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Review

Biosensors

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CONTENTS

1. INTRODUCTION

The last few years have seen unprecedented interest in the development of a newly emerging area of pleuridisciplinary technology, biosensors, lying at the confluence of biotechnology, materials science and electronics[1–4]. Biosensors are analytical devices that respond selectively to analytes in an appropriate sample and convert their concentration into an electrical signal via a combination of a biological recognition system and a physico-chemical transducer. Biosensors promise to provide a powerful and inexpensive alternative to conventional analytical strategies for assaying chemical species in complex matrices; they do this by being able to discriminate the target analyte from a host of inert and potentially interfering species without the requirement for separating and, subsequently, identifying all the constituents of the sample.

The requirement for accurate chemical intelligence is particularly conspicuous in human health care but is becoming increasingly important in veterinary medicine, the agri-food, horticultural, pharmaceutical and petrochemical industries, environmental surveillance, defence and security[3]. For example, it is now generally recognised that inexpensive and reliable sensors for monitoring key metabolites, hormones, drugs, gases or ions in the ward, surgery, home, work place, outpatients department and central laboratory are essential for the delivery of effective patient care. Biosensor technology is eminently suitable for satisfying the needs of "alternate" site diagnosis and is particularly apposite in circumstances where there are advantages in obtaining immediate analytical results; for example, in assessing cancer markers in tissue proximal to an excised tumour within an operating theatre or in assessing the nature of

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the drug in patients suspected of an overdose. In circumstances such as these, the sensor output could be qualitative, whereas for therapeutic drug monitoring, where the "window" between therapeutic and toxic effects could be relatively narrow, the sensor must display high precision even at the expense of longer assay times. These differences in performance criteria for biosensors also extend to other parameters such as price; for example, biosensors designed for monitoring glucose in the home by diabetics will be extremely price-sensitive whilst similar devices for critical care units and industrial bioreactors could be quite price-insensitive. Thus, the features required for particular sensors will depend on the individual application, although in all cases the device should be sufficiently specific, sensitive and reliable to permit analysis of the target species.

2. BIOSENSOR ARCHITECTURES

All biosensors exploit a close harmony between a selective biorecognition system and a transducer which translates a physico-chemical perturbation associated with the biorecognition process into a usable signal[1,2]. Generally speaking, the biorecognition system is typically an enzyme, sequence of enzymes, lectin, antibody, membrane receptor protein, organelle, bacterial, plant or animal cell or whole slice of plant or mammalian tissue. This component of the sensor is responsible for the selective recognition of the analyte, the generation of the physico-chemical signal monitored on the transducer and, ultimately, the sensitivity of the final device[5,6]. Discrimination ratios of 10^7–10^8 or greater may be required for the biology to recognise the target molecule in the presence of a complex matrix of other substances. The action of these "bioreceptors" can be categorised into three principal types: firstly, biocatalytic systems such as enzymes, organelles, whole cells or tissue slices where the selective binding sites "turn over". Such systems are more appropriate for monitoring analytes such as metabolites in the mM–μM concentration range, are reusable and, thus, display a capability for continuous sensing in real time. Secondly, "irreversible" binding systems which exploit antibody, binding protein or receptor systems where interactive sites can become saturated and where such devices are more applicable to "single use" disposable devices. These devices tend to be more applicable to analytes such as hormones, steroids, drugs, microbial toxins, cancer markers and viruses where concentrations lie in the μM–pM range. Finally, amplified systems represent a hybrid configuration between biocatalytic and "irreversible" systems and exploit an antibody, DNA/RNA probe or other appropriate high-affinity binding systems as the initial biorecognition event followed by a suitable amplification, cycling or cascade system linked to an appropriate transducer. Such systems are capable of monitoring analytes in the pM–aM concentration range and lower. Selective biosensors have now been developed in all three categories where recent advances in immobilisation technology have provided improved stabilisation, localisation and activity of the sensing surfaces[7].

