Biosensor

Abstract

Biosensor has rapidly become essential analytical tools, since they offer higher performance in terms of sensitivity and selectively than any other currently available diagnostic device. The development of biosensor technology represents a crucial task for environmental pollution management, there is a considerable need to project and realize biosensors with the best features for commercialization, such as selectivity, sensitivity, stability, reproducibility and low cost. With appropriate progress testing and commercialization, biosensors will have an important impact on environmental monitoring, reducing costs and increasing the efficiency of certain applications. The same multiple approach might be used for development of biosensor platforms suitable for use in fields as diverse as environmental and agrifood to industry, research security and defense, medical and clinical. This review paper focused on the various types of biosensors and applications in environmental monitoring.

Introduction

A biosensor device is defined by its biological, or bioinspired receptor unit with unique specificities toward corresponding analytes. These analytes are often of biological origin like DNAs of bacteria or viruses, or proteins which are generated from the immune system (antibodies, antigens) of infected or contaminated living organisms. Such analytes can also be simple molecules like glucose or pollutants when a biological receptor unit with particular specificity is available. One of many other challenges in biosensor development is the efficient signal capture of the biological recognition event (transduction). Such transducers translate the interaction of the analyte with the biological element into electrochemical, electro chemiluminescent, magnetic, gravimetric, or optical signals. In order to increase sensitivities and to lower detection limits down to even individual molecules, nanomaterials are promising candidates due to the possibility to immobilize an enhanced quantity of bioreceptor units at reduced volumes and even to act itself as transduction element. [1]

In most cases, accurate analyses of biological materials are expensive and need to be performed in external laboratories equipped with more sophisticated instrumentation. Most of these analyses require previous purification that require too much time relative to the processing time, making their on-line implementation impossible for control purposes. However, in living organisms, biological components like antibodies and enzymes work as natural sensing and controlling "devices." The ability of isolating and purifying these proteins and other biological elements such as cells or organelles has allowed their integration with physicochemical transduction devices to produce biosensors. The most widely accepted definition of a biosensors is: "a self-contained analytical device that incorporates a biologically active material in intimate contact with an appropriate transduction element for the purpose of detecting (reversibly and selectively) the concentration or activity of chemical species in any type of sample."[2] The first biosensor, an enzyme-based glucose sensor, was developed by Clark and Lyons. [3] Since then, hundreds of biosensors have been developed in many research laboratories around the world. Over 200 research papers about biosensors have been published each year for the past three years. [4]

Biosensors are analytical devices incorporating a biological sensing element. They harness the exquisite sensitivity and specificity of biology in conjunction with physicochemical transducers to deliver complex bioanalytical measurements with simple, easy-to-use formats.[5] Potential uses embrace virtually every conceivable analytical task, ranging from medical diagnostics through drug discovery, food safety, process control and environmental monitoring, to defense and security applications. The basic concept of the biosensor was first elucidated by Leyland C. Clark in 1962, in his seminal description of an ''enzyme electrode''. Building on his earlier invention of the Clark oxygen electrode, he reasoned that electrochemical detection of oxygen or hydrogen peroxide could be used as the basis for broad range of bioanalytical instruments, by the incorporation

of appropriate immobilized enzymes. The classic example was immobilized glucose oxidase (GOx), which converted a simple platinum electrode into a powerful analytical instrument for the detection of glucose in human samples from people with diabetes. Two decades later, optical transducers were harnessed in conjunction with antibodies to create real time bioaffinity monitors. These immunosensors laid the foundation for the second major evolutionary line of biosensing instrumentation. Both the enzyme electrode and the bioaffinity sensors originally found utility in laboratory instruments, but advances in manufacturing coupled with mediated electrochemistry, launched the enzyme-based systems along a new and highly successful trajectory of home use, which was to lead to a turnover currently in excess of US$13 billion and engaged the full attention of the world's major diagnostics companies.1 Hence, electrochemistry has come to dominate distributed diagnostics, while optical techniques have found their niche principally in R&D. To complete the picture concerning transduction strategies, advances in acoustic resonance devices are certainly worthy of note, but both thermometric and magnetic transduction have failed to have any serious practical impact to date. Growth in the field of biosensors has been phenomenal. When the principal journal in the field, Biosensors and Bioelectronics was launched in 1985 by Elsevier, it published about thirty biosensor papers that year out of a total published in the world of approximately one hundred. Last year, there were about four and a half thousand papers published on biosensors, and this represents more than 10% of all papers ever published on the subject (Fig. 1). Incidentally, it is interesting to note that an increasing share of this literature is published by Asian countries, which now account for approximately half the papers published on this and closely related subjects. A diminishing proportion of this work now originates from Europe, while the output from the USA remains steady. At this point, an apology is in order, since it is obviously impossible to do justice to this volume of material, this review will present a highly personal view of some of the key technology drivers, applications and potentials of biosensor technology. Despite the vast numbers of papers published, the field of biosensors may be viewed as comprising essentially two broad categories of instrumentation: (a) sophisticated, high-throughput laboratory machines capable of rapid, accurate and convenient measurement of complex biological interactions and components; (b) easy-to-use, portable devices for use by non-specialists for decentralized, in situ or home analysis. The former are expensive and the latter are mass produced and inexpensive. [6]

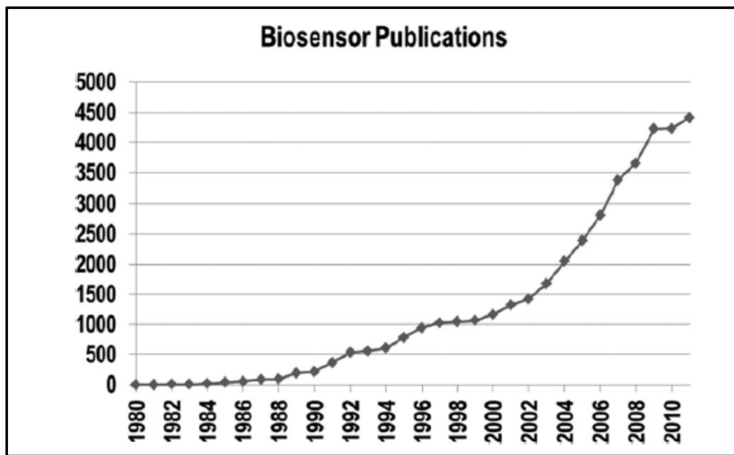

Fig. 1 Graph of a search on the term ‘‘biosensor*’’ during the period 1980 to 2011, using the Web of Knowledge.

Types of Biosensors

Biosensors can be grouped according to their biological element or their transduction element. Biological elements include enzymes, antibodies, micro-organisms, biological tissue, and organelles. Antibody-based biosensors are also called immunosensors. When the binding of the sensing element and the analyte is the detected event, the instrument is described as an affinity sensor. When the interaction between the biological element and the analyte is accompanied or followed by a chemical change in which the concentration of one of the substrates or products is measured the instrument is described as a metabolism sensor. Finally, when the signal is produced after binding the analyte without chemically changing it but by converting an auxiliary substrate, the biosensor is called a catalytic sensor. [7] The method of transduction depends on the type of physicochemical change resulting from the sensing event. Often, an important ancillary part of a biosensor is a membrane that covers the biological sensing element and has the main functions of selective permeation and diffusion control of analyte, protection against mechanical stresses, and support for the biological element. The most commonly used sensing elements and transducers are described below.

Sensing Elements

Enzyme

Enzymes are proteins with high catalytic activity and selectivity towards substrates (see the article Enzyme Kinetics). They have been used for decades to assay the concentration of diverse analytes. [8] Their commercial availability at high purity levels makes them very attractive for mass production of enzyme sensors. Their main limitations are that pH, ionic strength, chemical inhibitors, and temperature affect their activity. Most enzymes lose their activity when exposed to temperatures above 608C. Most of the enzymes used in biosensor fabrication are oxidases that consume dissolved oxygen and produce hydrogen peroxide [see Fig. 1(a)]. Enzymes have been immobilized at the surface of the transducer by adsorption, covalent attachment, entrapment in a gel or an electrochemically generated polymer, in bilipid membranes or in solution behind a selective membrane. Several reviews of enzyme immobilization have been published. [9-15] Enzymes are commonly coupled to electrochemical and fiber optic transducers.

