Journal of Fluorescence, Vol. 14, No. 5, September 2004 (©2004)

Current Problems and Potential Techniques in In Vivo Glucose Monitoring

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Received November 24, 2003; revised February 24, 2004; accepted February 25, 2004

Accurate in vivo monitoring of glucose concentration would be a valuable asset, particularly for management of diabetes and preterm infants during critical care. In vivo glucose monitoring devices can be divided into two categories: implanted and non-invasive. Extensive research into in vivo glucose monitoring over recent decades has not resulted in the widespread use of clinically reliable monitoring systems. For implanted devices, poor biocompatibility of the materials used for fabrication remains a major challenge, whilst progress in the commercial development of non-invasive devices is hampered by the problem of multiple interference between the detected signals and the biological components. In this review, the methods available for in in-vivo glucose monitoring are described and the associated problems are discussed.

KEY WORDS: Glucose sensor; in vivo monitoring; non-invasive; implantable; transducers; biocompatibility.

INTRODUCTION

The accurate estimation of blood glucose concentration is vitally important in the management of preterm infants during critical care and is used extensively in the management of diabetes mellitus. In diabetes mellitus it is now well recognised that the most serious complications, leading to renal failure, blindness, neuropathy and peripheral vascular disease, can be reduced by tight control of blood glucose [1]. On the other hand tight control leads to an increased risk of hypoglycaemia which can also have serious consequences including sudden death.

A measure of control is normally achieved through diet and intermittent insulin injections. Intermittent blood glucose monitoring is used to assess the level of control. More recently a variety of continuous blood glucose monitoring systems have been launched onto the market with the aim of providing non-invasive or minimally invasive frequent intermittent measurements [2,3]. It is hoped that the trend information provided will enable patients to improve control especially in those with brittle be disease. However these systems are not yet reliable enough to provide the necessary monitoring information for the development of an artificial pancreas which would hopefully provide a near physiological level of glucose control. This is the limiting step because implantable insulin pumps are available.

Preterm infants are at high risk of hypoglycaemia because of low glycogen stores and feeding difficulties, particularly the most immature infants who are likely to be receiving intensive care. Other groups of infants at risk from hypoglycaemia include those who have suffered from fetal growth retardation, infants of diabetic mothers and those with rare metabolic problems leading to lack of glucocorticoids or excessive insulin production. In turn, infant neuroglycopenia results in a range of symptoms including jitteriness, apneoic pauses and fits, but in preterm infants hypoglycaemia is often clinically undetectable, especially during critical care. Prolonged episodes of neuroglycopenia may result in permanent brain injury.

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1053-0509/04/0900-0513/0

Therefore frequent intermittent estimations are required using a heel prick to obtain blood in those patients who are not cannulated. It is not known whether preterm infants are at risk of serious levels of hypoglycaemia between measurements, because no reliable method of continuous measurement has been developed and applied to this group of infants. As many of these infants are cannulated for blood pressure monitoring and blood gas sampling, an in dwelling glucose sensor would make a valuable addition to oxygen, carbon dioxide and pH sensors. Although the pH arterial sensors are available [4], the use is not widespread because of problems with cost and biocompatibility of the sensors and catheters used.

The purpose of this paper is to review the technology that is under development and currently available, describing the advantages and disadvantages of each technique. Many problems still have to be overcome before continuous monitoring is available to neonates and an artificial pancreas in diabetes. These problems are identified.

OVERVIEW OF TECHNIQUES USED FOR GLUCOSE MEASUREMENT

There are several techniques for the measurement of glucose concentration.

1. The "dipstick" analysis in blood or urine. The change in colour in the glucose oxidase/peroxidase reaction is either estimated by the eye or optically detected and electronically processed to give a value.
2. The laboratory analysis of patient samples by electrochemical sensors [5,6].
3. Invasive devices that may be placed intravascularly or under the skin. These have the potential to monitor blood glucose continuously and would be the type of device required for the development of an artificial pancreas.
4. Non-invasive in vivo sensors that would be best suited to frequent but intermittent measurements in order to detect a trend.

