $10^{-18}$ m of target | Highly sensitive; usually requires no labeling step; multiple target detection at same electrode |
| DNA-specific redox indicator detection              | $10^{-15}$ m of target | Moderate to high sensitivity; well suited to multiple-target detection; samples remain unaltered |
| Nanoparticle-based electrochemistry amplification   | $10^{-15}$–$10^{-21}$ m | Extremely sensitive; well suited to multiple-target detection; highly multiplexable |
| Nanowire and nanoporous based detection             | $10^{-15}$–$10^{-21}$ m | Extremely sensitive; well suited to multiple-target detection; highly multiplexable |
| DNA-assisted charge transport                       | $\approx 10^{-15}$ m | Highly sensitive and simple assay; requires no labeling; suited for mismatch detection; sequence independent; multiplexable; applicable to DNA–protein sensing |

chemical surface modification [13]. Use of PS as a biodegradable material for in-vivo diagnostics and slow or on-demand release of vaccine is currently been investigated, as it can be used as a sensitive biosensor for proteins, antigens, and DNA. The PS biosensors were developed using bulk silicon crystal, by partial electrochemical dissolution in hydrofluoric acid solutions. Depending upon the etching conditions, PS has a very complex, anisotropic, and nanocrystalline architecture of high surface area. The PS surface is hydrophobic, and functionalization of the surface renders it as a biomaterial. The PS biocompatibility can be improved by a suitable change of the surface parameters. It is determined by two main properties: the normal electrochemical potential and the surface energy with respect to the living body. Studies of the electrochemical potential of a number of materials used in medicine have shown that only carbon, gold, and platinum have values close to those of living tissue: $+0.330$, $+0.332$, and $+0.334$ mV, respectively. The surface energy of these elements ranges from 20 to 30 erg/cm², which likewise corresponds to the values for living tissue. Generally, the carbon layer structure is formed by mixing sp² and sp³ bonding. A high fraction of sp³ bonds gives a highly hydrophobic surface, which is responsible for the cell attachment. For biological applications, we have developed technology to obtain different PS layers with 35–50% porosity on Si-p+(100) and (111), followed by different treatments for surface structure modification/stabilization, by which the modified surface acquires biomaterial properties. Table 1 lists different types of sensor platforms, their sensitivities, and merit analysis.

3. CNT based gas sensors

Nanotubes can be used as wire between two metal electrodes and the conductance between the electrodes can be measured as a function of gate bias voltage. Since the electrical characteristics are strong function of its atomic structure, mechanical deformation, and chemical doping can induce changes in conductance, thus making such devices as small and sensitive sensors to their chemical and mechanical environment. Chemical sensors based on an individual or ensembles of SWNTs detected 200 ppm of NO₂ and <2% of NH₃ in a few seconds [14]. Hence, sensors made from SWNT have high sensitivity and fast response time even at room temperature. The first principle calculations using DFT on several molecules, such as CO, NH₃, NO₂, O₂, and H₂O, show the direction of the charge transfer and hence, the doping of the semiconductor tube [15], which results in change in conductivity. For H₂O, a simulated molecular configuration shows repulsive interaction and no charge transfer is observed in the presence of water molecule [16], which offers an important option of using SWNTs in water as biochemical sensors. Gas sensors operate by a variety of fundamentally different mechanisms. Ionization sensors work by detecting the ionization characteristics of distinct gases, however, they are limited by the size and high voltage operation and large power consumption. The CNTs exhibit excellent field emission characteristics due to the existence of a very large field at the tips even at very low voltages, which could produce compact, battery powered gas ionization sensors. The field emission based ionization gas sensors are expected to show good sensitivity and selectivity, and are unaffected by the factors such as temperature, humidity, and gas flow. The CNTs have demonstrated electric field induced change in bandgap, which is interesting as it will lead to detection of ionic species without inducing charge transfer or doping of the SWNTs.