Antibodies

Antibodies are proteins that show outstanding selectivity. They are produced by b-lymphocytes in response to antigenic structures, that is, substances foreign to the organism. Molecules larger than about 10 kDa can stimulate an immune response. Smaller molecules like vitamins or steroids can be antigenic (also called haptens) but they do not cause an immune response unless they are conjugated to larger ones like bovine serum albumin. Many

antibodies are commercially available and commonly used in immunoassays. Antibodies are usually immobilized on the surface of the transducer by covalent attachment by conjugation of amino, carboxyl, aldehyde, or sulfhydryl groups. The surface of the transducer must be previously functionalized with an amino, carboxyl, hydroxyl, or other group. A review of conjugation techniques can be found elsewhere. [16] Antibodies share similar limitations with enzymes. Furthermore, binding may not be reversible and regeneration of the surface may require drastic changes in conditions like low pH, high ionic strength, detergents, etc. Therefore, efforts are being made to produce low cost, single use sensors. Probably the main potential advantage of immunosensors over traditional immunoassays is that they could allow faster and in-field measurements. Immunosensors usually employ optical or acoustic transducers.

Microbes

The use micro-organisms as biological elements in biosensors is based on the measurement of their metabolism, in many cases accompanied by the consumption of oxygen or carbon dioxide, and is, in most cases, measured electrochemically. [17] Microbial cells have the advantage of being cheaper than enzymes or antibodies, can be more stable, and can carry out several complex reactions involving enzymes and cofactors. Conversely, they are less selective than enzymes, they have longer response and recovery times, [18] and may require more frequent calibration. Micro-organisms have been immobilized, for example, in nylon nets, [19] cellulose nitrate membranes, [20] or acetyl cellulose. [21] Other biological elements such as animal of vegetable tissue and membranes as well as organelles and nucleic acids have been researched but are out of the scope of this article. A summary of some biological elements and transducers used in the fabrication of biosensors is presented in Table 1.

Table 1 Biological elements and transducers commonly used in the fabrication of biosensors

Biological elements	Transducers
Enzymes	Electrochemical
Antibodies	Amperometric
Receptors	Potentiometric
Cells	Ion selective
Membranes	Field effect transistors
Tissues	Conductimetric
Organisms	Optical
Organelles	Fiber optic (optrode)
Nucleic acids	Surface plasmon resonance (SPR)
Organic molecules	Fiber optic SPR
	Calorimetric
	Heat conduction
	Isothermal
	Isoperibol
	Acoustic
	Surface acoustic wave
	Piezocrystal microbalance

Electrochemical

Amperometric and potentiometric transducers are the most commonly used electrochemical transducers. In amperometric transducers, the potential between the two electrodes is set and the current produced by the oxidation or reduction of electroactive species is measured and correlated to the concentration of the analyte of interest. Most electrodes are made of metals like platinum, gold, silver, and stainless steel, or carbon-based materials that are inert at the potentials at which the electrochemical reaction takes place. However, because some species react at potentials where other species are present, either a selective membrane is used or an electron mediator that reacts at lower potential is incorporated into the immobilization matrix or to the sample containing the analyte. Potentiometric transducers measure the potential of electrochemical cells with very low current. Field effect transistors (FET) are potentiometric devices based on the measurement of potential at an insulator–electrolyte interface. The metal gate of a FET can be substituted by an ion selective membrane to make a pH transducer (pH ISFET). Enzymes have been immobilized on the surface of such pH ISFET to produce enzyme sensitized field effect transistors (ENFET). A complete description of such sensors can be found elsewhere. [22]

Optical

Fiber optic probes on the tip of which enzymes and dyes (often fluorescent) have been co-immobilized are used. These probes consist of at least two fibers. One is connected to a light source of a given wave length range that produces the excitation wave. The other, connected to a photodiode, detects the change in optical density at the appropriate wavelength [see Fig. 1(b)]. Surface plasmon resonance transducers, which measure minute changes in refractive index at and near the surface of the sensing element, have been proposed. Surface plasmon resonance (SPR) transducers have been proposed. SPR measurement is based on the detection of the attenuated total reflection of light in a prism with one side coated with a metal. When a p-polarized incident light passes through the prism and strikes the metal at an adequate angle, it induces a resonant charge wave at the metal/dielectric interface that propagates a few microns. The total reflection is measured with a photodetector, as a function of the incident angle. For example, when an antigen binds to an antibody that is immobilized on the exposed surface of the metal the measured reflectivity increases. This increase in reflectivity can then be correlated to the concentration of antigen. The basic theory of SPR excitation and some examples of its application to biosensors are presented elsewhere. [23, 24] A few SPR biosensors have been commercialized but no compact inexpensive portable device is available yet.

Acoustic

Electroacoustic devices used in biosensors are based on the detection of a change of mass density, elastic, viscoelastic, electric, or dielectric properties of a membrane made of chemically interactive materials in contact with a piezoelectric material. Bulk acoustic wave (BAW) and surface acoustic wave (SAW) propagation transducers are commonly used. In the first, a crystal resonator, usually quartz, is connected to an amplifier to form an oscillator whose resonant frequency is a function of the properties of two membranes attached to it. The latter is based on the propagation of SAWs along a layer of a substrate covered by the membrane whose properties affect the propagation loss and phase velocity of the wave. SAWs are produced and measured by metal interdigital transducers deposited on the piezoelectric substrate as shown in Fig. 1(c). [25]

Calorimetric. Calorimetric transducers measure the heat of a biochemical reaction at the sensing element. These devices can be classified according to the way heat is transferred. Isothermal calorimeters maintain the reaction cell at constant temperature using Joule heating or Peltier cooling and the amount of energy required is measured. Heat conduction calorimeters measure the temperature difference between the reaction vessel and an isothermal heat sink surrounding it. Using highly conducting materials ensure quick heat transferred between the reaction cell and the heat sink. Finally, the most commonly used is the isoperibol calorimeter that also measures the temperature difference between the reaction cell and an isothermal jacket surrounding it. However, in this case the reaction cell is thermally insulated (adiabatic). This calorimeter has the advantage of being easily coupled to flow injection analysis systems. [26]

Bioaffinity Monitoring

The first category of biosensor mentioned above was pioneered by Ingemar Lundstro¨m and his team at Linko¨ping University together with the BIAcoret Company, then a spin out from Pharmacia and now owned by GE. The early history of this device is excellently presented by the BIAcore engineers in a contemporary review[2] and the essential philosophies of their design can been seen in subsequent generations of machines based both on Surface Plasmon Resonance (SPR) and other detection technologies such as the resonant mirror, diffraction gratings and interferometry. The SPR machines harness a well-established physical phenomenon exhibited by totally internally reflected light at an optical interface coated with a thin layer of metal or semiconductor. The incident resonant angle at which surface plasmons are excited by plane polarized light is critically dependent on the Refractive Index at the interface of the metal (normally gold) layer with a sample. The addition of an affinity element, such as an antibody, nucleic acid sequence or biological receptor, enables biological binding interactions to be monitored in real time. The companies producing these instruments have paid considerable attention to optimizing the immobilization matrix on the sensing chip, refining the microfluidics and creating user friendly software. The net result has been a series of highly successful research machines that have found particular utility in drug discovery and life science research.[27] Some of the strongest patent protection was routed in the immobilization chemistry, using thiol immobilization of carboxyl groups to anchor carboxy-methylated dextrans to the surface of the sensing chip, followed by ethyl(dimethylaminopropyl) carbodiimide (EDC) and N hydroxysuccinimide (NHS) activation of the dextran matrix to immobilize the desired ligand. This endowed the surface with exceptionally high receptor loading capacity, good biocompatibility and charge effects that helpedreduce non-specific binding. Given the somewhat specialist market for these instruments, prices have remained high for both the disposable chips and the instrument while the resulting turnover remains moderate i.e. a total in the region of US$100 million per annum. However, this simple number belies the economic importance of these biosensors given that they are a very significant enabling technology for biological research and the pharmaceutical industry. Recently, there has been a proliferation of machines in this sector seeking to capitalize on the pressure to reduce animal experimentation and to exploit advances in the understanding of areas such as G protein-coupled receptors (GPCRs) and pluripotent cells. The 800 or so GPCRs known in humans have long been a key target for the pharma industry given their central role in cell physiology and pathophysiology. Around 40% of all marketed drugs target GPCRs and biosensor-based screening platforms play a vital role in further elucidation of GPCR signaling. The original SPR instruments now face stiff completion from other optical instruments based on resonant waveguide gratings, such as the Corning EPICt, and a range of electrical impedance based systems such as CellKeyt (Molecular Devices) and XCELLigencet (Roche). These label-free techniques have facilitated the discovery and characterization of agonists, antagonists and inverse agonists alike and offer an