The techniques for the first and second category are fully developed and have been applied successfully in the clinical setting. Their main limitation is the fact that they require blood to be drawn on each occasion and consequently are only suitable for infrequent intermittent monitoring. The implementation of devices in the third and fourth categories is far from complete although some of these devices are commercially available. These devices are considered further below and an overview is presented in Fig. 1.

Electrochemical Transducer

The electrochemical transducer is the most promising technique for invasive glucose monitoring and may be implanted intravascularly or subcutaneously. The principal

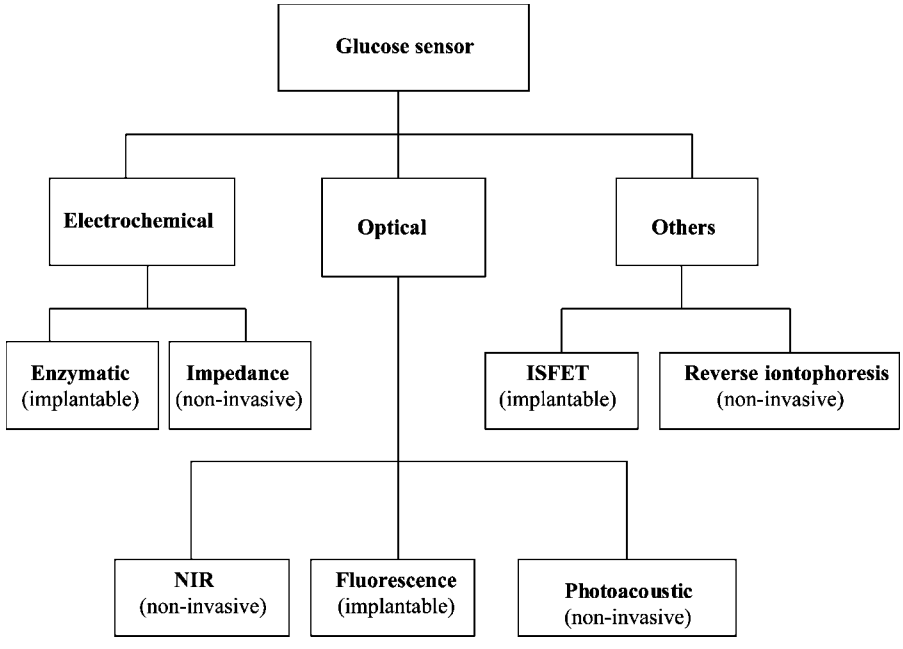

Fig. 1. The various techniques investigated for glucose monitoring in vivo.

behind the electrochemical sensor has been developed over a longer period of time than any other sensor. The first electrode of its type was constructed in the 1960s by Clark and Lyons [7]. In addition to the electrode itself, the sensor is constructed using three components: an inner layer which is a permselective polymer used to eliminate interference molecules, an enzyme layer, and an outer layer that acts as a diffusion barrier for glucose. Most importantly the outer layer also has to provide a biocompatible interface with the surrounding environment [8]. The sensor function is based on the concentration sensitive reaction between oxygen and glucose catalysed by glucose oxidase (GOD). Detection of the generated peroxide or consumed oxygen is achieved by the amperometric response on the electrodes. The advantage of the electrochemical transducer for glucose monitoring is its simple construction and ease of fabrication. It can be fabricated into a sensing catheter smaller than 0.6 mm outer diameter in order to make placement possible in very small blood vessels [9]. Various peroxide-based needle-type amperometric glucose sensors have been manufactured and miniaturised [10,11]. Such sensors are very stable, accurate and sensitive in vitro. However, once the sensors are implanted intravascularly, the stability and reliability reduces dramatically owing in large part to fouling of the sensor surface by proteinaceous material. When sensors are implanted intravascularly, the intensive biological interaction between the sensor surface and the blood stream will interfere strongly with the sensor performance. The sensor surface can be easily impaired by protein adsorption followed by platelet activation, adhesion and the formation of thrombus, leading to partial or complete malfunction of the sensors.

