Electrochemical biosensors at the nanoscale

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The general mechanism of chemical sensing is based on molecular recognition linked to different transduction strategies based on electrochemical, optical, gravimetric or thermal effects that can convert the signal to digital information. Electrochemical sensors support accurate, fast, and inexpensive analytical methods with the advantages of being easily embedded and integrated into electronics, and having the greatest potential impact in the areas of healthcare, environmental monitoring (*e.g.* electronic noses), food packaging and many other applications (E. Bakker and Y. Qin, *Anal. Chem.*, 2006, **78**, 3965).[1] Nanoscale electrochemical biosensors offer a new scope and opportunity in analytical chemistry. The reduction in the size of electrochemical biosensors to nanoscale dimensions expands their analytical capability, allowing the exploration of nanoscopic domains, measurements of local concentration profiles, detection in microfluidic systems and *in vivo* monitoring of neurochemical events by detection of stimulated dopamine release (R. Kennedy, L. Huang, M. Atkinson and P. Dush, *Anal. Chem.*, 1993, **65**, 1882).[2] This article reviews both state of art developments in electrochemical nanosensing, and the industrial outlook.

Electrochemical sensors measure the change in current (amperometry and voltammetry), voltage (potentiometry), impedance or conductance resulting from a chemical reaction that either transfers or separates electric charge with reasonable selectivity and sensitivity. They are thus classified as amperometric/voltammetric, potentiometric, impedance and conductometric sensors based upon their analytical principles of operation. *Amperometric and voltammetric* sensors measure the current based on the heterogeneous electron transfer reaction such as the oxidation or reduction of the electroactive analyte species. The current is proportional to the concentration of the analyte. *Potentiometric* sensors, which include ion-selective field effect transistors (ISFETs) and ion-selective electrodes (ISEs), relate the measured potential to the analyte concentration when the electrode reaction is at equilibrium (*i.e.* no current will flow through the electrode). In general, the potential difference shows a linear relationship with the logarithm of the activity of the analyte, as given by the Nernst equation. Electrochemical *impedance* spectroscopy measures the response (current and its phase) of an electrochemical system to an oscillating potential as a function of frequency. *Conductometric* sensors comprising both resistive and capacitive sensors quantify the change in electrical

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properties between two electrodes. Resistive sensors measure the resistivity change due to chemical reactions, while capacitive sensors detect the capacitance change due to a change in dielectric-constant. The different electrochemical techniques are based on different detection mechanisms and so provide information on different aspects of an electrochemical reaction and yield various information for each specific requirement. Potentiometric sensors need to perform under equilibrium, which takes time for the systems to reach. However, they do not require the detected samples to be electroactive, which is essential for amperometric, voltammetric and conductometric sensors as they generate currents for measurement. Electrochemical impedance spectroscopy investigates interfacial reaction mechanisms and allows the measurement of several electrochemical reactions that take place at very different rates and provides a more thorough understanding of an electrochemical system than any other electrochemical technique. Full reviews of electrochemistry and its role in biosensing can be found elsewhere, this review will focus on the interface between nanotechnology and biosensors.

Impact of nanostructure on biosensing

As early as the 1970s, electrochemical methods such as cyclic voltammetry were applied to *in vivo* analysis of brain tissue.[3] It has also been known for almost a decade that nanotubes and nanowires made of carbon and other materials make good sensors.[4,5] However, only in the past couple of years have research groups started to explore an integrated approach combining electronics and biology to build biosensors on the nanoscale. Since the fundamental processes of life occur at the nanoscale, nanosensors can leverage principles and materials common to biological systems. This enables access to microenvironments not accessible to larger electrodes, such as cells. Thus nanosensors enable application of analytical capabilities to the exploration of nanoscopic domains, detection of single cell secretion[6] and single molecule,[7] measurements of local concentration profiles, detection in microfluidic systems and *in vivo* monitoring of neurochemical events by detection of the stimulated dopamine release.[8] Devices based on nanowires are emerging as powerful platforms for the direct detection of biological and chemical species, including determining low concentrations of proteins and viruses.[9]

