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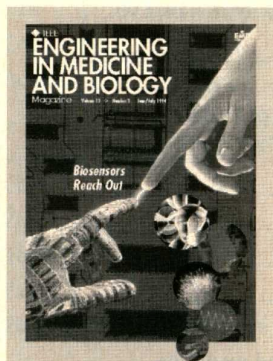


*Biosensors
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Cover: Advanced biomedical sensors are reaching commercialization, providing superior health care at lower cost.

Up Front

The senses, even when aided by all the means and appliances of science and art, reveal to us only a part, and probably only a small part of the properties, phenomena, and relations, of the substances and agencies, which go to make up the material universe.

*Elisha Bartlett [1804-1855]
U.S. Physician, Essay on the Philosophy of Medical Science [1848]*

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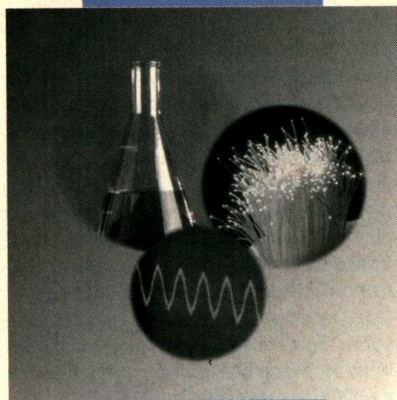
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Continuous Analyte Monitoring to Aid Clinical Practice

Restoration of the normal chemical balance in the body is a key target in the treatment of illness. In the healthy human, this balance is maintained by a number of biological sensors and actuators, which enable the complex functions of, for example, the liver, kidney and pancreas. In clinical practice, samples are routinely taken from the patient for analysis in order to assess the status of the component homeostatic mechanisms of the body and then determine an appropriate therapy. However, the analysis step often causes a delay in treatment, and in many illnesses it would be of great value to clinicians to obtain continuous real-time monitoring of the analytes of interest. Indeed, in surgery and intensive care, the analyte concentration may vary over a period of minutes, which can have considerable clinical significance. It would be useful in this situation to observe gross changes in analyte concentration in near real-time.

The area of sensors for *in vivo* monitoring encapsulates one of the most challenging applications of analytical technology, together with both relief of human suffering and the possibility of commercial success. The present article aims to review the current state of development of sensors for *in vivo* monitoring, focusing on the last two years. Two books provide detail of recent progress towards reliable continuous *in vivo* monitoring [1,2] and form the background for this article.

Analyte Selection

The clinician needs continuous sensing only where the data obtained will improve the treatment of illness, and where the benefit outweighs any risk, cost or discomfort associated with the sensing procedure. In order for *in vivo* sensing to be worthwhile, the target analytes must vary rapidly and unpredictably, and a therapeutic action must be available. These criteria might be satisfied for certain analytes such as gases (e.g., O_2 , CO_2 , halothane), ions (e.g., H^+ , K^+ , Na^+ , Cl^- , HCO_3^-), metabolites (e.g., glucose, lactate, urea) and hormones (e.g., insulin). A survey to elucidate clinicians' requirements found that the responders considered diabetes to be the clinical problem for which *in vivo* sensors would be of most use [3]. They