extremely convenient tool for drug discovery.[28]Such binding assays are complimented by whole cell-based biosensors, which originated as simple mono-cultures immobilized on a sensor to measure Biochemical Oxygen Demand (BOD) and evolved into sophisticated microarrays produced using micro-contract printing. Rapid developments in stem-cell technology provide another stimulus to this technology. Long-term research seeking to produce artificial organs has a more immediate application in the use of appropriately instrumented synthetic organs for drug testing. These alternatives to animal testing have become a prerequisite for the cosmetics industry and are also likely to have a major impact on pharmaceutical development in the future. SPR devices have also risen to the demand of array-based sensing with the introduction of SPR imaging, which permits multiple assay spots to be monitored simultaneously. [29] Commercial instrument on the market are capable of measuring 900 spots and light activated peptide synthesis can deliver 2500 assay regions. The target now is to reach 7500 simultaneous assays for applications such as epitope mapping and metabolomics. As might have been expected, there have been various attempts to reduce the instrument costs associated with SPR and similar measurement technologies. A notable attempt was the introduction of the inexpensive SPR chip by Texas Instruments (USA) and several companies have developed instruments in the mid-range price bracket of tens of thousands of US dollars, as compared to the hundreds of thousands required to purchase the high-end machines. So far, however, these cheaper alternatives have met with limited success, with the typical user preferring the benefits of sophisticated fluidic handling, ease-of-use and high throughput. In the biosensors research community, however, a small revolution has been developing with the widespread adoption of localized surface Plasmon resonance (LSPR) based on gold nanoparticles.[30] LSPR requires only a light source and a spectrophotometer to measure the wavelength change in the reflected light from a nanostructured material or surface. While the penetration depth of the plasmon field for SPR is between 200–1000 nm, it is 15–30 nm for LSPR. Hence bulk effects have less influence. LSPR is also responsible for the electromagnetic field enhancement that underpins surface-enhanced Raman spectroscopy, which can identify specific target molecules based on their unique vibrational signatures. LSP is created when free electrons on metal nanoparticles are excited by electromagnetic radiation thus causing polarization of the charges of the particle-free conduction electrons. Depending upon number of free electrons, the dielectric function and dielectric coefficient of local medium gives rise to intense optical extinction. The optical extinction is based on a combination of absorption and scattering, where absorption depends upon the concentration of particles and scattering is proportional to the square of the particle concentration. The highest absorption for oscillating dipoles occurs at their resonance frequency, which for gold nanoparticles, lies in the non-infrared region of the light spectrum. The exact LSPR spectrum is dependent upon nanoparticle shape, size, inter-particle distance, dielectric properties and, most importantly, on the dielectric properties of surrounding medium. This latter property plays the key role in the development of biological sensors, along with the ability to tailor the spectral signature of the spectrum by modifying the shape and size of the nanoparticles. Most frequently,

we see gold nanorods used for these systems, with thiol modification of the gold surface followed by EDC/NHS immobilization chemistry to add antibodies, aptamers or other modified nucleic acids. Various gold nanostructures have been explored including nano-rods, nano-rings, nano-crescents and even nanoholes. Some of the most elegant recent plasmonic materials work has been on angular nanostructures such as cubes and pyramids, where the slope of the dependence of the LSPR peak on size, increases as the edges become sharper. Bipyramids thus yield higher sensitivity affinity assays than nanorods and nanospheres and can be stabilised for biosensing purposes with the aid of bilayer lipid membranes. While label-free detection is extremely appealing due to its inherent simplicity, labels and dyes continue to offer high sensitivity. Dye label-enhanced SPR, for example, can extend the capability of a conventional instrument to detect and quantify the binding of several molecules simultaneously. Multi-colour analysis at a single wavelength can be used in genomics for partial sequencing or for proteomic studies. An elegant single-cell fluorescent approach that is amenable to decentralized application, using fluorescently-coded microspheres, was very recently published by Fran Ligler's group from the Naval Research Laboratory in Washington.[31] They used a spinning magnetic trap to automate clinical sample processing prior to quantitative pathogen detection with a microflow cytometer. Another highly novel fluorescent biosensor for the detection of vimentin serine phosphorylation was recently described by Jeong et al.8 They described the use of multicolour ''Quenchbodies'' which depend on the removal of a quenching effect from the intrinsic tryptophan residues in a carboxytetramethylrhodamine dye attached to the N-terminal region of a single-chain antibody when antigens bind. Such new work adds to a wide body of literature describing very sensitive optical assays, often combined with waveguide devices, to deliver practical systems for distributed diagnostics and environmental monitoring. Last, but not least in the label-free affinity assay armoury, come piezoelectric and micromechanical devices. The quartz crystal microbalance is a well-established tool that correlates mass changes with frequency of oscillation in air or gases and monitors complex viscoelastic changes in liquids when a biological receptor binds its complimentary partner. Instrumental design has been refined to the point that reliable commercial systems are now available on the market both for research and for measuring, for example, drugs of abuse and explosives for border control (e.g. Biosensor Applications, Solna, Sweden). These machines have capitalized on the experience gained from designing the SPR instruments to offer advanced microfluidics, friendly user interfaces and easy-touse sample application. Surface acoustic wave devices have also been used for both gas sensor and biosensor applications, seeking particularly to take advantage of the higher sensitivity in liquids offered, in principle, by operating at higher frequencies. The comparative advantages of mechanical biosensors were recently reviewed by Arlett et al. [32] and they highlight the challenges faced by systems such as oscillating-beam devices microfabricated in silicon, especially with respect to nonspecific binding. Clever applications that circumvent such limitations are one expedient, such as measuring antibiotic activity by following the comparative growth of microorganisms immobilized on an array of oscillating beams; here, nonspecific binding is no longer

a major hurdle since the measurement is comparative. At the extreme end of experimental elegance using a label-free technique comes a delightful paper from Silberberg et al. This team was able to functionalize a 200 nm needle with antibodies, insert it into a living cell and measure the unbinding forces using atomic force microscopy. This is hardly an example of distributed diagnostics, but does illustrate how far we have come with biosensors for research applications. These researchers were able to use their technique to examine the effect of cytoskeleton disrupting drugs and to observe effects that were hardly detectable using optical or fluorescence methods. [33]

Biosensors for Diabetes: A Special Case

Blood glucose measurement for the management of diabetes comprises approximately 85% of the world market for biosensors. This remarkable success both demonstrates the utility of the technology and begs the question whether this is the only opportunity really worth pursuing. The answer depends to a large extent on aspirations. A large company normally needs to see a market in excess of US$100 million before investing in a high-risk development programme. Besides glucose and pregnancy testing, other large-scale consumer markets for inexpensive biosensors have so far proved elusive. However, smaller companies have either been content to pursue opportunities with smaller market sizes or to develop niche instrumentation for R&D. Indeed, most of the innovation in biosensors has been by start-ups, which have been willing to take the risk of breaking new ground in the hope of a trade sale in the medium term or a modest long-term income. The classic example of this was the development of mediated amperometric glucose sensors for home use by people with diabetes. The first such device to reach the market was launched by a small start-up, initially comprising three people and called Genetics International and later MediSense, which was incorporated in Boston, USA and had its R&D labs initially in Cranfield and then Abingdon in the UK. It capitalized on the joint work of two groups at Cranfield and Oxford Universities to bring ferrocene-mediated electrochemistry of GOx [34] to the market in 1987. The company and the technology were finally sold to Abbott (USA) in 1996. Next on the market, with a ferricyanide-mediated GOx version, was Boehringer Mannheim (latterly Roche) leveraging technology purchased from a small company in the USA called Tall Oaks. This was closely followed by a Japanese launch from another SME, Kyoto Daiichi Kagaku (latterly Arkray), using a similar mediated format, but with capillary fill to draw in the blood from a pin-prick sample. Many of the original team from MediSense, then joined Inverness Medical and produced a fourth mediated system, which was purchased by Johnson & Johnson Life scan. The so called ‘‘big four’’ (Roche, Johnson & Johnson, Abbott and Bayer) in the glucose sensing market, which between them dominated 90% of sales, was completed when Bayer imported the capillary-fill mediated devices from Japan. This classical case history shows how a small group of a dozen scientists operating out of a small, lightly equipped laboratory could sow the seeds for a multi-billion dollar industry. So what were the essential elements that we can see, in retrospect, drove this technology to commercial success? First and foremost, is the extraordinary need and demand from the diabetic community? The mid 1990’s saw the unequivocal proof that careful monitoring and control of blood sugar could reduce the horrific side effects associated with chronic diabetes, i.e. blindness, amputation, kidney and organ failure, with the publication of the Diabetes Control and Complications Trial (1993). The need to monitor blood sugar up to several times a day, drove people with diabetes to seek the most convenient technology, and the chronic nature of the disease turned them into a highly discerning customers. Hence, relatively modest improvements were widely appreciated and drove the race to supply the best technology to this lucrative market.