There are several benefits of using nanoscale electrodes in electrochemical sensors. Firstly, since current (i) is proportional to the electrode area, nanoelectrodes will further reduce the Ohmic (iR) drop distortion and can be used to detect electrochemical reactions in poorly conducting media, even in the absence of a supporting electrolyte. Secondly, double-layer capacitances are proportional to electrode area, and so are greatly reduced for nanoelectrodes which have small surface area, resulting in electrochemical cells with small RC (R: resistance, C: capacitance) time constants. Such nanoelectrode electrochemical cells enable high speed voltammetric experiments that can probe the kinetics of very fast electron transfer and coupling reactions or the dynamics of processes such as exocytosis.[10] Thirdly, the rate of mass transport to and from the electrode (and the related current density) increases as the electrode size decreases. Models also predict substantially higher mass transfer rates for nanoelectrodes due to radial (nonplanar) diffusion, which would enable ultrafast electrochemical measurements, compared to measurements using bulk electrodes that operate *via* planar diffusion.[11] As a consequence of the increase in mass transport rates and the reduced charging currents, nanoelectrodes will exhibit excellent signal to background noise (S/N) ratio in comparison to their macroscopic counterparts. Fig. 1 illustrates the different scope (macroscopic and nanoscopic) of electrodes and their cyclic voltammograms, which are determined partially by the mass transport of electroactive compounds (*e.g.* ferrocene) to the electrodes. Planar diffusion is the main mass transport mechanism when using traditional macroscopic electrodes and redox reactions are limited by mass transport. This contributes to the peak shape of the cyclic voltammogram in Fig. 1a. With such large electrodes the cyclic voltammogram is affected by *iR* drop, despite the supporting electrolyte. When the size decreases down to the nanometer level, radial diffusion becomes dominant. As the

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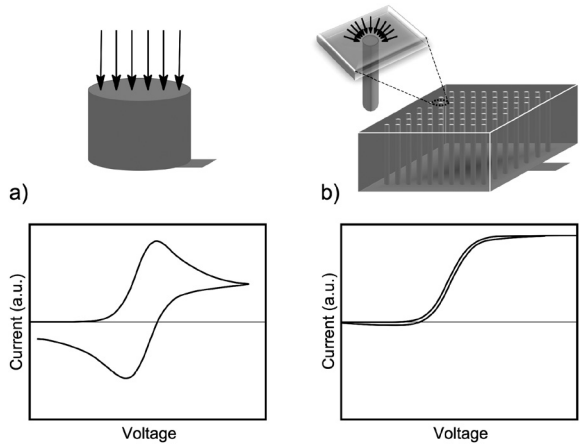

Fig. 1 a) Macroscopic electrode with planar diffusion and its corresponding cyclic voltammogram. b) One of the nanoelectrodes from the nanoelectrode array with radial diffusion and its steady state voltammogram when cycling with slow rate. Both cases were conducted in redox solutions (*e.g.* ferrocene in aqueous solutions).

sigmoid curve of the voltammogram (Fig. 1b) shows, for nanoelectrodes, the contribution of convective transport is negligible and steady state or quasi-steady state currents are rapidly attained. In this case, redox reactions are limited by the rate of electron transfer. Small sensors have high concentration gradients and do not significantly deplete the solution, which also benefits many kinetic studies.

Besides the use of individual nanoelectrodes, where device miniaturization provides benefits, the use of nanoscale components in large scale devices has advantages. Single sensors are normally used to measure a single analyte. Devices that combine multiple individual sensors can either provide a multianalyte measurement capability (sensor arrays) or be used to obtain spatial distribution measurements for a single analyte (nanoelectrode ensembles).