3. BIOCATALYTIC SYSTEMS

The majority of successful biosensors exploit enzymes as the biological recognition/response system linkd to transducers capable of responding to protons,

ions, gases, heat, light, mass or electrons generated during the catalytic cycle. Conceptually, the simplest systems catalyse the generation or uptake of protons or other ions that can be coupled to an appropriate potentiometric sensor. In this type of device, local equilibrium is establishd at the sensor surface and leads to the generation of a potential proportional to the logarithm of the analyte activity. The most universal potentiometric biosensors are enzyme electrodes, where an appropriate enzyme is immobilised over an ion-selective electrode[8]. Enzyme electrodes for the estimation of glucose, urea, antibiotics, L-amino acids and a plethora of other substances have now been realised and commercial devices have been in the market place for over a decade. However, more recently, considerable effort has been directed towards the miniaturisation of enzyme electrodes. This has been achieved by exploiting monolithic silicon fabrication technology coupled with appropriate enzyme immobilisation techniques to produce highly selective microsensors[9,10]. Enzyme-sensitised field-effect transistors (ENFETs) for urea, penicillin, glucose, acetylcholine and ATP have all been fabricated from ion-selective field-effect transistors (ISFETs) by combining an enzyme-loaded gel with the ion-selective membrane over the gate region of the field-effect transistor (FET). Considerable interest has been shown in this device technology because of their small size and potentially low production costs. Furthermore, the approach is also amenable to producing monolithic multi-analyte biosensors with photolithographically patterned enzyme-loaded gels for the simultaneous monitoring of K^+, urea and glucose[11–13]. However, despite exciting advances in technology, attempts to commercialise enzyme-modified FET biosensors have been plagued by poor device sensitivity and response times, difficulties in assaying analytes in "real" samples and prohibitively high encapsulation and fabrication costs. Thus, despite some elegant solutions to these problems, many researchers are sceptical as to whether potentiometric ENFETs could ever be exploited without dramatic improvements in the technology.

Current measuring or amperometric devices exploit electron exchange between biocatalytic systems and electrodes and offer a wider scope of applications than potentiometric techniques[14]. They give a current response which is directly proportional to analyte concentration, a normal dynamic range and a normal response to errors in the measurement of current[15]. First-generation amperometric devices monitored oxygen consumption or hydrogen peroxide production associated with the oxidation of substrates by a number of oxidases. Unfortunately, such devices suffer from a dependence on ambient oxygen concentrations and interference by contaminating electroactive species found in crude samples at the high electrode potentials required for electron exchange. Second-generation devices have largely circumvented these problems by substituting an artificial electron mediator for oxygen in order to facilitate electron shuttling between the enzyme and electrode[16]. Ideally, such mediators should participate in enzymatic redox reactions, exhibit rapid electron-exchange rates, be stable and non-toxic, be amenable to immobilisation alongside the enzyme system and display redox potentials sufficiently removed from other electroactive species present in samples to avoid interference. Mediators such as quinones[17], hexacyanoferrate[18], phenazine methosulphate[19], ferrocene[16], tetrathiafulvalene[20] and tetracyanoquinodimethane[21] have all been used to couple the redox enzyme glucose oxidase to suitable electrodes. However, despite the commercialisation of mediated-enzyme sensors, biosensor technology is rapidly moving into the realms of

third-generation devices in which reduced enzymes react "directly" with the electrode itself. For example, conducting organic salts such as N-methylphenazinium tetracyanoquinodimethane (NMP^+TCNQ^-) appear to promote electron exchange with reduced enzymes[15,22]. However, the precise mechanism of electron transfer between the active site of the enzyme and the conducting organic salts is still a matter for conjecture.

More recently, the entrapment of redox enzymes in electrically conducting organic polymers has been suggested as a means of promoting close liaison between the enzyme and the electrode surface[23]. Initial studies have shown that glucose oxidase may be incorporated into polypyrrole[23,24], poly-N-methylpyrrole[25] and polyaniline[26]. This technique of incorporating enzymes into electrodepositable-conducting polymer films also permits the localisation of biologically active molecules on electrodes of any size or geometry[27] and is particularly appropriate for the fabrication of multi-analyte microamperometric biosensors[28]. Recent work in our laboratory with monolithic silicon devices showed the feasibility of constructing miniature multi-analyte enzyme sensors. The microelectronic devices comprised five pairs of serpentined and interdigitated gold electrodes (1000–3500 nm thick) deposited over Ti and Pt metal layers (*ca.* 100 nm thick) on a thermally oxidised silicon "chip" mounted on a ceramic carrier[28]. The microelectrodes were bonded to pads located at the periphery of the wafer and comprised two large electrodes (*ca.* 500 × 500 μm each) bonded in series and used as a counter electrode and three smaller electrodes, each of dimensions 200 × 500 μm. The middle pair of electrodes were converted to an Ag/AgCl reference electrode whilst the outer two electrodes were exploited to deposit electrochemically in polypyrrole, glucose oxidase and galactose oxidase respectively. Glucose and galactose were assayed by monitoring the oxidation of hydrogen peroxide at the respective enzyme microelectrodes held at a fixed potential of +0.7 V (*versus* Ag/AgCl). Fig. 1 shows the calibration curves for glucose and galactose of the microfabricated dual-enzyme biosensor.