After several years of effort to improve the performance of glucose sensors working intravascularly, research has shifted its focus to monitoring glucose concentration in interstitial fluid (ISF). This involves either implantation of the sensor subcutaneously or implantation of a hollow fibre in subcutaneous tissue through which a buffer solution is circulated. Measurement of the perfused medium is via a semipermeable membrane with a glucose sensor placed outside the body [12–14]. ISF has a milder biological interaction with the microdialysis or sensor surface compared to the blood stream. Consequently the major difficulties related to sensor stability due to blood clot and embolism are circumvented. At this point in time only one product, which utilises a subcutaneous needle sensor for glucose measurement (CGMS from Medtronic/MiniMed), is available on the market. Sensors based on microdialysis are in clinical trials. The major problem in measuring glucose concentration in ISF is that there is a 'lag time' [15] in the equilibration between ISF and blood glucose when glucose levels change. The reported mean lag time is 6.7 min [16]. Thus frequent calibration with blood glucose may be still required. In addition, the interaction of biological components with implanted devices is not completely eliminated. When these devices are used continuously, fibrosis forms around the device, which could adversely affect the lag time by changing the diffusion properties of glucose to the sensing part or semi-permeable membrane. For intensive care patients, especially preterm infants, this lag time may lead to a critical delay in treatment when blood glucose levels fall.

Optical Transducers

Absorption of Visible and Infrared Light

Near-infrared absorption spectroscopy (NIRS) is a non-invasive technique with potential for glucose monitoring [17], which is based on the fact there is an 'optical window' in which human tissue is transparent to light in the NIR region. Consequently the technique has been used for the non-invasive monitoring of a range of biochemicals, particularly oxygenated and de-oxygenated haemoglobin in the brain of preterm infants [18]. The ability of a molecule, for example glucose, to give rise to specific absorption at certain wavelengths is dependent upon the structure of the molecule. The glucose absorption peaks are small but, using several wavelengths and employing multivariate techniques and calibration methods it has been possible to produce good correlation between reference blood glucose and predicted glucose concentration. Attempts have been made to monitor glucose concentrations using both reflection and transmitted light in a wide spectral range. Commercial devices making use of a wide spectrum have been reported. In one such unit, light in the range 500–1000 nm is transmitted across the tip of a finger (like pulse oximetry), and the received light is processed to estimate the glucose concentration. So far, performance of NIR has only achieved moderate success because of multiple interference from variations in tissue hydration, blood flow, environmental temperature, light scattering and the presence of non-glucose metabolites which also absorb NIR [19]. The effects of scattering are important for either transmission or reflection modes. Phase or time resolved techniques [18] are used to overcome this problem and enable glucose concentration to be determined more accurately. Finger, forearm, tongue and ear tend to be the normal probe application sites for reflection/transmission measurements.

Fluorescence Techniques

Optical fibres have been used for measuring or monitoring a number of physiological parameters such as, oxygen saturation, pH, temperature and biochemical compounds using a variety of fluorescent dyes. Complete

electrical safety and the absence of electromagnetic interference are two key advantages. Such measurement of glucose applies the affinity sensor principle. This is based on the competitive binding of glucose and a fluorescein-labeled analogue with receptor sites specific for glucose and the labelled ligand. The sensor consists of two parts: the fibre end for transmitting and receiving light and the biochemical sensor which is connected by a substrate, Concanavalin A (ConA), a glucose specific binding protein [20]. Fluorescein-labeled dextran is frequently selected as the competitively labelled ligand. Once glucose replaces the dextran, the intensity of unbound fluorescein-labeled dextran is measured and related to glucose concentration. Such fluorescence sensors can be inserted in the subcutaneous tissue and both activated and interrogated from outside the body. A fluorescence affinity sensor using the ConA system, which is implanted just under the skin, has been reported [21]. When irradiated through the skin with a light of an appropriate wavelength, a portion of the fluorescence generated by the implanted sensor is detectable on the skin surface. The light source is in the UV range. A surface mounted photodetector is used to measure the glucose concentration. In a further development of this technique [22], ConA was conjugated with fluorochrome, Alexa647TM, shifting the emission and excitation wavelength to near infrared range (670 nm). This improves tissue penetration and avoids much of the interference from strong light absorbers such as haemoglobin. This is a promising technique and laboratory in vitro studies have shown close correspondence between the sensor output and blood glucose levels. Long term stability needs to be improved for application in human subjects.