Despite the benefits of nanoelectrodes, the current will decrease as the surface area of the electrode decreases. Nanoelectrode ensembles successfully address both the issue of decreasing electrode size, by using nanoporous templates[12,13] and the issue of increasing overall current by multiplexing the nanoelectrodes.[14] Nanoelectrode ensembles created in templates can have densities upward of 10^{11} electrodes/cm^2.[15] The ideal case is to have the electrodes at an intermediate density to maintain radial diffusion while using the electrode area as efficiently as possible.[16] Detection limits are one of the key factors for chemical sensors. When combined with innovative signal transduction technology, micro and nanosystems have recently achieved specific biomolecular detection at femtomolar concentrations.[17,18] The design of biomolecular sensors for ultrahigh sensitivity applications has to take into account the limits imposed by analyte transport in fluidic systems. The detection limit reported to date for nanoscale biosensors is in the range of femtomolar concentrations which is very likely limited by analyte transport rather than signal transduction.[19] The detection limit in a voltammetric experiment is related to the ratio of signal to noise (S/N). The signal is the Faradaic current that occurs at the electrode during the redox reaction of the analyte. Background noise is predominantly caused by a double-layer charging current at the electrode–solution interface and is proportional to the area of the conductive portion of the electrode. For most electrodes, the conductive area is equal to the total area, but for nanoelectrode ensembles the conductive area may be only a small portion of the entire electrode surface. Therefore, since the signal is the same but the background is several orders of magnitude lower, the S/N for nanoelectrode ensembles is significantly higher than for conventional electrodes. The S/N increase has been exploited to detect many interesting species in chemical sensors, for example, electrochemical studies of cytochrome c (cyt c) usually require a promoter to avoid electrode poisoning due to adsorption. The use of nanoelectrode ensembles with a differential pulsed voltammetry method enabled the concentration of cyt c to be reduced low enough to avoid poisoning, therefore allowing detection without a promoter.[20] Random arrays of single DNA molecules can be sequenced without amplification using such high density approaches as well.[21] Applications of nanoelectrode ensembles in chemical sensing have taken advantage of increased diffusion rates, improved S/N ratio and a lower detection limit for several electrochemical species.[22,23] Sensor response time is also an important factor. Ion screening can significantly increase the average incubation time needed to achieve the same sensor response in nanosensors.[24] Hence, it is important to develop functionalization schemes with low ion concentrations not only due to ion screening (magnitude of sensor response) but also to reduce the time taken to obtain a detectable signal change. Advances in nanofabrication allow the manipulation of biological samples in a controlled way, so enabling integration with nanostructures. There are many strategies to embed receptor groups that recognize specific molecules in the transducer layer to guarantee selectivity and sensitivity.[25] A paradigm for nanobiosensors that can be miniaturized and integrated to produce intelligent analysis systems in numerous biotechnology applications has been sought for years[26–29] and the tremendous potential of highly sensitive electronic detection of biomolecules by nanoscale biosensors has been demonstrated for genomics[30,31] and proteomic[25,32] applications.

Arrays of sensors enable new analytical approaches. The components of a sensor array need not be identical and each can provide its own signal. The most optimistic scenarios depict very high density sensing arrays containing thousands to millions of individually addressable and accessible nanoscale elements. Nanoarrays, which consist of probe biomolecules immobilized on a chemically modified surface, have attracted much attention for the development of nanobiosensors.[27,33] Assuming that the requisite chemistries for performing molecular recognition of thousands of different species are developed, such arrays will have the capability for performing a high level of multiplexed sensing or analysis. They will have tremendous functionality while using only small amounts of sensor material, and thus could provide a universal platform for cost-effective analysis. This vision relies on the capability to put increasing functionality into smaller and smaller species as is exemplified by the computer chip industry. Electronic components such as CCD chips, CMOS devices and high-density integrated circuits provide the ability to collect enormous amounts of data on short time scales. Various materials have been proposed to act as transducers in such

nanoarrays. Carbon nanotubes (CNTs) are a leading candidate and a number of different methods for the immobilization of biomolecules on CNTs have been reported.[34] As electrochemical technology is amenable to miniaturisation, it is anticipated that future economic and portable sensing electronics will rely on nanoscale electrochemical sensors.[35]

Amperometric and voltammetric nanosensors

Both amperometric and voltammetric sensors measure the Faradaic current generated by electroactive analyte species. However, in amperometric sensors, the potential is fixed at a constant value and the concentration of the electroactive species is determined by the Faradaic current. Voltammetry is the application of a potential ramp with the subsequent measurement of current as a chemical species reacts at the electrode.