A recent extension of this approach has included the co-entrapment of mediators into the polypyrrole films by electrodeposition of polypyrrole copolymers bearing redox mediators with glucose oxidase[29]. This technique of electrodepositing enzymes

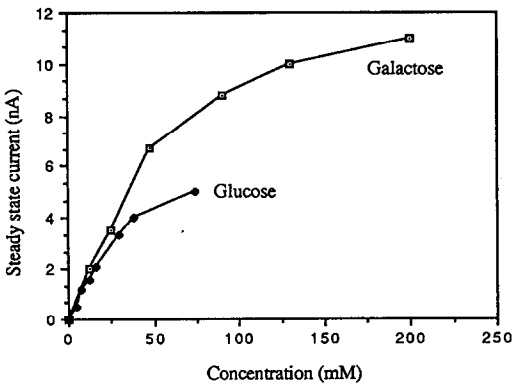

Fig. 1. Steady-state current responses of a polypyrrole-entrapped glucose oxidase–galactose oxidase dual-analyte microamperometric sensor to glucose and galatose.

within polymers modified with redox mediators provides an elegant means of generating reagentless enzyme systems at amperometric electrodes. Other developments include the covalent electro-immobilisation of gluose oxidase in conducting polymers[30] and the claimed "direct" electron transfer between a reduced oxidase and the conducting polymer[31]. Furthermore, covalent attachment of redox mediators such as ferrocene analogues[32] or ruthenium pentammine complexes to glucose oxidase itself has provided electron relays between the active site and the electrode and thus provided a route for direct electron exchange. Finally, covalent attachment of the flavin cofactor of an oxidase to an electronically conducting support via a conducting link has provided an alternative route for electronic communication between the enzyme and the electrode[34].

The technique of enzyme entrapment in conducting polymers has also been exploited for the construction of microconductimetric devices[28]. The development and operation of an inexpensive, rapid and accurate microconductimetric biosensor that exploits the change in conductance by the catalytic action of enzymes immobilised proximal to a planar microelectronoic conductance cell has been described previously[35]. More recently, it has been possible to construct a five-electrode microelectronic device dual-measurement principle device which is capable of detecting glucose amperometrically and urea conductimetrically with both enzymes entrapped in polypyrrole[28].

4. "IRREVERSIBLE" SENSORS

Biocatalytic systems based on enzymes can display poor stability, limited selectivity towards some key analytes and insufficient sensitivity when the analyte is present at very low concentrations. Nevertheless, highly selective and sensitive devices based on immunological recognition systems can be devised to circumvent these shortcomings. The development of "direct" immunosensors, which require only the addition of the analyte to elicit a response, has proved a worthwhile, but challenging, objective. Early attempts at constructing an immunologically sensitised field-effect transistor (IMMUNOFET) have not proven entirely promising despite the use of a number of innovative approaches to membranes[36]. Indeed, it is now generally considered highly unlikely that an immunologically sensitive potentiometric device will ever be constructed in view of the unlikelihood of realising an ideally polarised interface at which measurements could be made.

In principle, direct sensing of antigens by antibodies could be achieved by exploiting sensitive mass to frequency transducers based on piezoelectric materials[37]. For example, a piezoelectric immunosensor has been developed based on ST-cut surface acoustic wave (SAW) quartz crystals comprising interdigitated transducers between which was deposited a goat antibody by covalent immobilisation to the silanized surface. However, despite the elegance of the approach, difficulties associated with damping on immersion in aqueous solutions and with non-specific adsorption and sensitivity were experienced. Fortunately, it is possible to use other acoustic wave modes than the Rayleigh mode operation at 10 MHz used in these early studies which may resolve some of these deficiencies. Thus, exploitation of more sophisticated approaches to SAW technology may breathe a new lease of life into biosensors based on piezoelectric technology for application in aqueous media.