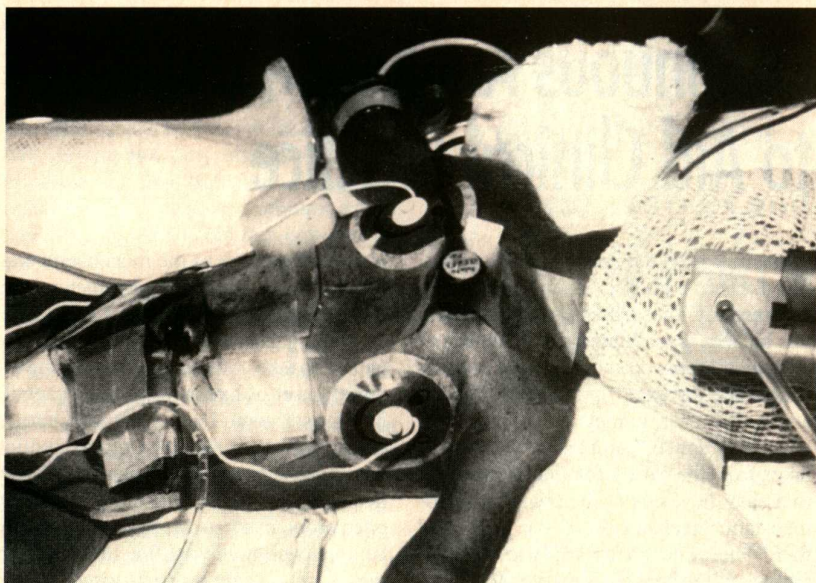
identified glucose as the most useful analyte to sense, preferably by means of a continuous measurement at a subcutaneous site. This sensing would enable the patient to take corrective action during the initial onset of hyper- or hypoglycaemia. The sensor could be incorporated into an implantable artificial pancreas, which would provide automated insulin delivery to the patient. The second most commonly cited problem where *in vivo* sensors would be applicable was vital function monitoring in critical care by measuring the concentration of analytes such as O_2 , CO_2 , and H^+ . A smaller number of respondents saw urea sensors as being useful for monitoring dialysis in the management of patients suffering from renal failure. A significant number of clinicians saw value in monitoring K^+ , which is of importance in critical care.

Device Development

There are many special factors which must be considered in the development of useful *in vivo* sensing devices. The basic technical performance (range, response time, sensitivity, limit of detection, drift, interference, accuracy, and precision) must be assessed first *in vitro*, and later *in vivo*. In the latter, results obtained are generally poorer due to the hostile environment. There are particular technical considerations associated with *in vivo* use, such as implantation method, site, and effect; and calibration method, reliability, longevity, safety, and ease of use. In addition, clinical, social, and economic factors must be considered, and also the impact on quality of life. The performance of the device must eventually be assessed in properly designed clinical trials to determine clinical effectiveness.

Continuous *in vivo* monitoring can be approached by either invasive analytical methods (e.g., chemical sensors implanted in the body) or non-invasive techniques such as near infra red spectroscopy (NIRS) (Fig 1). Non-invasive techniques are generally preferable to chemical sensors [4]. However, the former are not always able to deliver the required specificity, and instrumentation costs preclude their use for many *in vivo* applications. A notable exception is the pulse

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1. Non-invasive NIRS monitoring of oxy- and de-oxy hemoglobin concentration in the brain of a neonate. Also shown are monitoring of transcutaneous oxygen partial pressure and invasive umbilical pH (courtesy of Professor P Rolfe, University of Keele, Stoke on Trent, UK).

oximeter, which is widely used in surgery and in intensive care units, for measuring arterial haemoglobin - oxygen saturation continuously and non-invasively. This device uses the pulsatile variations in optical density of tissues in the red and infrared wavelengths to compute arterial oxygen saturation without calibration [5]. Transcutaneous oxygen and carbon dioxide electrodes are used for monitoring babies, but adult skin tends to be unsuitably thick. A new "device" is being marketed by Futuress Inc. in the US for non-invasive monitoring of analytes such as glucose. This device uses pattern recognition to predict analyte concentration. The need for caution in the routine application of this empirically-based instrument is illustrated by the manufacturer's specific reference to it as "a research tool", and it is notable that no peer reviewed, critical evaluation of the utility and accuracy of the technique has appeared to date.

Continuous monitoring of analytes to aid clinical practice can be achieved by using *ex vivo* or *in vivo* sensors. *Ex vivo* sensors are used to monitor blood in arterial or venous shunts, or in samples which are continuously taken from the body by means of a catheter. Glucose sensors, for example, have been incorporated in a bedside artificial pancreas able to correct hyperglycaemia over a few days (e.g., an instrument such as the Biostator. Analytes such as pH, $p\text{CO}_2$, $p\text{O}_2$, and K^+ have been effectively monitored using *ex vivo* sen-

sors, and several bedside instruments have been marketed. An on-line system has been developed for continuous monitoring of urea in blood during dialysis, facilitating optimisation of the treatment [6].