Amperometric sensors are usually applied to the measurement of a specific biological component (*e.g.* glucose) since they are operated at a constant potential. As an example, a new tool for sensitive biosensing and molecular diagnostics has been reported using a self-assembled peptide nanotube attached to a gold electrode as an amperometric biosensor for the determination of glucose.[36] Among voltammetric methods, electrochemical DNA biosensors show considerable promise for obtaining sequence-specific information in a simpler, faster, cheaper and portable manner, compared with traditional hybridization assays.[37] As a result, DNA biosensors based on nanostructured materials have attracted both scientific and industrial interest.[38] The most common approach to electrochemically detecting DNA hybridization is to use pulsed, square wave voltammetry as square pulses can significantly reduce any adverse charging effects and provides very reproducible signals. Nanoelectrode ensembles have been used to detect multiple DNA hybridizations. Such ensembles can be made by deposition of gold onto polycarbonate track-etch template membranes, and then O_2 plasma etching to yield 3D brush-like electrodes.[39] This change in geometry allowed more DNA to bind to each electrode, decreasing the nanoelectrode ensembles detection limit to the attomole level.[40] Well oriented nanowell array matrix for integrated digital nanobiosensors have also been developed as shown in Fig. 2.[41] The nanowell geometry minimizes unwanted nonspecific binding and decreases the noise signal, since the resist layer can restrict some biomaterials from attaching to the gold electrodes in the well. This restricted geometry significantly enhanced the sensitivity of these electrochemical DNA chips.

DNA hybridization can also be measured by cyclic voltammetry using Ru(III)/Ru(II) and ferrocyanide redox couples as signal amplifiers.[42] In this work, Ru(III) was reduced to Ru(II) by electron transfer through dsDNA, which was formed by hybridization of target single-stranded DNA (ssDNA) to self-assembled ssDNA probes on the electrode surface. Ru(III) was catalytically regenerated from Ru(II) by conversion of excess of Fe(II) to Fe(III) in solution, which amplifies the response by a factor greater than 10. Other electrochemical methods including monitoring the surface charge increase after hybridization measured by chronocoulometry[43] and impedance[44] have also been reported.

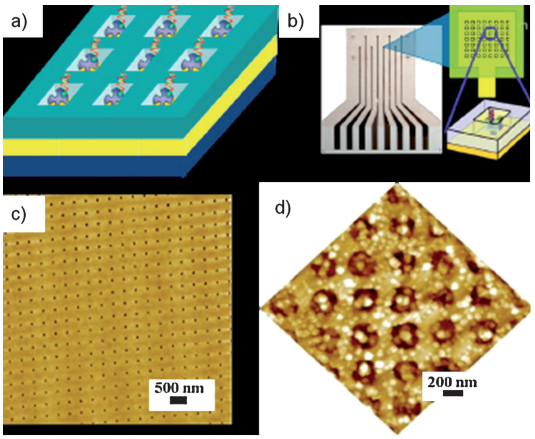

Fig. 2 a) Schematic diagram of the oriented nanowell (ONW) array geometry on a gold electrode designed to minimize the unwanted, nonspecific binding or aggregation of biomaterials. b) An ONW array electrode composed of eight Au pads. The Au electrode on each pad was covered with a resist blocking layer. c) An AFM image of an ONW array with a 200 × 200 probe grid on a gold electrode 800 μm in diameter. In fabricating the ONW array electrode, 100 × 100 nm^2 wells were generated on the resist surface at 500 nm intervals. d) An AFM image of probe ssDNA immobilized by streptavidin–biotin binding on an ONW array. Although there are also bright spots on the resist layer, these DNA/streptavidin complexes do not contribute to the electrochemical signal.[41] *Reproduced by the kind permission of the American Institute of Physics.*

Aligned multiwalled carbon nanotubes (MWCNTs) can be used as nanoelectrode ensembles. MWCNTs were grown on a Si wafer from lithographically-patterned Ni catalyst spots using plasma-enhanced chemical vapour deposition (PECVD).[45] The wafer and MWNTs were then coated with an insulating layer of SiO_2, which was later polished to expose the nanotube tips. These tips were electrochemically etched, leaving hydroxyl and carboxylic acids groups that could be used to covalently bind analytes such as DNA. DNA hybridization was similarly detected using the catalytic redox species of $Ru(bpy)_3^{2+}$ to oxidize guanine residues in double-stranded DNA (dsDNA). Using alternating current (AC) voltammetry and an 18 base-pair probe sequence, 300 base-pair PCR amplicon targets were detected with a sensitivity approaching that of laser-based fluorescence techniques.[46] Similar to the MWCNT nanoelectrode ensembles, vertically aligned diamond nanowires have been applied to the electrochemical sensing of DNA.[47] DNA hybridization was detected by comparing the cyclic voltammograms, differential pulse voltammograms and electrochemical impedance spectra collected before and after the DNA hybridization event. A sensitivity limit of about 2 pM was reported with no degradation of the DNA on the nanowires after 30 cycles of DNA hybridization/denaturation, which is comparable with the chemical stability observed for diamond-based optical DNA biosensors.