And what was that technology? The academic literature largely credits ferrocene electrochemistry with the setting the scene for home-use amperometric biosensors and it is true that the patent claiming this technology is arguably the only one that has truly stood the test of time. Interestingly, the original UKfiling focussed on chloranil as a mediator and ferrocene was only added as a supplementary filing some months after the original filing date. It is also true that ferricyanide predated ferrocene as a mediator for amperometric biosensors (in laboratory analysers produced by Hoffman La Roche in the late 1970's) and subsequently became the mediator of choice for the larger proportion of commercially successful devices. A second technology driver was clearly machine manufacture. Prior to the MediSense device, all commercial biosensors had been hand fabricated, and this was hardly a suitable means to produce the billions of disposable sensors required per year. The MediSense team pioneered the adaptation of screen printing for the production of disposable biosensors printed on a polyethylene terephthalate (PET) substrate. Despite its huge enabling role, this production technology was never specifically patented for this use by the team, although it did feature as part of claims in numerous subsequent patents from both MediSense and its competitors. Thirdly, there was the instrument design. The original MediSense ExacTecht was accommodated in a pen-shaped device and is still one of the smallest instruments produced. Indeed it proved to be too small, requiring more dexterity and visual acumen than the market wished to accommodate. A fourth element of eventual success, which did not come from the original MediSense team, was the concept of capillary fill. This idea was patented by another Bedfordshire-based lab in the UK, Unilever, and provided a more convenient means of applying a small pin-prick blood sample to a sensing strip. More importantly, however, it reduced problems associated with evaporation from tiny samples, which changes the volume of liquid and cause a chilling effect; both these parameters have highly significant effects on the accuracy of the device. The ''Shanks'' capillary fill patent subsequently earned Unilever a small fortune in royalties, despite the company itself never launching a commercial glucose sensor into this market.[5] Many more innovations followed and these are documented principally in patents rather than the conventional academic literature. These include clever designs to automatically switch on the instrument when a strip is inserted, ways to correct for variations in ambient temperature, methods to detect when the sample is introduced into the device and to show when the device is completely full, and sometimes by integration of these parameters, ways to compensate for the variable haemoglobin content of blood samples, often by the indirect measurement of viscosity. The mediated amperometric glucose sensor is now considered a mature product by many companies, which means that further development of the chemistry has stalled and the main focus is on the extraction of profit from the investment already made. Engineering advances continue, however, and there has been a major drive towards further integration of the sampling, sensing and data processing elements of these devices. Commercial products are now available on the market with sensors packaged in disks, drums and, most recently, as a continuous tape, so that the user no longer needs to unpack and load a separate strip every time they wish to make a measurement. The

pain associated with sampling has long been an obstruction to patient compliance and various attempts have been made to reduce the discomfort associated with taking a blood sample and to improve the transfer of sample to the machine. A product was briefly offered on the market by Pelikan Technologies (Palo Alto, USA), which sampled blood in a near-painless fashion using an electromagnetic sampling system, but it proved too expensive to sell in sufficient numbers. The current state-of-the-art relies on being able to make measurements on very small blood samples (1–3 mL), cam-driven lancets and convenient ergonomics, such as the co-location of the lancing device. The other important recent refinement has been the imaginative harnessing of information technology. This has ranged from the creation of numerous helpful apps to the integration of glucose sensing devices with the iPhonet in the form of a plug-in accessory. Arguably the most imaginative of these ideas was Bayer's DIGITt, which interfaced a glucose meter with a Nintendo games station to encourage children with diabetes to monitor their blood sugar by rewarding them with points to obtain items and unlock new game levels.

Fig. 2 Glucometer

Printed Biosensors and Biosensing Systems

The adaptation of screen printing for the production of enzyme electrodes, by the MediSense team in the early 1980's, proved to be a decisive element in the success of mediated electrochemical devices in the home blood glucose monitoring market. Until then, biosensors had been wholly hand made, and could not possibly address a market requiring billions of devices a year. Coupled with the use of proprietary mediators and patented capillary-fill designs, machine fabrication of enzyme electrodes enabled the paradigm changing switch from reflectance photometry to the electrochemical devices that now dominate this market. Today, approximately half of the electrodes used in disposable glucose sensors are screen printed using curable polymer inks, while the remainder are produced using a combination of vapor deposition of thin layers of metal such as palladium, followed by laser ablation to pattern these into individual electrodes. Additional printing steps, drop-on-delivery and/or lamination results in a final sensor, which can cost around 2–6 US cents per strip to make, when produced by the millions. Today, organic chemistry is having a renewed influence on biosensor design beyond the innovative formulation of polymers for printing inks. Printed electronics has emerged as an exciting alternative to silicon technology for the production of very low-cost electronic devices, amply illustrated by the proliferation of RFID tags in our shops and supermarkets.[35] Added to this comes advances in organic electronics, which attempt to replace conventional, top-down microfabrication with a bottom-up molecular approach. This heady mixture of organic chemistry and mass production challenges the now conventional paradigm of a disposable strip coupled to a pocket sized meter. Even in bulk, the average meters cost between US$7–10 to make, with some more elaborate versions costing as much as US$90; the current market model generally requires the manufacturer to effectively give this meter way as a loss leader. So why not print the whole device? All-printed Mn–ZnO batteries with a capacity of 1–10 mA h can already be made to power microsensors and for other applications such as RFID tags, transdermal drug delivery, cosmetic patches and smart packaging. These are available as commercial products such as the SoftBatteryt from Enfucell (Finland). Monochrome emissive or reflective digital displays can be printed on both paper and plastic, requiring around 1–3 V for a reflective display and 110 V for an emissive display. A recent report describes a much simpler way to manufacture active matrix displays that is particularly suited to this application. Electrochromic display pixels are printed together with their corresponding organic electrochemical transistors on opposite sides of a PET substrate, thus both components share a common electrolyte and conductive polymer. [36]

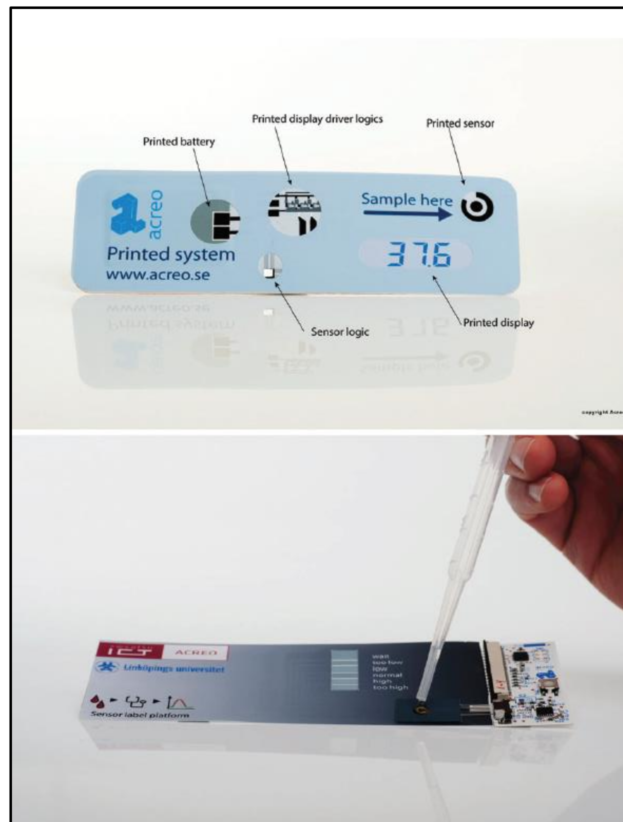

Fig. 3 All-printed biosensing system. Top: shows the concept of an all-printed biosensing system, where not only is the amperometric sensor printed, but all the associated elements such as battery, display and circuitry are printed on a single sheet of PET and then laminated in an appropriate casing. Below: shows the reality to date, a prototype functioning system resulting from collaboration between Acreo AB and Linko¨ping University. Glucose concentration can be measured in a few seconds and observed via the printed display using this device, powered by a printed battery. Rudimentary silicon circuitry can be seen to the right of the picture, but this could be readily integrated into a tiny, inexpensive silicon chip. This device is being used as a concept demonstrator to develop a range of new products, principally for medical diagnostics.

Non-volatile and flexible memories based on ferroelectric polymers offer retention times of more than 10 years and read/write cycles of 109 and can be mass produced using roll-to-roll techniques and integrated with logic elements, sensors, batteries and displays. In keeping with the trend to interface biosensing systems with telecommunication, thin film aluminium or copper antennae are also available, operating in the 1 kHz–1 GHz range. All-printed diodes are at an advanced stage of development and electrochemically and electrolyte gated OFETs operating at 0.5–1.5 V offer switch times of 106 to 102 s. A state-of-the-art combination of these technologies allows us to formulate an all-printed sensing instrument where everything is produced on a simple sheet of PET laminated into a plastic case to form a credit-card like device. In practice, current printed circuit technologies fall a little short of requirements and some element of silicon technology is still required to process the signals, but this can be reduced to the size of a minute chip barely visible to the eye. An example of such a working demonstrator is shown in Fig. 3.