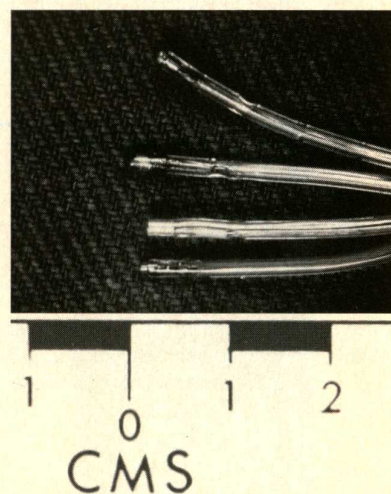
The implantation or sampling site must be one where the analyte of interest can usefully be measured, and which minimises any associated risk. Blood provides a homogenous sample, which is known to be representative of the general state of health of the body. In critical care settings, intravascular sensors inserted into a catheter have been used to monitor various analytes in blood, e.g., pH, $p\text{CO}_2$, $p\text{O}_2$ (see Fig 2). Tissue is a less reliable site for analyte monitoring, since actual analyte concentrations vary between parts of the body and due to local reactions, e.g., inflammation. However, it is generally a safer site for sampling than the intravascular site, since there is less risk of infection and the hazard of blood clot formation is avoided. The subcutaneous site is therefore generally preferable for routine monitoring. For glucose, it has been shown that the subcutaneous concentration parallels that in blood (see [7]).

Chemical Sensors

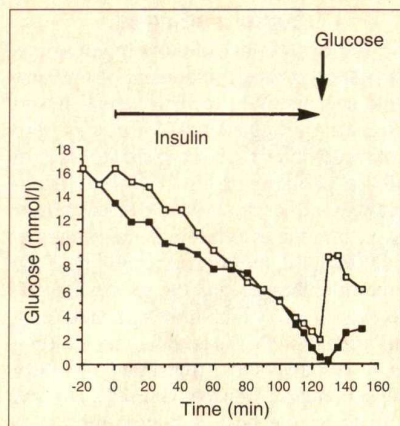
A chemical sensor comprises a molecular recognition system in intimate contact with a physico-chemical transducer that is capable of producing a continuous signal proportional to the analyte concentration. A sensor with a bio-

logical recognition element (such as an enzyme) is termed a biosensor [8], and these devices offer many advantages for *in vivo* monitoring. The transducer, which converts the output of the recognition element into a processable signal, may be electrochemical, optical, mechanical, or thermometric. For *in vivo* monitoring, the electrode is the most commonly used transducer. There are two types of electrodes, those which consume the analyte (e.g., amperometric and biocatalytic sensors), and those which will equilibrate with the sample (e.g., potentiometric and bioaffinity sensors).

Enzyme electrodes based on oxidoreductases have been designed to detect electroactive products, such as hydrogen peroxide, at a metallised electrode. Glucose oxidase is commonly used to detect glucose by measuring electrochemically either the amount of hydrogen peroxide produced or the amount of oxygen consumed. The recent elucidation of the 3D structure of this enzyme [9] has enhanced the prospects for improved design of such sensors. Progress has also been made in the development of sensors for lactate [10], an important metabolite which reflects oxygen supply in tissues. Lactate has been continuously monitored *ex vivo* using a portable system [11]. Mediators transfer electrons between the enzyme and the electrode, and examples such as ferrocene and its derivatives have been used commercially to reduce problems of interference by other electroactive species present in real samples [12]. However, these mediator molecules are small and tend to diffuse out of the sensor, precluding their

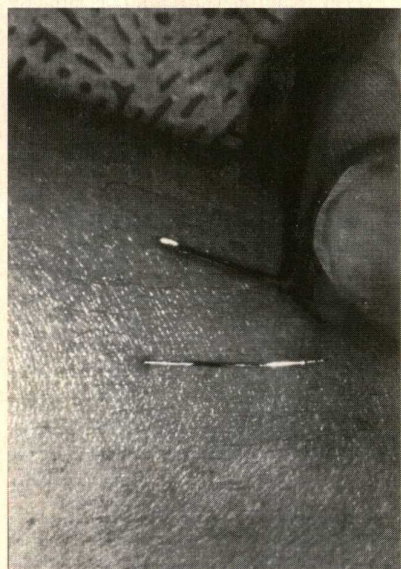


2. A selection of electrochemical intravascular sensors (courtesy of Professor P Rolfe, University of Keele, Stoke on Trent, UK).