Voltammetric nanosensors have also been used to detect other biomolecules. A biosensor based on a nanoneedle consisting of a MWNT attached to the end of an etched tungsten tip was developed.[48] The nanoneedles prepared in this work are 30 nm in diameter and 2–3 μm in length as shown in Fig. 3.

Dopamine and glutamate, which are important neurotransmitters, were successfully detected using these nanoneedles. Bare

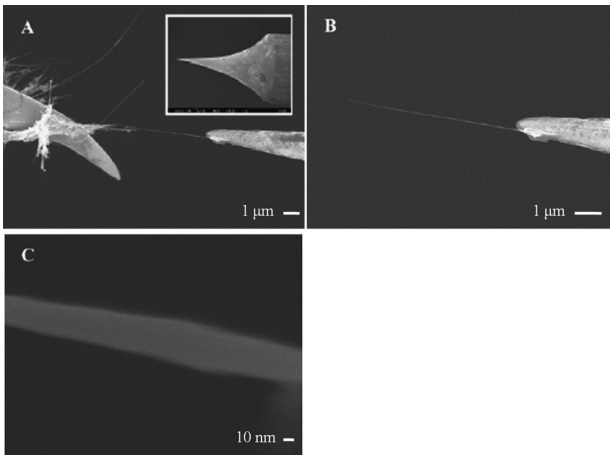

Fig. 3 SEM images of MWCNT nanoneedle electrode. (A) Contact of a single MWCNT with tungsten tip from MWCNT bundle. Inset is a tungsten tip after etching. (B) Attachment of a single MWCNT to tungsten tip after a sufficient voltage is applied. (C) MWCNT image magnified from (B).[48] *Reproduced by the kind permission of the American Chemical Society.*

nanoneedles measured the concentration of dopamine in the range from 100 to 1000 μM by differential pulsing voltammetry, and enzyme-modified nanoneedles were able to report glutamate concentration in the 100–500 μM range using potentiostatic amperometry. Single walled carbon nanotube (SWCNT)-arrayed electrodes can also be formed directly on Pt surfaces, and thus arrayed on a chip.[49] Vertically aligned CNTs are always configured with one end in contact with underlying electrode and the other end exposed in electrolyte solution. The aligned CNTs usually need to be supported by impregnating with SiO_2 or conducting polymers when immersed in solutions.[50;51] The electrochemical measurements of amino acids revealed that the peak current intensities using such SWCNT-arrayed electrodes are about 100-fold higher than those using bare Pt arrayed microelectrodes.

Significant electrocatalysis has been found in CNTs used as electrodes in voltammetry, which promote electron transfer rate.[52–54] CNT modified glassy carbon electrodes show enhanced sensitivity and stability when used to oxidise phenolic compounds.[55] The additional resolution conferred by CNT modified electrodes has been exploited to develop a biosensor for dopamine (DA) that works in the presence of ascorbic acid (AA) and uric acid.[56–59] At a conventional glassy carbon electrode, the oxidation potentials of AA and uric acid (UA) are close to that of DA, resulting in an overlapping voltammetric response.[60] Nevertheless, selective detection of DA in presence of AA and UA on a glassy carbon electrode modified by an MWCNTs-ionic liquid gel has been reported.[61] Room temperature ionic liquid (RTIL) is a melting salt at room temperature, which can work under a large electrochemical window. The RTIL used was 1-octyl-3-methylimidazolium hexafluorophosphate ([OMIM][PF_6]). The anodic peaks of AA, UA and DA can be well separated in the mixture since the peak potential of AA is shifted to a more negative value, while that of UA is shifted to a more positive value. The detection limit of DA by using differential pulse voltammetric technique is *ca.* 0.1 μM in the presence of a large excess (more than 100 times excess) of AA in neutral pH solutions. In such approaches, CNTs were randomly deposited onto conductive surfaces in a mat configuration. CNT electrodes can also be screen printed for use in the electrochemical monitoring of DNA hybridization.[62] Particles as tiny as 60 nm have been printed with single-particle resolution to create nanopatterns ranging from simple lines to complex arrangements.[63] This method could advance the development of nanoscale biosensors. Biosensors created with printed electrodes or organic transistors could offer an inexpensive solution with the additional advantage of flexibility in their shapes.