Optical transducer techniques have achieved great progress in the fluorescence labelling protocol and also in the instrumentation. The fluorescence resonance energy transfer (FRET) technique has been applied [23,24] to measure glucose concentration, in which ConA is labelled with fluorescence donor molecules, while an acceptor is attached to the sugar. In the absence of glucose the ConA-sugar association results in short distances between donors and acceptors and thus efficient FRET. With the addition of glucose, the acceptor-labelled sugar is replaced by glucose at ConA binding sites, increasing the average donor-acceptor distance, decreasing the rate of FRET and hence decreasing the fluorescence decay rate. This technique has great potential for in vivo measurements if a more sensitive glucose indicator protein can be genetically engineered [25] and better carrier materials can be developed to make the sensor stable and leak-proof [24].

An alternative to measuring the intensity of fluorescence is the measurement of fluorescence decay lifetime or decay rate. This is a more promising and reliable technique [26] because fluorescence lifetime is virtually independent of any fluctuation in light source intensity and detector sensitivity. Also, these measurements are independent of probe concentration and probe bleaching or washout. However, an expensive pulsed light source is required. Thus bringing the technique to fruition is inhibited by the complexity and the high cost of the instrumentation required. Recently, it has been reported [27] that the advent of blue light-emitting diodes with high luminosity could replace the expensive pulsed light source, which has the potential to produce a reliable lifetime-based sensor device at cheap and affordable prices.

Other Transducers

Impedance Spectroscopy

This technique has been proposed by Pendragon Medical [28], and is based on the variations in the impedance pattern of the skin, due to changes in the composition of blood. An open resonant circuit fixed in the device is in contact with the skin surface. The presence of glucose molecules changes the impedance, which is measured to provide an indication of glucose concentration. It is claimed that the results can be related to blood glucose levels. However, differences between patients in the thickness of the skin and underlying tissue, affects the impedance pattern. Thus, calibration for each individual repeated over time is required. Clinical studies to evaluate the performance [29] are proposed.

Ion Selective Field Effect Transistor

Ion Selective Field Effect Transistor (ISFET) uses a semiconductor as a transducer. The sensor size can be miniaturised, giving potential for the development of an implantable glucose sensor. The glucose concentration is quantified by measuring pH variations, which are due to the dissociation action of gluconic acid according to the enzyme reaction and the electrolysis of hydrogen peroxide as shown by the equations below [30].

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconolactone} + H_2O_2$$

$$\text{Gluconolactone} + H_2O \longrightarrow \text{Gluconic acid}$$

$$\text{Gluconic acid} \longrightarrow \text{Gluconate} + H^+$$

$$H_2O_2 \xrightarrow{0.7v} 2H^+ + O_2 + 2e^-$$

Some of the initial problems with ISFET, such as low sensitivity and slow response, have been addressed. An

important parameter is the fast reproducibility with repeat measurements. Recent work [31] using an electrolysis method has achieved recovery times of less than 2 min as compared to 10–20 min obtained in the conventional method.

Reverse Iontophoresis

Glucose concentration can be measured by a new sampling technique termed reverse iontophoresis. In this technique transdermal extraction of interstitial fluid is obtained by applying two electrodes mounted on the skin surface [32]. Glucose is brought to the surface by electroosmostic flow of water and analysed out of the body by selected glucose sensors. This technique has the advantage of being non-invasive [33]. The potential problem is the low glucose concentration in the extracted fluid. This requires a highly sensitive glucose detection sensor, and it takes a long time to collect sufficient fluid for analysis. Local damage to the skin is possible when the sensor is used in the long term. A glucose sensor based on this principle has been market branded as GlucoWatch G2 Biographer. However, the lag time was reported as 18 min [34].