3. Glucose sensor responses (solid rectangles), and blood glucose concentrations (clear rectangles) in a representative diabetic subject in whom hypoglycemia was induced by the i.v. infusion of short-acting insulin.

application for continuous *in vivo* monitoring. A Nafion membrane has been used in glucose sensors implanted subcutaneously in normal humans [13], as an alternative means of eliminating interference by the analgesic acetaminophen. Progress has also been made in the immobilisation of mediators to the surface of the electrode [14], within conducting polymers [15], to the enzyme itself in combination with redox polymers [16], and by binding to poly(ethylene glycol) [17]. An alternative approach has been to reduce the potential



4. Sensor for monitoring subcutaneous glucose concentration. Also shown is the hypodermic needle used to implant the sensor (courtesy of Dr G Reach, INSERM U341, Hotel-Dieu, Paris, France).

at which hydrogen peroxide oxidation takes place. A platinised carbon-fibre microelectrode for glutamate has been designed to operate at a pulsed potential of 300 mV (vs. Ag/AgCl) [18].

Oxygen independence is an important consideration for *in vivo* sensors. In subcutaneous tissue, the concentration of oxygen can be considerably lower than that of glucose, and hence oxygen can be a rate-limiting substrate. Membranes with differential permeabilities to oxygen and glucose have been incorporated in sensor design to overcome this limitation [19]. An oxygen-independent glucose sensor has been developed by incorporation of quinoprotein glucose dehydrogenase into a redox gel to produce a high current density electrode (1.8 mA cm^{-2}) [20], although problems with enzyme stability remain. The use of synzymes, artificial enzymes or other analogues of biological activity can be tailored to result in a rapid, thermodynamically-controlled, and reversible system, demonstrating a route towards the design of stable sensors [21]. In another approach, an oxygen-independent glucose sensor has been constructed utilising polypyrrole incorporated in track-etch membranes as the mediator [22]. The authors suggest that direct electron transfer may occur between glucose oxidase and the conducting polymer. The design also has the advantages that the sensor does not suffer mediator leakage, and that it will be resistant to damage during sensor manipulation.

Direct electron transfer between redox enzymes and an electrode could be a way of avoiding interference due to untargeted redox reactions. Direct electron transfer has been reported between covalently immobilised cytochrome c and a modified gold electrode. This technique could be used for *in vivo* detection of the production of superoxide radicals [23], which are implicated in tissue damage in certain disease states, including rheumatoid arthritis.

In Vivo Monitoring in Man

Subcutaneously implanted sensors have been used to monitor blood glucose in man for up to ten days [1] (Fig. 3). A promising needle-type subcutaneous glucose sensor and associated instrumentation has been developed in France [24] (Fig 4); a World Patent Application has recently been published (WO91/15993). The design of the disposable sensor element is suitable for insertion and removal by the patient. In common with other biosensors, the sensitivity *in vivo* is considerably lower than *in vitro*. Ideally, the sensor would be connected to a wearable

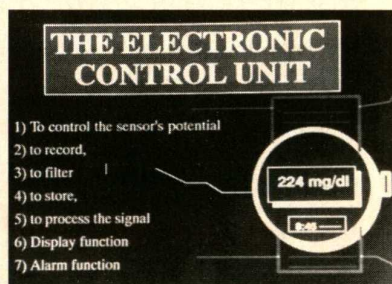
miniaturised monitoring unit with the form of a wrist-watch [7] (Fig 5).

Researchers at Eli Lilly & Co. have recently reported on their major programme to develop subcutaneous glucose sensors [25]. They describe the mass production of flexible electroenzymatic devices using thin/thick clean room processing techniques, and promising results in normal human subjects monitored over a 72 hour period. However, the device is too large (c. $2.5 \text{ cm} \times 1.12 \text{ mm}$) to permit insertion by patients, a limitation that reduces the prospects for commercial success of the present design.