Impedance nanosensors

Electrochemical impedance spectroscopy (EIS) has proven to be one of the most powerful tools for the investigation of interfacial reaction mechanisms.[64,65] EIS measures the response (current and phase) of an electrochemical system to an oscillating potential as a function of frequency. This technique offers several advantages over chronoamperometry and cyclic voltammetry because the effects of solution resistance, double layer charging and currents due to diffusion or to other processes occurring in the monolayer can be observed more explicitly.[66] EIS allows the measurement of several electrochemical reactions that take place at very different rates and provides a more thorough understanding of an electrochemical system than any other electrochemical technique.

E. coli detection has been reported using an interdigitated array microelectrode based electrochemical impedance immunosensor.[67] Basu *et al.* modified an array of Au nanowires with anti *Escherichia coli* antibody and through titration using different *E. coli* cell concentrations, a detection limit of 10 cells over a 0.173 cm^2 area was achieved by EIS.[68] The underlying detection in this case ultimately depends upon the antigen–antibody complex formation, which changes the surface capacitance properties of the sensor. In this case, *E. coli* served as an antigen and the detection limit for the capacitance change was from 10 nF to 1 pF. Using the same technique, interfacial interactions between immobilized DNA probes and DNA-specific sequence binding drugs can also be investigated. Impedance sensing of DNA binding drugs using gold substrates modified with gold nanoparticles has been reported.[69] In this study, impedance measurements were made based on the charge transfer kinetics of the $[Fe(CN)_6]^{3-/4-}$ redox couple. Compared to bare gold surfaces, the immobilization of DNA and then the DNA–drug interaction on electrode surfaces altered the capacitance and the interfacial electron resistance and thus diminished the charge transfer kinetics by reducing the active area of the electrode or by preventing the redox species from approaching the electrode. The electron-transfer resistance was measured as a function of the drug concentration on selected specific binding DNA probe-modified electrodes. The charge-transfer resistance on the gold nanoparticle-modified surfaces increases with increasing drug concentration from 15 nM to 1 μM and from 40 nM to 1 μM for mithramycin and netropsin, whereas the detection limits of mithramycin and netropsin using bare gold electrodes were 300 and 500 nM, respectively. Using gold nanoparticle deposited substrates, impedance spectroscopy resulted in a 20–40 fold increase in the detection limit. Arrays of

gold nanoparticles deposited on electrodes were demonstrated to control the probe immobilization subtly to ensure suitable adsorbed DNA orientation and accessibility of DNA binding drugs for ultrasensitive analysis.[69]

Gold nanoparticles can provide the ultra-sensitive detection of nucleic acids and proteins. When Si nanowires were coated with them, the nanocomplex exhibits high sensitivity for pesticide detection when used as an electrochemical nanosensor.[70] Applications of other metal nanoparticles such as platinum, silver and palladium in electroanalysis have been thoroughly reviewed elsewhere.[71]

Conductance nanosensors

Direct measurement of conductance change can also be used in electrochemical sensors. Gold nanoparticles are often used to enhance the signal. In the conductance sensing strategy, gold nanoparticle probes are coated with a nucleotide sequence that complements one end of a target sequence in the sample and another sequence that hybridises to the other end, are attached to a surface between two electrodes. If the target sequence is present, it bonds the nanoprobes to the surface and when treated with a silver solution, they create a bridge between the electrodes and produce a current. Though the commercialization of nanosensors is still in its infancy, a range of products are now reaching the market. Verigene® is a platform based on the above mechanism to automate the analysis and detection of nucleic acids and proteins. Nanosphere Inc. has already generated revenue from the sale of such nanobiosensors (Verigene®).