4. Field emission in CNTs: X-ray generation

SWNTs and MWNTs are found to be excellent field emitters [17] at relatively low operating voltage, as compared to the thermionic emission mechanism, where electrons are emitted from a heated filament (hot cathode). As compared to Spindt-type and diamond tip emitters, the electron emission from CNTs offer a robust and viable alternative due to large aspect ratio, structural integrity, high electrical and thermal conductivity, chemical stability, and a possibility of large-scale production. Recently, there have been studies to utilize CNT as field emitters for display panels, cold-cathode for X-rays generation, and for photonic devices [18–21]. We investigated electron emission from
carbon nanotubes in a vacuum and the onset of emission occurred at an electric field of 1.45 V/\mu m, which is similar to the values reported in literature. An emission current of 0.35 mA was observed at an electric field of 3.8 V/\mu m. This represents a very large current density for such small dimensions, and is quite close to the theoretical limit for resistive heating. The CNTs can emit very large electron current for extended period of time without any catastrophic failure. Recently, higher current densities approaching $10^{15} \text{A/cm}^2$ were announced by Nano Proprietary Inc. CNTs field emitting surfaces can be printed or even painted with nanotubes, or even grown in-situ. Both catalytically and arc produced MWCNTs function essentially identically. Recently, low threshold field $\sim 1.6–2.6 \text{V/\mu m}$ was reported for MWCNTs and boron doped MWCNTs embedded in a polystyrene matrix [22]. Freestanding CNTs often provide non-uniform current distribution and limited current stability due to mutual shielding, residual pressure and heating effect. To improve the field emission stability, approaches such as synthesis of massive arrays of mono-dispersed CNTs in porous silica template by MOCVD and growth using single mask self-aligned process with an integrated gate electrode are used [23]. Due to low threshold voltage of the electron emission and higher current density, several prototype devices and device configurations, such as field emission display devices, X-ray sources for medical applications, and photonic devices.

5. Next generation sensors, detectors, and applications

X-ray radiation is extensively used in many industrial and medical applications. The emission of X-rays takes place using resistively heated filament in excess of 1200°C to emit electrons, which are accelerated and bombarded on a target. In comparison, electron emission employing field emission is more attractive as the electron emission takes place at room temperature and is controllable using rather small voltage. X-ray tubes, which can generate sufficient X-ray flux for diagnostic imaging applications, have been reported [20]. Such devices can produce focused electron beams with very small energy spread, programmable pulse width, and repetition rate, leading to possibility of portable and miniature X-ray sources. Research is currently in progress to fabricate nano-materials by integrating nano-fabrication and chemical functionalization, particularly nano-electrode assemblies interfaced with biomolecules for the development of biosensors. DNA molecules are electro active at certain potentials that can be used to identify the hybridization process. These sensors will be integrated into the next-generation ‘gene-chips’, especially where detection of less than an attomolecule, such as amino acids or DNA, is critical. Recent discovery of quantum confined particles or quantum dots (QDs) having unique optical and electronic properties, such as size and composition-tunable fluorescence emission from visible to infrared wavelength, large absorption coefficient across a wide spectral range and very high level of brightness and photo-stability, will lead to the development of multifunctional nanoparticles probes for cancer targeting and real-time in-vivo imaging in living cells. The broad excitation profiles and narrow, symmetric emission spectra in high quality QDs are well suited to optical multiplexing, in which multiple colors and intensities are combined to encode genes, proteins, and small molecule libraries [24]. In-vivo studies on mice show that QDs accumulate at tumor sites. Such QDs were encapsulated with triblock copolymers and treated chemically with tumor-targeting ligands having drug-delivery functionalities [25]. These results suggest new possibilities for ultra sensitive and multiplexed imaging of molecular targets. Other nano-technology projects include a nanoscale barcode for genome-wide screening such as disease susceptibility and therapeutic responses, blood fingerprinting, and development of a technology capable of directing nerve growth through scar tissue for spinal chord regeneration, to name a few.

One of our recent project concerns duchene muscular dystrophy (DMD), which is one of nine types of muscular dystrophy, a severely degenerative, lethal genetic disease primarily affecting voluntary muscles, caused by absence of dystrophin, a protein that helps keep muscle cells intact. New experiments on mice with DMD show that gene therapy can reverse some symptoms of the disease even in old mice. A major limitation to gene therapy so far has been absence of a method by which a new gene could be efficiently delivered to the dystrophic muscles. We are investigating the regenerative potential of satellite cells immobilized with a new dystrophin gene on nanostructured templates, which can be delivered to all the affected muscles of mice with muscular dystrophy. The satellite cells, normally associated with healthy muscle fibers are inactive, unless there is muscle damage caused by mutation on the gene encoding dystrophin. The repair and regeneration process of damaged muscles is mediated by fusing satellite cells to the damaged muscle fibers. Hence, the use of therapeutic templates based on nanostructured materials can be inserted for targeted gene delivery.

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