Photoacoustic Technique

Photoacoustic spectroscopy (PAS) is analogous to NIRS. In PAS, infrared light is absorbed by a sample resulting in heat generation. The heat energy causes pressure variations in the gas containing sample cell. If the excitation is pulsatile, a travelling acoustic wave will be produced. The pressure pulse propagates and can be measured by a piezoelectric detector. The detected energy is related to the absorption coefficient of the sample, i.e. concentration [35]. In vitro and in vivo results with PAS have been reported and a good correlation with blood glucose measurements has been demonstrated [36]. The technique was shown to be able to measure mili-molar concentrations of glucose. However, the repeatability and sensitivity of the photoacoustic measurement of glucose need to be improved. This work is in the early stages of development. The full in vivo application is being explored.

DISCUSSION

In general, the techniques described above can be utilized to fabricate two main types of glucose sensors for in vivo monitoring, implanted and non-invasive. Semi-invasive sensors are also being developed and these are currently attracting attention both in the academic and the commercial arena.

Implanted glucose sensors are usually based on the electrochemical transducer. Although the study of an implanted glucose sensor can be traced back to the 1960s [37,38], two major obstacles remain. Firstly, maintaining the stability of the glucose oxidase layer for the required lifetime of the sensor as clearly surplus enzyme activity is a prerequisite for success. Secondly, it is necessary to maintain a highly biocompatible outer layer to prevent fouling of the surface of the sensor. Consequently there is no reliable glucose sensor currently available which is suitable for long term intravascular implantation. However these problems are being addressed.

Recent research on the stability of GOD has focused on the fabrication procedure and materials. Several groups have used a sol-gel system or a hybrid sol-gel system to immobilize GOD as one of the main problems has been leaching of the enzyme over time [39]. An improved stability of enzyme has been claimed by the application of a bifunctional silane and covalently immobilised GOD in a film derived from a TMOS/PBS/silane system [40]. Hybrid films of sol-gel silica with dextran sulfate were also demonstrated to have good biocompatibility [41]. Another very promising procedure was reported [42], which involved the deposition of GOD on an electrode by electrochemical mediation followed by immobilisation using electropolymerization of phenol and electrochemically cross linked to form a stability-reinforced membrane. This hybrid film improved both the stability of GOD and the permselectivity of the membrane, which resulted in a highly sensitive glucose response with a working lifetime of more than 50 days in vitro. However, in vivo tests have not yet been conducted.

Improving the biocompatibility of implanted and semi-invasive glucose sensors is the other pre-requisite for success. Although poor biocompatibility is most obviously a problem for intravascular sensors, even subcutaneously implanted devices with prolonged contact with interstitial fluid will provoke an inflammatory response. This leads to encapsulation of the devices with various cellular components, which can alter the mass transport of the analyte to the surface or change the surface concentration. It is predicted that encapsulation can also affect the lag time of the sensor's response to varying glucose levels. This serves to exacerbate the intrinsic 'lag time' problem [43]. In the case of microdialysis, the permeability of the dialysis membrane can also be changed.

Creating anti-fouling or anti-coagulate sensors should resolve the problem of signal drift and permeability change. This may be achieved in one of three ways. Firstly it is possible to develop an anti-biofouling polymer as the

outermost membrane of the sensor. Copolymers bearing an electrically neutral phosphorylcholine head group that is able to reject protein adsorption [44–47] have been synthesised. These mimic the outer lipid layer structure of the red cell membrane thus creating a highly biocompatible polymer membrane. In vitro and in vivo animal tests have demonstrated better performance when a biocompatible polymer was used as the outer layer for a needle-type glucose sensor [47,48] and other type biosensors [49,50]. Unfortunately it has not yet been possible to attach the biocompatible polymer firmly to the surface of the sensor, in order to prevent stripping during insertion or from the shearing forces of blood in the case of an intravascular device. Secondly, natural anti-coagulant biomolecules, such as heparin, can be grafted or simply coated to the sensor or device surfaces. However the attached heparin is gradually released from the surface and platelets do eventually adhere and activate [51]. Nevertheless this solution has been used in a few commercial products [4].