Implantable Biosensors and Non-Invasive Monitoring

An alternative evolutionary line to the disposable strip sensor is the implantable biosensor. This was envisaged by Leyland C. Clark in his original paper in 1962 and realized in a practical form, as a needle-type subcutaneous electrode by Shichiri et al., as early as 1982. However, it was not until 2005 that people with diabetes could purchase these devices for personal use. [37] Medtronic (USA) was the first company to sell a sensor for continuous glucose monitoring and this was marketed as the Guardiant. The sensor is self-implanted in the abdomen and uses classical amperometric detection of hydrogen peroxide, produced as a result of the oxidation of glucose catalyzed by GOx. Initially, these sensors needed to be changed every 3 days and delivered one reading every 5 minutes, following a 2 hour run-in period. In 2006, another US company, Dexcom, launched a sensor with a 7 day use life. Implantable sensors have been received well by many people with Type I diabetes (insulin dependent diabetes) and others who have difficulty managing their disease, but they are severely technically restricted. The US Federal Drug Administration (FDA) still requires that a finger stick blood sample be taken before acting on the result from a continuous sensor to administer insulin and the technically exciting possibility of hooking up a continuous sensor to a commercially-available automated insulin infusion pump is not permitted. In Europe, some degree of automation has just recently been approved, allowing the Medtronic device to be used to shut off insulin if the blood sugar drops too low, thus avoiding the risk of hypoglycaemia, which is one of the greatest fears of people with Type I diabetes. These sensors, while extremely useful, are still not reliable enough; at the heart of the problem lies the difficultly of implanting an active device in the body. Perfect biocompatibility has never been achieved and, at best, implants are tolerated by the body. Add to this, the requirement to maintain consistent diffusion of an analyte such as glucose across a membrane, while the multifarious defense mechanisms of the body coat the sensor in proteins and, eventually, a fibrous capsule, and the analytical challenge can begin to be appreciated. Amperometric sensors are dynamic, catalytic devices that operate by consuming their target substrate. If the passage of that analyte is modified unpredictably by the body, it becomes extremely difficult to produce a reliable, continuous signal that reflects the true, systemic concentration of glucose. These fundamental problems are unfortunately often overlooked by enthusiastic proponents of biosensors and bioelectronics systems, such as biological fuel cells, which are equally problematic when operated in vivo. Even more contentious, is the area of non-invasive alternatives for blood glucose measurement. While limited commercial success has been achieved with minimally invasive techniques such as reverse iontophoresis, which enables small volumes of interstitial fluid to be extracted through the skin, non-invasive technique such as near-infra red spectroscopy, have so far proven dismal commercial failures. However, such is the demand from marketers, who have clearly identified that avoiding having to take a blood sample would be a paradigm changing development, many millions and possibly billions of dollars have been spent in pursuit of this dream. We conducted a survey three years ago

and identified 96 companies that believed they would be on the market with a non-invasive glucose sensing device within two years, none have succeeded in delivering on this aspiration. Recently, the Qualcomm Tricorder X PRIZE has been announced, offering a US$10 million in prizes for ‘‘Integrated diagnostic technology, [which] once available on a consumer mobile device that is easy to use, will allow individuals to incorporate health knowledge and decision making into their daily lives.’’ The challenge includes diabetes diagnosis as a target and expects the device to be non-invasive. Numerous technologies have already been proposed and developed over the past 20 years, including breath analysis (including particle analysis in breath), skin volatiles, Raman spectroscopy, optical coherence tomography, impedance analysis and smart tattoos. However, none present sufficiently reliable clinical data for this author to be confident in predicting their likely success. Given the size and importance of the diabetes market, paradigm-changing technologies are quick to grab the headlines. If non-invasive monitoring became available it would signal a major shift in the diagnostics industry, while an even more disruptive technology potentially exists with regenerative medicine and pluripotent cells. Over the next decade, teams are seeking to demonstrate that stem cell-based cell replacement therapy is an effective and safe treatment for diabetes that may, in time, make glucose monitoring completely redundant. Companies such as ViaCyte (San Diego, California) and BetaLogics (Vancouver, Canada) are working towards the use of stem cells differentiated into pancreatic beta cell precursors, which can then be subcutaneously or abdominally implanted. It has been shown that such precursor cells mature into endocrine cells that secrete insulin and other hormones in a regulated manner to control blood glucose levels in mice. If successful in humans, this would effectively represent a ‘‘cure’’ for diabetes that would render the artificial pancreas (a biosensor interfaced to an insulin pump) redundant.

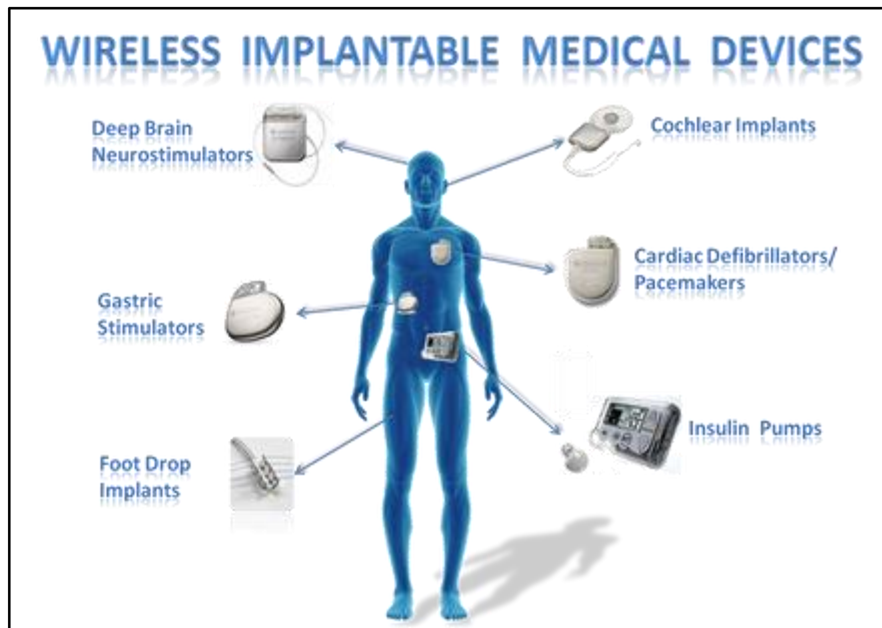

Fig. 4 Implantable Biosensors

Emerging Technologies

Much has been written about glucose sensors due to their dominance in the field of biosensors, but this example should really be viewed largely a model that can be copied for hundreds and potentially thousands of alternative analytes. Admittedly, GOx has proved a remarkable useful, versatile and robust enzyme for incorporation into biosensors, but it has been superseded by alternative, engineered proteins based on quinoprotein glucose dehydrogenase in recent years. A wide range of other catalysts and affinity elements are now available to the biosensor technologist to create a diverse range of portable and lab-based instruments using electrochemical, optical or other transducer technologies. Genetic manipulation of catalytic proteins has created hybrid enzymes combining the best redox centers with protein shells exhibiting the greatest affinity and specificity for the desired metabolite. Meanwhile, the art of stabilization has improved, using polyelectrolytes and sugars to stabilize dry reagents and yield long, stable shelf lives. While electrochemical biosensors have made most impact in the form of enzyme electrodes, electrochemical immunoassay has delivered some remarkable results and a few minor commercial products. Once the initial concept of using an enzyme label had been established, workers began to experiment with various biochemical amplification schemes. By increasing incubation times and reducing volumes, very low concentrations of analyte could be recorded in the femtomolar and even attomolar range. These and other label-based techniques lend themselves to incorporation into the lateral-flow formats that have been so successfully realized for pregnancy test strips. Commercial applications in this area were severely curtailed for 20 years due to the aggressive position taken by the original patent owners, but now that these have expired, we see numerous systems being launched with electrochemical, optical and even magnetic transducers, although the later has failed to live up to its exciting early promise. A second important electrochemical immunoassay technology exploited the Field Effect Transistor (FET) by monitoring the charge effects resulting from biochemical interactions at the gate of the transistor. This original work in the late 1970's and early 80's has undergone something of a revival, stimulated by the recent availability of nanomaterials exhibiting extraordinary electronic properties such as the nanowire, enabling highly sensitive detection of bioaffinity interactions.[38,39] The mainstay affinity element, the antibody, has likewise been modified to reduce excess baggage (antibody fragments) and improve selectivity (monoclonal antibodies). Moreover, alternatives in the form of affibodies, peptides, aptamers and molecularly imprinted polymers have emerged as viable ways to construct affinity sensors. Aptamers, in particular, have attracted considerable attention since their simultaneous discovery in 1990 by Larry Gold and Jack Szostak's teams and automation of the technique by Andy Ellington's lab. While much attention is focused on aptamers as an alternative to antibodies for therapeutic applications, these randomly selected binding sequences of nucleic acid also provide a powerful analytical tool. The now classical process of systematic evolution by exponential enrichment (SELEX) provides novel binding partners that can be attached to