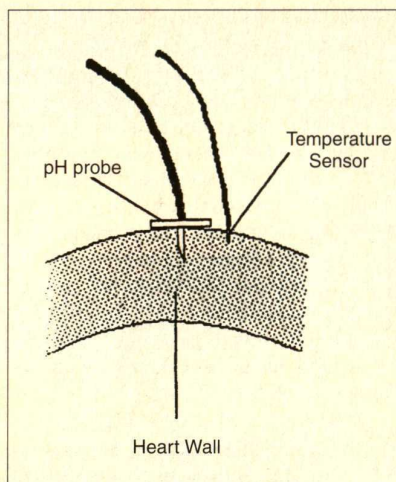
Ion-sensitive field-effect transistors (ISFETs) have been used to monitor intramycardial pH in twelve operations in man [26] (Fig. 6). The use of such sensors is expected to reduce morbidity in patients undergoing cardiac surgery. An ISFET glucose sensor with a new membrane structure demonstrated an extended linear range, up to 300 mg dl^{-1} , in serum, and could be useful for the development of a portable glucose monitor [27]. The resistance of a membrane containing charged proteins, which was deposited on an ISFET, has been determined by means of a simple A.C. impedance measurement [28]. The measurement method proved adequate and might also be suitable for other types of sensors, e.g., enzyme sensors.

A non-invasive transcutaneous blood glucose monitoring technique has been reported, combining an ISFET with a vacuum device to collect transcutaneous suction effusion fluid [29]. A 7.5 min interval measurement has been established [30]. The method has been used to monitor human blood glucose levels, and a portable system may be feasible.

Optical sensors are usually based on optical fibres, or on planar waveguide films. Advantages cited for such optical



5. Wrist watch design for glucose monitor. Reach G, Moatti-Sirat D and Poutout V: Continuous glucose sensing for the treatment of diabetes mellitus. *Analysis* 21: M35-M39 1993. Reproduced with permission.

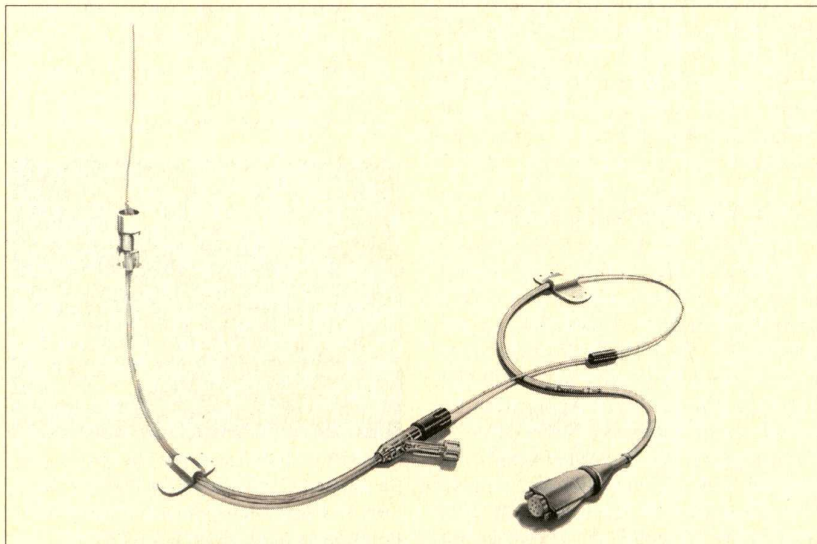


6. In vivo myocardial pH monitoring.
Analysis 21: M43-M46 1993. Reproduced with permission.

sensors for *in vivo* measurements include their small diameter, the potential for sensitivity, and the fact that no external light source is necessary. However, there are some problems with instability and with inexpensive mass production. Cardiovascular Devices Inc. (USA) have had *ex vivo* devices on the market for some time. The clinical performance of a fluorescent optode-based blood gas monitoring system for arterial pH, $p\text{CO}_2$, and $p\text{O}_2$ has been assessed in a multicentre trial involving 117 adult intensive care patients over a 1 to 4 day period. The triple sensors were introduced via a standard radial artery catheter. The results demonstrated stability, consistency, and accuracy comparable

to modern blood gas analysers [31]. New intravascular devices are appearing, e.g., Biomedical Sensors Ltd (UK) recently launched an *in vivo* catheter device which has optical sensing of pH and $p\text{CO}_2$, combined with temperature measurement and electrochemical sensing of $p\text{O}_2$ (Fig. 7). In an interesting new development sensors have been incorporated onto an ophthalmoscope, providing sensor data at the same time as an image. The device comprises an array of small fibres, each in contact with part of a chemically-sensitive membrane covering the tip of the bundle, effecting an array of sensors via the pixels [32] (Fig 8).