The interaction of gas molecules with CNTs can lead to conductance changes, allowing detection limits of a few parts-per-million (ppm) for gases such as NO_2, NH_3, N_2 and O_2.[72,73] Even individual gas molecules (*e.g.* NO_2) can be detected by graphene sensors. The adsorbed molecules change the local carrier concentration in graphene one by one electron, which leads to step-like changes in resistance.[74] Changes in the conductance of random networks of CNTs paired with electronics on a semiconductor chip or plastic detection device have recently been extended in sensors for medical diagnostics.[75] These devices are arrayed on silicon and plastic substrates using scalable and inexpensive manufacturing techniques. The arrays enable multiplexed, multi-analyte detection and pattern analysis for ultra-sensitive, specific and reproducible results. Changes in the electronic characteristics of the devices correspond to the amount of analyte present. Based on this design, Nanomix (www.nano.com) is developing an asthma monitor, which is a small, inexpensive unit that measures the level of nitric oxide in exhaled breath. An even more tiny and sensitive CNT-based chemical sensor can detect low parts-per-billion (ppb) concentration of gases.[76] It can also go from detecting one gas to another within half a minute by coating the CNTs with chemicals that allow the nanotubes to rapidly switch their response. Such device is made of two parts. The first is an ultrasmall gas chromatograph to separate different gases by time intervals. The output of the chromatograph feeds into the nanotube sensor. The sensor contains CNTs spanning the space between tiny gold electrodes. The adsorption of different gas molecules onto the nanotubes changes their electrical conductivity by a known amount enabling the gases to be identified. Conductance biosensors have also been developed with oxide nanowires such as ZnO nanowires.[77] When ZnO nanowire field effect transistors (FETs) were functionalized with biotin they easily detected streptavidin binding.[78] Such sensors can provide sensitive, label-free, real-time detection of a wide range of biological species. Detecting the change in electrical signal leads to smaller, cheaper and more sensitive sensors. The difficulty has been finding an easy way to integrate the nanowires with electronics. Most recently, researchers at Penn State University have come up with a way to guide single nanowires into place on a silicon chip using an electric field.[79] Once the nanowires are in place, the electrodes are deposited on top to make arrays of sensing devices.

Charles Lieber's group at Harvard University has produced highly sensitive biosensors based on field effect transistors by assembling p- and n-silicon semiconductor nanowires as planar gate electrodes. The binding of biomolecules to the surface of the nanowire leads to the depletion or accumulation of carriers in the bulk of the nanometer-diameter structure, as opposed to only the planar surface, as seen for planar devices. These semiconductor nanowires effectively increase the active surface area, so providing sufficient sensitivity to enable the detection of single viruses and of single molecules in solution.[9,32,80,81] The underlying concept for detection using these nanowires is based on the classical electrical behavior of FETs, which exhibit a conductivity change in response to variations in the field or potential at their surface. Thus, in the case of p-silicon, applying a positive gate voltage depletes carriers and reduces the conductance, whereas applying a negative gate voltage leads to an accumulation of carriers and increases the conductance. In this sense, the binding of a charged species to the gate dielectric is analogous to the effect of applying a voltage with a gate electrode. Lieber's group also showed that these nanowires can act in a non-invasive manner to detect, simulate and inhibit neuronal signals with a good spatial and temporal resolution.[82] In the same way, single- and multi-walled CNTs have also been configured as planar FETs to explore with high sensitivity the detection of gases[72] and biomolecules such as antibodies and cytochrome c.[83–87] It was shown that the label-free detection of DNA hybridization and the discrimination of single-nucleotide polymorphism was possible using this configuration.[88] The CHEMFET biosensors based on CNTs have been reviewed by Kim *et al.*[89] One practical limitation for the *in vivo* use of these sensor-based FETs is that the detection sensitivity depends on solution ionic strength. As blood serum samples have a high ionic strength, any diagnostic procedure will require a prior desalting step to be used before the analysis in order to achieve the highest sensitivity.

Potentiometric nanosensors

Potentiometric sensors measure the potential when there is no net current flowing in a system as all the driving forces are in balance. Under these conditions the measured potential is related to the concentration (activity) of the electroactive species by application of the Nernst equation. Potentiometry with ion selective electrodes (ISEs) is an accurate, fast, and inexpensive analytical method.[90–94] An example is the novel iridium oxide nano pH sensor designed to work in the range of pH 3–14 (Fig. 4)[95].

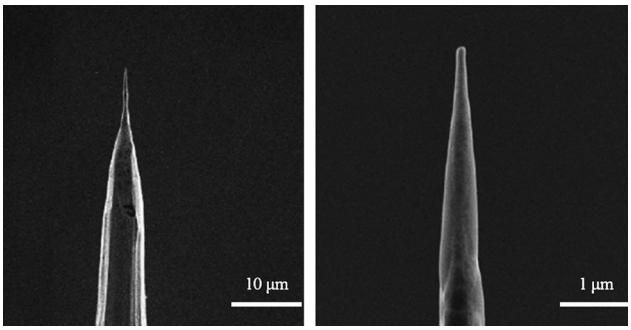

Fig. 4 Example of an iridium nanotip. Bars: left 10 μm, right 1 μm.[95] *Reproduced by the kind permission of the American Chemical Society.*