electrochemical, optical, piezoelectric or other micromechanical transducers, such as oscillating beam structures, to provide a new range of versatile affinity sensors. These chemically synthesized receptors can be readily modified with a tail for immobilization to gold or carbon surfaces and can display conformational changes which can be harnessed in the transduction process. They are particularly useful when good antibodies are scarce or difficult to produce, such as in the case of detecting toxins. Although now widely used to make material for both medical and environmental sensors, the SELEX process itself is less than perfect for the purpose. Due to volume constraints, SELEX routinely only searches about 1014 of a possible 1018 20–60 mers. In addition, nonspecific recognition of the support may occur and the polymerase chain reaction used in the process tends not to amplify secondary structures. In order to help improve the design of aptamers, we recently reported a retrospective computational docking study of the thrombin aptamer (TBA) 50 -GGTTGGTGTGGTTGG-30. [40] TBA interacts specifically with the fibrinogen recognition exosite through the two TT loops. We used this computational approach to confirm the results observed in SELEX as a first step in reducing the number of structures screened in the first pool of potential binding agents. In this way, we hope to both reduce the time taken to find an appropriate aptamer and increase the chances of finding the best possible sequence. Another non-immunoglobulin protein that can be used in sensors is the affibody, based for example, on the immunoglobulin binding B domain of protein A from the bacteria Staphylococcus aureus. Genetic engineering of this region results in an analogue known as the Z domain, further modification of which, via mutagenesis, leads to the production of a range of high affinity molecules that can be used as alternatives to antibodies [41] in affinity sensors and assays. For example, we have obtained promising results using such affibodies to detect the overexpression of human epidermal growth factor receptor 2 (HER2), which is observed in 20–30% of breast cancer cases. Such semi-synthetic receptors may also be used in combination to create hybrid sensors where the initial capture molecule maybe, for example an aptamer, but a second recognition element such as an enzyme-labelled monoclonal antibody is used in a sandwich format to enhance the specificity and sensitivity of detection. Such combinations can also be combined with magnetic beads to aid separation from complex media such as blood, milk or turbid environmental samples. Progressing from semi-synthetic to fully synthetic analogues of biological receptors could furnish a new generation of sensors that display the desired sensitivity and specificity of biosensors, but lack their consequential instability and, in some instances, irreproducibility. A variety of synthetic receptors have been explored for this purpose, but one of the most promising approaches is the use of molecularly-imprinted polymers.[42] Pioneered in the 1970's by Gu¨nther Wolf and Klaus Mosbach, in covalent and non-covalent forms, respectively, this form of template-assisted synthesis may have finally come of age. The basic concept is one of allowing selected functional monomers to self-assemble around a target analyte, followed by polymerization and subsequent removal of the template. Reversible interactions between the template and the polymerisable functional monomer may involve reversible covalent bonds, covalently attached polymerisable binding groups that are activated for non-covalent

interaction by template cleavage, electrostatic interactions and/or hydrophobic or van der Waals interactions. The resulting structure contains cavities which reflect both the shape and chemical functionality of the target species. Early workers produced bulk polymers that had to be ground up prior to use, thus destroying some recognition sites and providing variable access to others. In addition, it is often difficult to remove the template from bulk polymers and they possess a range of binding sites displaying a variety of binding constants. While having some utility as an affinity separation medium, these polymers lacked the sophistication required for more direct analytical applications. Key advances in the field have been the use of grafting to produce relatively thin molecularly imprinted layers on the surface of transducers, nano-structuring and the development of a range of techniques to create imprinted polymer nanoparticles, including precipitation, mini-emulsion, micro-emulsion and core–shell emulsion polymerization, and the core–shell grafting approach.[43] These imprinted polymer nanoparticles effectively behave as plastic antibodies, offering close to a single binding site per particle and being small enough to be soluble. However, to find wide applicability, it is essential to have a reliable manufacturing process that results in particles with consistent properties. A very recent paper reports such a technique based on an automatic solid-phase photo-reactor. [44] At the core of the reactor is the immobilized template, which can be a small molecule, peptide or whole protein. This ensures that surface-confined imprints are formed only on one face of the nanoparticles and that the template can be reused for the synthesis of subsequent batches of imprinted polymer. The reactor integrates template-directed synthesis with affinity separation, a combination which ensures the production of monoclonal-type molecularly-imprinted nanoparticles. Batches of 100 mg of particles can be produced in each six-hour cycle with templates including melamine (KD = 7 1010 M), vancomycin (KD = 1.9 1010 M), a model peptide (5.5 1012 M) and various proteins (KD = 1011–109 M for trypsin, pepsin, amylase, peroxidase). The affinity measurements stated were obtained using a SPR sensor (Biacore 3000). This generic approach to the automated synthesis of polymer nanoparticles provides material of ‘‘monoclonal’’ quality produced in a consistent and reproducible manner, suitable for use as a direct replacement for antibodies and in a variety of applications. It offers speed of synthesis and multiple batches of polymer nanoparticles can be produced in 24 h under continuous computer control. Template re-use and in-built affinity separation also ensure consistent, economical and high-quality of production. Another relatively new imprinting approach that is particularly useful for electrochemical sensors uses electropolymerisation of a thin film of the recognition element directly on the sensor surface.[45] The ability to precisely control the thickness of the layer and the fact that it is generated in situ, makes this an attractive alternative. In some so far unpublished work, we showed that a troponin sensor could be prepared by electropolymerisation of o-phenylenediamine on a gold electrode in the presence of troponin as a template. Measurements were performed in the presence of 5 mMol L1 K3Fe(CN)6 and the current generated was inversely proportional to the analyte concentration, since the measurement depends on occlusion of the electrode surface by the template. The resulting molecularly imprinted troponin biosensor could be used to detect

cardiac injury, offering benefits in terms of cost effectiveness, storage stability, sensitivity and selectivity. Even sequence-specific imprints of gene point mutations can be made using electropolymerisation.[46] Tiwari et al. described an ss-DNA biosensor, fabricated by electropolymerisation on an indium-tin oxide coated glass substrate, using single-stranded oligodeoxyribonucleotides (ss-ODNs) as the template and o-phenylenediamine as the functional monomer. A linear response to 0.01 to 300 fM was obtained with a response time of 14 seconds, thus providing a method for the detection of p53 gene point mutation using sequence-specific polymer electrode. Molecular imprinting originally gained traction using small molecules as templates, but more recent work has proven the usefulness of the technique for larger molecules, using strategies such as epitope imprinting. In common with other alternatives to antibodies, such as aptamers and peptide arrays, imprinting offers the ability to tackle highly toxic analytes and other targets that it is difficult to raise antibodies to. It also provides a route to tackle problematic assays, such as the detection of autoantibodies associated with autoimmune diseases, since these antibodies would simply bind nonspecifically any assay antibody included as part of a protocol. Most imprinted sensors have sought to mimic the immunosensor, but an intriguing possibility is the construction of an artificial enzyme electrode, where the natural enzyme is replaced by an imprinted polymer. Berti et al. reported on quasi-monodimensional polyaniline nanostructures for enhanced molecularly imprinted polymer-based sensing. An imprinted mimic of the tyrosinase active site was combined with a nanostructured NPEDMA polymer layer, which mediated the conduction of electrons between the catalytic sites and the electrode. The polymer was nanostructured by sputtering a gold layer on one side of an alumina nanoporous membrane as a mould. Electrochemical polymerization of the aniline monomer was achieved on the sputtered membrane by cycling the voltage between 0.4 V and +1.0 V (vs. Ag/AgCl) at a scan rate of 50 mV s1 in a solution of NPEDMA (2.4 mM) in 50 mM HClO4 (15 cycles). The aluminium mould was then sacrificed by dissolving it in 3 M NaOH for three minutes. The system exhibited Michaelis–Menton kinetics and competitive inhibition properties similar to those of the enzyme tyrosinase (polyphenol oxidase). [47] Numerous assays and sensors have been described in the literature in recent years, but to the author's knowledge, no molecularly-imprinted sensors are yet on the market, although one company in the UK is known to have developed and manufactured in quantity a sensor for the anaesthetic gas, Propofol.