Microdialysis is an *in vivo* sampling technique which enables continuous removal of metabolites from the body without the need to remove any fluid. Microdialysis probes (Fig. 9) have been inserted into subcutaneous tissue as an alternative to implanting the sensor itself [33] and several groups have obtained promising results using a coupled sensor for on-line monitoring of glucose. For example, glucose profiles in both healthy and diabetic humans have been monitored for up to 27 hours by combining the microdialysis technique with a wearable amperometric glucose sensor [34]. This approach is being explored by the Italian company, Amplifon SpA. Alternatively, an enzyme-reactor for electrochemical monitoring can be coupled to the outlet of a microdialysis probe [35]. A 150 μm ID microdialysis fibre has been used to entrap enzymes near to the tip of a carbon-fibre microelectrode in a design intended towards *in vivo* analysis of neurotransmitter dynamics [36].



7. Intravascular catheter device for monitoring pH, $p\text{CO}_2$, $p\text{O}_2$ and temperature
(courtesy of Biomedical Sensors Ltd, High Wycombe, Bucks).

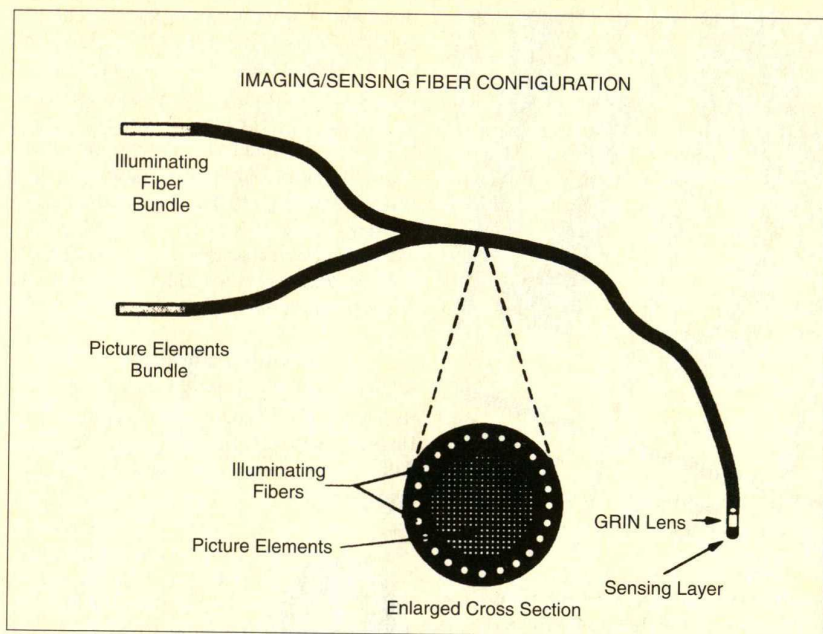
Sensor Packaging

The operational lifetime required is a key issue in the development of implantable sensors. Whenever a sensor is implanted into the body or comes into contact with clinical samples, the issue of biocompatibility arises. The host or sample may affect the function of the sensor (e.g., protein absorption, cellular deposition (Fig. 10), altering permeability of the outer membrane), and the sensor may affect the site in which it is implanted (e.g., inflammation of tissue, infection, clotting in a vascular site). Implanted chemical sensors must produce no harmful adverse effects on the host, and they must also maintain chemical communication with the blood or tissue for the period of use. Hence the materials used in the construction of the sensor, particularly the outer membrane, are a critical consideration in determining the overall performance and longevity of an *in vivo* chemical sensor. The materials used to coat the device must be effectively inert, and be stable for the period of operation. The deposition and growth on the outer covering layer of surface-active colloidal species, notably proteins, lipids and cells, must be limited by choice of materials and by consideration in the overall design of the sensor. It is also necessary for the materials to have the desired analyte permeability properties, as well as being able to exclude interferent species. One convenient strategy is to utilise polymeric covering and barrier layers within existing devices. These strategies for "packaging" can also limit leaching of potentially toxic electrode components and mask antigenic components [37]. A further consideration for an *in vivo* sensor is avoidance of mechanical damage to the sensor surface during implantation and operation.