The nanotips were first covered by an insulating layer of polymer. Milling with a focused ion beam (gallium ions) enables the opening of the apex, leading to a sensing area with 100 nm diameter. After the oxidation of the iridium tip in an oxygen atmosphere, the nanotip gives a stable pH response and a typical Nernst behavior with slope of the calibration curve 59.2 mV/pH. Based on this potentiometric method for the measurement of pH, other important biosensors can be developed.[96] The most frequently used enzyme in biosensors is glucose oxidase (GOD) due to the importance of glucose analysis in blood for diabetic patients and other biomedical applications.[97] pH transducers can be applied in potentiometric glucose sensors based on the following equations.[98]

$$\beta - \text{glucose} + O_2 \xrightarrow{\text{GOD}} \beta - \text{gluconic acid} + H_2O_2 \quad (1)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (2)$$

The number of protons produced in equation (2) are proportional to the amount of hydrogen peroxide formed (equation (1)), which can be used to determine glucose concentration. Blood glucose monitoring by diabetics is the outstanding commercial application of electrochemical biosensors, representing 85% of the market for healthcare biosensors and an estimated market size of $3.6 billion by 2014.

Rapid progress in microelectronics has enabled the development of semiconductor-based enzyme biosensors such as the ion selective field effect transistor (ISFET).[99–101] An extension of this device, the region ion sensitive field effect transistor (RISFET) has been constructed and demonstrated on two different sensor chips that could measure glucose with good linearity in the range of 0–0.6 mM and 0–0.3 mM with a detection limit of 0.1 and 0.04 mM, respectively.[102]

Conclusions and outlook

In general, nanotechnology refers to materials that have structure on the 1–100 nm scale in at least one dimension. Advances in such areas of materials and fabrication techniques are particularly relevant for emerging nanosensors. Standard medical detection technologies provide quantitative but costly results. Electrochemical techniques have been proven to be suitable for miniaturisation and high-throughput manufacture, allowing economies of scale to make them cost-effective in comparison to other analytical techniques. According to the Business Communications Company Inc. (BCCI), the global nanosensor market was worth 190 M USD in 2004 and is forecast to rise with an average annual growth rate of 25.5% to reach in excess of 592 USD by the end of 2009.[103]

Electrochemical nanosensors demonstrate immense surface area-to-volume ratios that allow improved sensitivity, portability, small size for reduced power consumption and heat generation and a reduced cost of materials. The power consumption of many sensor devices has now been reduced to the point that they can be powered from vibration and thermally driven electrical generators. In addition, the tiny active surface area of nanosensors limits the amount of analyte required per single assay. This will reduce the fabrication cost by cutting down on the volume of expensive materials (*e.g.* gold) needed for a given sensor. Integrating the electrochemical nanosensors with wireless technology will enable the sensors to be interrogated remotely. The recent adoption of Bluetooth Low Energy Technology as the wireless standard for healthcare and sports and fitness devices by the Continua Health Alliance[104] will ensure that multiple nanosensors can be monitored simultaneously with minimum power usage. This will enable the user go beyond current technology and directly monitor changes in biomarkers during exercise. Fig. 5 shows a concept of such a device.

The Morph concept device represents the future of mobile devices. An array of nanowire-based sensors are incorporated that can sample the sweat and blood of the user while the display gives a real-time output of these parameters that can be uploaded to a cloud-based computer for further analysis. Early versions of such a device are beginning to appear. For instance PalmSens has developed a wrist-mounted device for voltammetric and amperometric sensing (http://www.palmsens.com/).

The dimensions associated with nanotechnology are comparable to those of biological macromolecules such as proteins and nucleic acids. Electrochemical biosensors at the nanoscale allow such biological systems to be evaluated directly and non-destructively. The enlargement of the operating range of the electrochemical nanobiosensor and the combination with other sensor techniques will open up new avenues for the development

Fig. 5 The Nokia Morph concept mobile device (http://www.nokia.com/A4852062) showing its biosensor capability based on nanowire sensors and a sample output of key physiological markers for sporting performance.

of mobile devices that enable real-time monitoring of the user and their environment and direct coupling of this sensor data to new response mechanisms such as targeted drug release or immediate point-of-care diagnosis.

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