The impact of Nanotechnology

Reflecting on the last decade of biosensor development, one can clear see the impact of nanotechnology. One of the first new nanomaterials to impact on amperometric biosensors was the carbon nanotube (CNT), which was blended into a number of formulations to improve current densities and overall performance of enzyme electrodes and enzyme-labelled immunosensors. [48] Amperometric enzyme electrodes benefited from enhanced reactivity of NADH and hydrogen peroxide at CNT-modified electrodes and aligned CNT ‘‘forests’’ appeared to facilitate direct electron transfer with the redox centres of enzymes. The most widely used nanomaterial in industry overall to date, however, is the silver nanoparticle. These have also been harnessed as a simple electrochemical label in a highly sensitive amperometric immunoassay intended for distributed diagnostics and as an inexpensive solution for immunoassays performed in developing countries. In this electrochemical sandwich immunoassay, silver nanoparticles are used as a robust label, which can be solubilized after the binding reaction has occurred, using thiocyanate, to form a silver chelate. This benign chemistry replaces earlier versions using aggressive chemical oxidants such as nitric acid. Once solubilized, the silver concentration can be very sensitively determined using stripping voltammetry on a single-use screen-printed carbon electrode. The silver colloid aggregates due to the presence of thiocyanate and the negatively charged aggregates are attracted to the positive potential of the carbon electrode during the pre-treatment. Once in direct contact with the electrode surface, the silver is oxidized at 0.6 V to form soluble silver ions, which are immediately complexed by the thiocyanate and detected by the ensuing anodic stripping voltammetry. Hence, the analyte concentration yields a signal which is directly proportional to the anodic stripping voltammetry peak of silver. In one example, the cardiac marker myoglobin, was measured down to 3 ng mL1, which was comparable with the conventional enzyme-linked immunosorbent assay (ELISA). Samples volumes of less than 50 mL could be handled and the assay worked in turbid solutions without the need for sample clean-up. [49] A variety of other nanoparticle-based strategies have been described in the literature for electrochemical affinity assays and we have recently reviewed them.[50] Some of the main types of transduction include conductometric, stripping voltammetry, multi-labelling with quantum dots and molecular beacons. In conductometric configurations, nanoparticles of metal or conducting polymer are used to label binding interactions on microelectrode arrays and the conductance or impedance of the system is measured. Both gold and silver nanoparticles can be used in stripping mode, as described above in more detail. Cadmium, zinc and copper quantum dots have been reported as multi-labels for affinity reactions, with each being separately detected via the electrochemistry of its ion. Finally, and arguably most elegantly, molecular-beacon architectures can be exploited to monitor conformational changes occurring upon binding of, for example, an aptamer to its target. In this design, a redox species such as a ferrocene, is anchored to the tail of the aptamer, while the other end of the nucleic acid sequence is tied to an electrode surface. Conformational changes on binding are recoded by virtue of

the proximity of the redox species to the electrochemical surface, effectively switching the signal on or off as the aptamer binds. The detection of structure-responsive recognition elements such as electrochemical molecular beacons and apta-beacons can be further enhanced by nano-structuring the receptor surface to improve receptor loading and signal strength. Nano-porous gold surfaces, for example, can be prepared by de-alloying of goldsacrificial metal alloys to facilitate high rates of catalysis and high immobilization densities. Most recently, nanostructured materials have been used to deliver label-free electrochemical immunoassays. Justin Gooding's group in Australia described a direct electrochemical immunosensor for detection of veterinary drug residues in undiluted milk. They used a displacement assay for with a mixed layer of oligo(phenylethynylene) molecular wire, to facilitate electrochemical communication, and oligo(ethylene glycol) to control the interaction of proteins and electroactive interferences with the electrode surface.[51] More recently, we reported on the use of a highly conductive N-doped graphene sheet-modified electrode, which exhibited significantly increased electron transfer and sensitivity towards the breast cancer marker CA 15-3. This label-free immunosensor delivered a low detection limit of 0.012 U mL1 and worked well over a broad linear range of 0.1–20 U mL1. [52] The other obvious target for electrochemical affinity sensors is DNA. The advent of the DNA chip has focused attention on alternatives to the fluorescence detection made famous in the Affymetrixt system. Several companies have successfully launched electrochemical arrays and the literature abounds with various electrochemical detection schemes. Many of the electrochemical DNA sensors reported have targeted the detection of single nucleotide polymorphisms (SNPs) associated with inherited diseases or short pieces of DNA associated with specific organisms. The idea of using a redox label attached to a complimentary strand of nucleic acid to render it electrochemically active on binding its partner or on displacement, is now well established. However, an interesting idea to enhance the sensitivity and specificity of such detection has recently been published and serves to illustrate a general and important principle. Nasef et al. described melting-curve analysis of ferrocene-functionalized DNA bound to its complimentary strand directly on an electrode, by following the electrochemistry of the redox tag with change in temperature. [53] This electrochemical method enhanced by temperature modulation, illustrates how a second level of analysis can not only yield additional information, but can greatly enhance the quality of information from the initial sensor response. Such a strategy is extremely useful and is a valuable generic tool that can be used as a solution to conundrums facing quite different systems, by seeking a second parameter that can be externally modulated and yields secondary information. A similar theme is illustrated by the development of switchable sensors. One illustration is the use of temperature responsive polymers to regenerate an affinity sensor surface. Poly(N-isopropylacrylamide), for example, is a temperature-responsive polymer, which at temperatures greater than 32 1C, undergoes a reversible lower critical solution-temperature phase transition from a swollen hydrated state to a shrunken, dehydrated state, losing approximately 90% of its mass. When used as an underlay on a sensor surface, this transition can be used to repel the binding partner and hence regenerate the sensing surface under

extremely mild conditions, effectively yielding a continuously usable immunosensor. Temperature responsive polymers can also be used to switch on and switch off a catalytic sensor, by controlling the access of substrate through the polymer matrix.[54] This approach allows a background reading to be taken, thus providing a convenient way to compensate for electrochemical or other interference from the sample. All these various electrochemical configurations potentially allow the simple, mass produced carbon and thin-film metal electrodes, developed originally to serve the diabetes market, to be adapted to a wide range of affinity sensors[55] for cardiac markers such as troponin and the opsonin, C-reactive protein, cancer markers such as prostate specific antigen (PSA), alpha foetal protein (AFP), human chorionic gonadotropin (hCG), CD34 etc., hormones and potentially for more challenging clinical analytes in the future, such as autoantibodies, myeloid proteins, markers for regenerative medicine and detection of organisms causing encephalopathies. Sensor technologists have increasingly dared to entertain the feasibility of single molecule detection in a convenient format and arguably the most widely discussed example is DNA nanopore technology for DNA sequencing, pioneered by Hagan Bayley and his team. [56] The concept of using an aperture in the order of 1 nm to determine the order of nucleotides in a sequence, can be traced back to the mid 1990's. Natural structures used for this purpose include transmembrane proteins, such as alpha-hemolysin, and synthetic approaches encompass capitalizing on advanced silicon-microfabrication technology, and most recently, the ubiquitous appeal of graphene sheets. The basic idea is to observe the ion current across a nanopore, which is extremely sensitive to the changes in the shape and size caused by single nucleotides passing through it. Bayley's team used tethered oligonucleotides, covalently attached to the lumen of the a-hemolysin pore, to detect single-base changes in DNA strands. Some of the most recent work in this area has explored the possibility of sequencing DNA by passing the molecule through nanopores in a sheet of graphene. [57] For example, explored the use of graphene sheets with nanopores of 5 to 10 nm. These ultra-thin sheets yielded larger blocked currents, but suffered from somewhat higher noise levels than traditional silicon nitride structures. While many of these techniques have now demonstrated single base resolution, the challenge remains to record the tiny ionic currents at the very high translocation rates associated with nucleotide passage through the pore. Only then can the exciting promise of rapid gene sequencing and other single molecule detection be delivered. Hybrid nanostructures, such as the combination of nanopores with nanowire detectors to detect field effects, is one interesting attempt to capitalize on earlier work using field-effect transistors to monitor affinity reaction, but overcomes some of the earlier limitations by operating in the very defined space of a nanopore.[58]