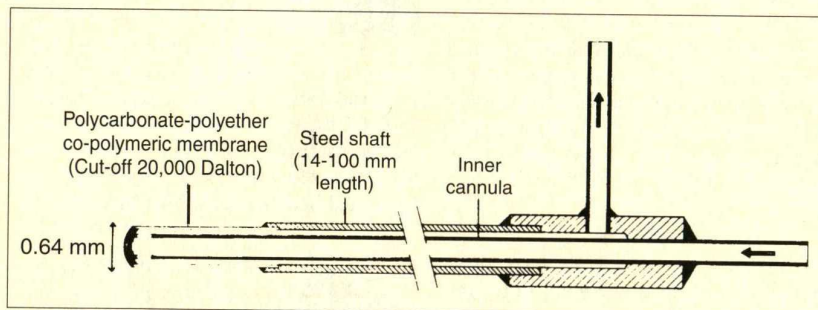
Sensor Fabrication

Research results have demonstrated promising designs for practical sensors for continuous clinical monitoring. However, many *in vivo* sensors are currently hand-fabricated. An important aspect is the ability to mass produce reliable, reproducible and economic sensors, and this will determine industrial uptake. Use of microelectronics technology minimises the size and price of sensors and permits the inclusion of full systems of signal processing as an integral part of the sensor.

Sensors used for *in vivo* monitoring are prone to drift and require calibration *in situ*. A new theoretical approach uses a set of nine sensors to create a sensor that responds to changes in the physical parameters governing sensitivity, rather than



8. Design of imaging sensor (courtesy of Professor D Walt, Tufts University, Medford, USA).



9. Microdialysis probe.



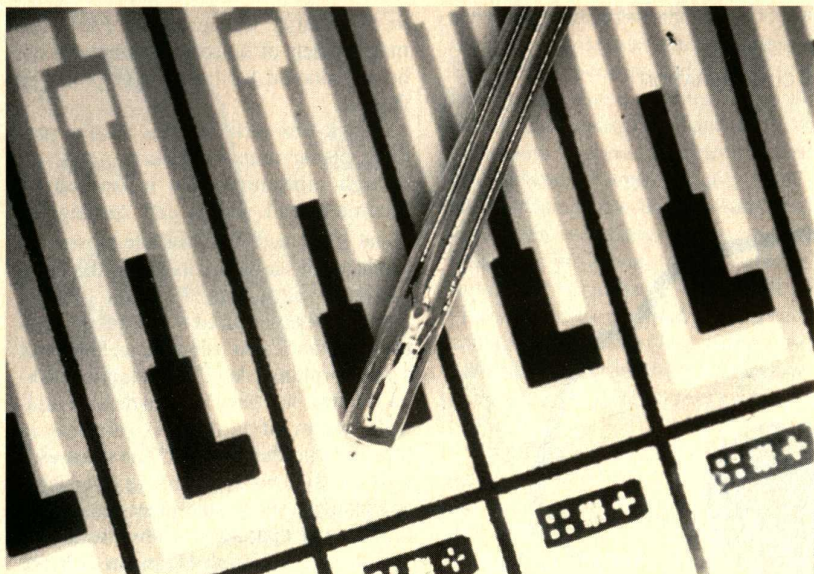
10. Explanted intravascular catheter with a sensor at the tip showing fouling of the surface (courtesy of Professor J Feijen, Twente University of Technology, Enschede, The Netherlands).

to the analyte concentration [38]. The matched sets of sensors required will not be a limitation for industrially-produced devices.

Thick-film technology is used to produce planar amperometric sensors, although miniaturisation is limited by minimum lead widths and spacings of about 100 μm . The disposable biosensors produced in large numbers for the commercially successful Exatech glucose monitor, which is used to test blood samples, are fabricated using the technique of screen printing. Another technique, ink-jet printing, has been used to deposit multiple layers of biological reagents in biosensor production [39]. The track-etch sensor [22] is not amenable to disposable applications, and a subsequent design uses uniform latex particles as a porous matrix which can be applied as an ink for polypyrrole coating and successive enzyme immobilisation [40].