Research into optical techniques for direct immunosensing probably holds the most promise for the future, since refractive index is one of the few physical parameters which varies on formation of immune complexes. Thus, optical techniques such as ellipsometry[38,39], evanescent wave immunoassay[40], dynamic light scattering[41] and surface plasmon resonance[42–44] have all been applied to the detection of immunological reactions. In the latter approach, antibodies are immobilised on thin metal films, usually gold or silver, deposited on the surface of glass prisms[42,43] or diffraction gratings[44]. If light of an appropriate wavelength is directed on the metal–glass interface at an incident angle within certain narrow limits, the delocalised electrons of the metal at the metal–external medium interface are excited into a collective motion, termed a "plasmon". The transfer of energy from the light beam to the surface electrons results in decrease in the intensity of the reflected beam. The angle at which the incident light excites the surface plasmon is extremely sensitive to the refractive index of the medium immediately adjacent to the metal surface and is thus influenced by immune reactions occurring at the surface[44]. Fig. 2 shows a typical plot of normalised reflectivity *versus* the angle of incidence of a helium–neon laser on sequentially binding protein A, anti-lysozyme antibody and hens egg lysozyme to a silver (150 nm) on chromium (3 nm) diffraction grating. At a fixed angle of incidence, immune interactions may be followed in real time directly at the device surface[44]. However, despite the obvious attractions of this relatively simple optical technique, non-specific adsorption of serum components to the sensor surface was found to be significant and would be expected to limit the sensitivity for estimating specific analytes in serum[45]. Nevertheless, new approaches aimed at investigating the nature of the adsorbed serum components, orientated antibody immobilisation procedures and engineering both the sensor surface and the antibody may reduce non-specific adsorption to an acceptable level[45].

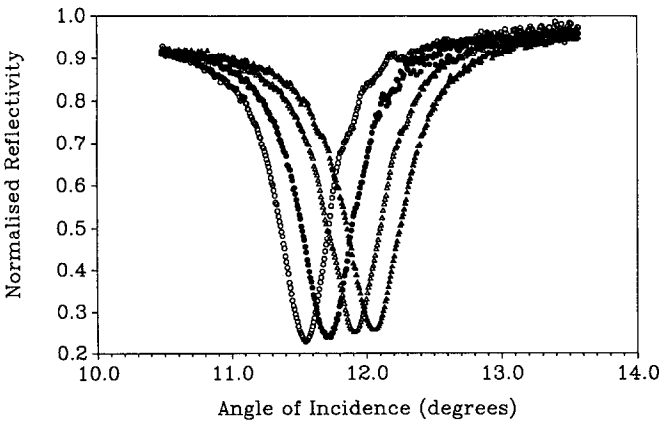

Fig. 2. Resonance curves obtained by sequential addition of protein A (333 μg ml^{-1}), anti-hens egg lysozyme antibody (333 μg ml^{-1}) and hens egg lysozyme (33 μg ml^{-1}) to a silver–chromium diffraction grating in 10 m*M* sodium phosphate buffer, pH 7.0. All measurements were made after washing the grating with buffer following incubation with the respective proteins. ○ = Buffer; ● = protein A; △ = anti-lysozyme; ▲ = lysozyme.

5. PERSPECTIVES AND CONCLUSIONS

It is now universally recognised that biosensors are likely to form a vital and pivotal feature of any future chemical surveillance and control system. Work over the past decade has now identified the problems facing the development of biosensors and future trends in the technology. There is a detectable trend to scrutinise the biological component in more detail than hitherto. For example, novel biorecognition systems isolated from newly discovered microbial, plant or microbial sources, from "extremophiles", from specifically engineered organisms by recombinant DNA techniques or exploiting the opportunities offered by artificial enzymes, catalytic antibodies (abzymes) or chemically imprinted polymers could lead to more selective and durable sensing elements. An alternative approach to biorecognition which could hold considerable promise in the longer term is the use of more complex chemoreceptor systems found in living organisms[46,47].

Novel biorecognition systems may still be sufficiently stable to permit reliable measurements over extended time regimes and thus require the development of intelligent interfaces to offset some of these limitations[4]. The interface could perform data acquisition and conrol, implement intelligent algorithms and communicate to the central controller. This system could recognise the performance characteristics of individual sensors, correlate, reject faulty signals, compensate for interferences and perform all necessary calibration steps. A multi-function chip comprising an array of biologically sensitive electrodes on a monolithic silicon device a few square millimetres in size could, at least in principle, encompass sufficient signal processing circuitry to address each sensor in turn and output the concentration of the analyte by comparing it with a calibration standard. Unfortunately, modern silicon microelectronics is considerably in advance of the biorecognition, protein and surface chemistry required to realise these concepts.

6. ABSTRACT

This review introduces biosensors as analytical devices that respond selectively to analytes in appropriate samples and convert their concentrations into electrical signals via a combination of a biological recognition system and a suitable transducer. The last decade has seen dramatic advances in the design of sensor configurations, the marriage of biological systems with modern monolithic silicon and optical technologies, the development of effective electron-exchange systems and the introduction of direct immunosensors.

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