The role of biosensors in healthcare

Simple, easy-to-use measurement devices for a diverse range of biologically relevant analytes have an intuitive appeal as portable or pocket-sized analyzers, and this has driven the diverse range of applications reported in the literature. However, both historical precedent and a critical analysis of potential markets leads to an indisputable conclusion that healthcare is and will continue to be the most important area for the application of biosensors. The maintenance of health is one of the most laudable technological objectives challenging science and technology and diagnosis is an essential prerequisite for treatment and prevention of disease. Moreover, related applications of biosensors, such as the maintenance of food safety and environmental monitoring can be aligned with this central objective. The developing world has a desperate need for robust diagnostics that can be deployed in the field by both healthcare professionals and volunteers. Infectious diseases account for around a quarter of worldwide deaths, although they are projected to decline as a percentage of total deaths over the coming 20 years, as other cause become more prevalent. In developing countries we are faced with diseases of poverty such as HIV/AIDS and tuberculosis, where the former kills 1.8 million people each year and the latter still affects around a third of the world's population and accounts for an estimated 1.4 million deaths, according to the WHO (2012), although the incidence has been falling globally at a rate of 2.2% in recent years. In addition there are 2.5 million deaths from diarrheal infections and almost 800 000 from malaria. Of the estimated 57 million global deaths in 2008, 36 million (63%) were due to non-communicable diseases. Population growth and increased longevity are leading to a rapid demographic change with a rapidly aging population. The world's population of people 60 years of age and older has doubled since 1980 and is forecast to reach 2 billion by 2050. It is projected that the annual number of deaths due to cardiovascular disease will increase from 17 million in 2008 to 25 million in 2030, with annual cancer deaths increasing from 7.6 million to 13 million. Overall, deaths from noninfectious diseases are projected to reach 55 million by 2030. Cardiovascular disease accounts for 48% of these deaths, followed by cancers (21%) and chronic respiratory diseases (12%). Diabetes is directly responsible for 3.5% of these deaths, with Asia now home to four of the five world's largest diabetic populations. The net result of all this is healthcare spending that threatens to spiral out of control. According to the WHO, the USA already spends 17.9% of its GDP on healthcare, while the European Union average is closer to 9.5% (UK and Sweden 9.6%). Technology needs to offer more economic solutions and distributed diagnostics enabled by biosensors and enhanced by consumer products available over-the-counter are a key part of the solution. This is also commercially attractive, with in vitro diagnostics already worth an estimated US$40 billion per year. While glucose biosensors for diabetes have had the most profound effect on disease management to date, biosensors for other metabolites promise utility for other non-communicable diseases such as kidney disease, which is increasingly being recognized as an emerging problem in a rapidly ageing population. Multifarious affinity

biosensors have been described to detect cardiac disease markers such as creatine kinase and troponin, while cancer markers and single cell cancer detection have attracted considerable recent literature. Last but by no means least, nucleic acid-based biosensors such as SNP detectors and gene chips, have played an increasingly significant role in personalized medicine. The latter area, exploiting companion diagnostics or theranostics, could offer an estimated world market worth around US$72 billion and encompasses systems combining diagnosis, therapy and monitoring e.g. a test that qualifies a patient for treatment with a particular drug. An early example is the HER-2/neu assay (Human Epidermal growth factor Receptor) required prior to treatment with Herceptin to determine aggressiveness of breast cancer. Another example is the predetermination of the presence or absence of the KRAS genetic mutation, since its presence results in no benefit from Vectibixt, a monoclonal antibody based therapy used for colorectal cancer. All this adds up to a prediction of a strong commercial future for biosensor technology. Fig. 3 shows the value of the biosensor market calculated from various primary and secondary commercial sources over the years and predicted for the future. The principal instrument designs so far delivered for healthcare applications have been pocket-sized portable systems, desk-top instruments, decentralized analyzers and high-throughput, automated machinery.

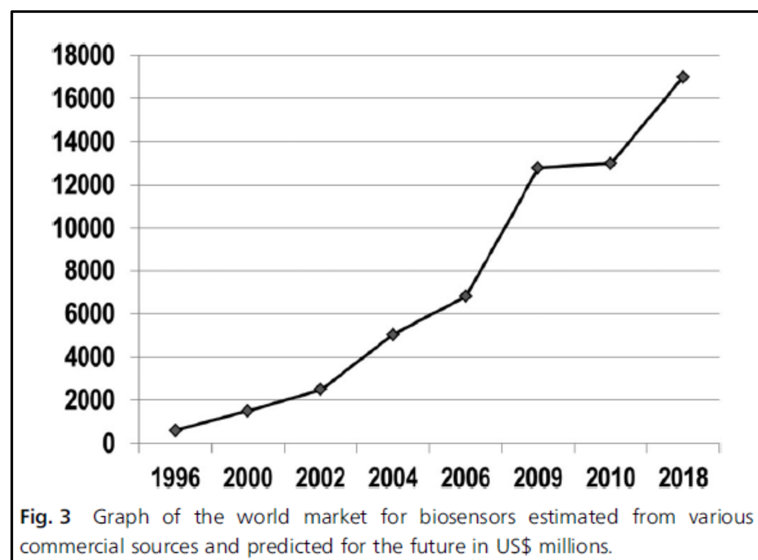

Fig. 3 Graph of the world market for biosensors estimated from various commercial sources and predicted for the future in US$ millions.

Fig. 3 Graph of the world market for biosensors estimated from various commercial sources and predicted for the future in US$ millions

They range from the home blood glucose monitor, through hospital analyzers, to automated drug-screening machines in use by the pharmaceutical companies. When clinical samples are used, they are usually blood samples obtained either as a pin prick or by a phlebotomist. As a more patient-focused approach predominates, however, new designs for wearable and distributed sensors are emerging. Implantable sensors are already a reality and used by many people with diabetes, as described above. Wearable biosensors present a number of technical challenges including the availability of a suitable sample that can be readily accessed without invading the body. Sweat,

tears, saliva and breath has all been intensively explored with limited success. Problems include lack of correlation with in vivo concentrations, inhomogeneity or variability of the sample and the design of reliable sampling systems. Saliva, for example, offers potentially reliable measurements of lactate and salivary amylase, along with an interesting array of other proteins, but is difficult to access conveniently and reliably, and is easily subject to contamination from food etc. Incidentally, salivary glucose bears little correlation to blood glucose, rendering this route ineffective for diabetes monitoring and control. Sweat shows a similarly poor correlation, despite being the target for a number of sensor approaches reported in the popular press. An arguably more serviceable route is via the eye using, for example, the contact lens as a sensing vehicle or mounting optical devices on glasses. Finally, the so far insurmountable hurdle of comprehensive non-invasive monitoring continues to attract substantial attention and even more significant investment. Press reports of solutions continue to abound and high-profile prizes, promise rich rewards on success. This furore of public attention can obscure serious research in the field, which is continuing, since there is no doubt that patients would prefer noninvasive techniques if they could be made to work reliably. Especially challenging is the rapidly increasing demand for technology to support the elderly. While the top three priorities to improve the quality of life for people at home are to reduce the risk of falling, provide orientation aids and counter loneliness, biosensors should arguably come next as a way to manage chronic multiple sicknesses and provide early warning of acute problems.

Conclusions

I hope that this brief overview has illustrated that biosensors have achieved considerable success both in the commercial and academic arenas and that the need for new, easy-to-use, home and decentralized diagnostics is greater than ever. The enormous success of the glucose sensor serves as a model for future possibilities and should not overshadow the multifarious other applications that this versatile technology can address. Theranostics (companion diagnostics) offers an important new financial model to drive the development of biosensors, since the principal customer is not the patient, but the pharmaceutical company seeking to deliver an efficacious therapeutic. Hence, the dynamic of the normal sales process is shifted towards a comprehensive set of treatment tools, rather than isolating the diagnostic device as a separate requirement that has to be purchased from a separate budget. This could have important commercial implications as pharmaceutical companies seek to counter a diminishing pipeline of blockbuster drugs and the intense competition from generic drugs. Another dynamic influencing demand is the empowerment of patients with personal health accounts. The impact of this freedom of patient access and mobility of data, enabled by information technology, is likely to stimulate demand for more analytical data and enable the patient or healthy subject to add data themselves, aided by a new range of over-the-counter biosensors. The expanding market generated by this boom in personal diagnostics will stimulate the development of new, inexpensive sensor platforms that can compete effectively to meet consumers' needs. Next generation diagnostics manufacturing is therefore targeting further integration to create complete sensing systems that can interface seamlessly with modern telecommunications. New technologies are likely to encompass all-printed systems capitalizing on the printed electronics revolution and systems with high compatibility with future mobile technology such as tablets and 4G phones. Emerging science, driving new sensors to deliver the molecular information that underpins all this, includes the development of semi-synthetic ligands that can deliver the exquisite sensitivity and specificity of biological systems without the inherent instability and redundancy associated with natural molecules. Currently aptamers, affibodies, peptide arrays and molecularly imprinted polymers are particularly promising research directions in this respect. Chances of success are enhanced by the potential utility of some of these materials for novel therapeutic, antimicrobial and drug release strategies, since these complimentary areas will drive investment in these approaches. New nanomaterials, conducting polymers and switchable systems offer exciting possibilities for hybrid devices. Research in synthetic biology provides an inspiration to mimic the art of compartmentalization practiced so well in living systems. Synthetic analogues facilitating seemingly incompatible sequences of biochemical reactions can be tailored to deliver complex diagnostic sequences using self-assembled systems and/or nanoreactors built using supramolecular structures. Realization of any of these paradigm-changing new products however, requires the effective harnessing of

emerging technology, inspired vision from clinical partners or others ‘‘users’’ and leading-edge engineering to design and produce functional systems in appropriate volumes at the right cost.

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