Thin-film technology developed in the field of microelectronics has been applied to the production of planar electrode arrays for electrochemical sensors, and for sandwich overlayers in optical waveguides. For example, a microelectrode composed of a 64 electrode array of 60 nm thick gold electrodes each 8 μm wide has been developed for monitoring electrogenic cells in culture [41]. Workers in Switzerland have developed this approach by incorporating an enzyme layer on a silicon wafer, to produce amperometric glucose biosensors. These sensors have been used for *in vivo* monitoring in rats [42] (Fig. 11). The same team has used the technique of photolithography to pattern membranes incorporating glucose oxidase over the working electrode in an amperometric biosensor. An amperometric O_2 sensor and an ISFET-based CO_2 device have also been fabricated using photolithographic patterning [43].

A planar, all-solid-state oxygen sensor has been produced using a photocrosslinkable hydrogel and a gas-permeable membrane to obtain a miniaturised device which can be produced by thin-film technology [44]. This amperometric sensor exhibits no oxygen consumption and hence the sensor signal is virtually independent of flow. The design also has the advantage that the reaction products reach a dynamic equilibrium in the hydrogel layer, overcoming lifetime limitations associated with buffer degradation. The electrode arrangement can be scaled down to 0.2 mm and is suitable for integration in a multi-sensor array.



11. Microfabricated glucose sensor, with a processed wafer as background. Lam-brechts M and Sansen W: *Biosensors: Microelectrochemical Devices*, Institute of Physics Publishing, Philadelphia, 1992. Reproduced with permission.

Thin-film technology has been used to produce optical sensors: polymer layers with specific optically-active molecules are covalently attached to the surface of the optical waveguide to provide chemical sensitivity. The Langmuir-Blodgett technique is suitable for the deposition of a thin film of biological reagent onto the flat surface of a waveguide [45]. An interesting development is the possibility of the production of complete miniaturised optical sensors on a silicon wafer.

Economic Considerations

The cost of health care is escalating in many countries as life expectancy improves and patient demands and expectations rise with new knowledge and developments. For example, the total expenditure for health care in the US as reported by the National Institute of Health was \$817 billion in 1991. Evaluation of the efficiency of new medical technology is of primary importance in the effort to control costs. The cost of illness is the sum of direct costs (medical care), indirect costs (associated loss of productivity of the individual), and reduction in the quality of life. The success of a sensor in clinical practice will depend on its capacity to provide improved benefit or lower cost, or ideally, a combination of both. There is enormous potential for such sensors to be introduced in some areas of medicine. In the US and Western Europe, up to 0.5% of the population have type 1 (juvenile onset) diabetes, a condition that

has major economic as well as health implications. The direct cost of diabetes consumes about 5% of the total health care budget in Western Europe and North America. It has been estimated that a reliable sensor used as a hypoglycaemia alarm could save US \$200M per year in direct costs alone [46].

Conclusion

Considerable progress has been made in the development of practical glucose sensors for *in vivo* use. In some designs, the sensor can be replaced by the patient, who "injects" a new sensor into a fresh site using a hypodermic needle. An open-loop hypoglycaemia sensor providing an alarm signal could be available soon, and it would be very useful in the management of diabetes. The sensor for this application would need to function for a minimum period of about 12 hours. Further developments in the glucose sensor area are expected to lead to a comfortable implantable sensor/pump device, which would provide closed loop control for a prolonged period. An acceptable system would be a sensor replaced every 2 to 3 days, coupled with a microprocessor-controlled pump replaced after 2 to 3 years [47]. Further engineering input is needed to design the monitoring and control unit containing the alarm and pump systems.

The world market in clinical analysis is about US \$6 billion, and covers about 10% of the total expenses for health care. It is in the field of continuous microsensors

that future competition for the clinical diagnostic market will take place. Funding *in vivo* projects is less attractive to industry than the more proven and well-established area of *in vitro* diagnostics. However, the benefits of *in vivo* monitoring to the health of the community, and the financial rewards to manufacturers are ultimately great. It is clearly of interest for industry to invest in the development and commercialisation of this high added-value technology.

Acknowledgments

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