The third approach is the development of an active outer layer, which can release anti-coagulant molecules continuously. Nitric oxide (NO) is a potent naturally occurring anti-platelet agent. A new hydrophobic NO-releasing polymer that exhibited greatly enhanced haemocompatability and improved analytical performance of biosensors [52] has been reported. The technique of controlled release active agents has been successfully applied by several companies to maintain the patency of stents used for the treatment of blocked coronary arteries. A sirolimus-eluting stent [53,54] demonstrated a great improvement in preventing the growth of scar tissue, which leads to restenosis.

Although biocompatability is less of a problem for semi-invasive sensors there still remains a number of challenges to be overcome before these sensors can be used widely in the clinical environment. It is essential to maintain stable analyte influx through the sensor surface to the sensing layer, which is the GOD layer in the electrochemical sensor, and the fluochromo capsule in the fluorescence sensor. It is also important to establish a precise relationship between changes in blood glucose and ISF glucose in both time and concentration dimensions [55]. An understanding of these relationships may help to overcome the physical lag time that occurs between a change in blood glucose concentration and a change in ISF glucose concentration.

Non-invasive monitoring is the most attractive technique for patients. Such techniques will eliminate biocompatibility problems completely. However there are still major problems to be overcome in relation to NIRS and PAS as described above particularly in relation to light scattering and sensing technology [56]. A major increase in the sensitivity of detectors either by a new-generation of instruments or by novel mathematical filtering techniques to maximize all aspects of the signal is required. Another significant obstacles to home monitoring are the

Table I. Estimation of Potential of Various Techniques for Glucose Monitoring In Vivo

Sensor type	Measurement mode	In vivo test	Market availability	Future potential
1.Electrochemical				
Needle type	Subcutaneous	Yes	Yes (CGMS)[a]	Optimistic, need to reduce frequency of calibration and improve biocompatibility
	Intravscular	Yes	No	Need strong biocompatible materials
Microdialysis	Subcutaneous	Yes	No	Optimistic, need to reduce frequency of calibration and improve biocompatibility
2. Optical				
Fluorescence	Subcutaneous	Yes	No	Optimistic, need more sensitive, stable glucose indicator proteins
NIRS/PAS	Non-invasive	Yes	No	Optimistic, need better algorithm between the spectrum and glucose concentration
3. Other				
Impedance Spectroscopy	Non-invasive	Yes	Yes (Pendragon)[b]	Trend indication
Reverse inotophores	Non-invasive	Yes	Yes (GlucoWatch Biographer)[c]	Trend indication
INSFET	Subcutaneous	Yes	No	Need improved response rate

[a] Manufactured by Medtronic MiniMed.
[b] Manufactured Pendragon Medical.
[c] Manufactured by Cygnus.

high cost of the instruments and the requirements of periodic calibration against a standard measurement. This may limit the use of these techniques to the hospital as is largely the case for bilirubinometers and pulse oximeters. Development of glucose sensing contact lenses [57], and impedance spectroscopy [29] represents new directions which are attractive to industry because of the ease of use and low cost. Whether these methods can produce precise enough quantification to give real value in the clinical situation remains to be further explored.

Calibration of the Sensors and User Issues

Most continuously monitored patient parameters (e.g. pO_2, pCO_2, SpO_2) must be periodically calibrated against a "gold standard" and glucose monitoring is no exception. This is especially the case when decisions about change in medication are to be made. Methods of calibration may vary from laboratory analysis (Quality Assurance) to methods carried out by the user as specified by the device manufacturer. Most sensors have a usable lifetime and the user, especially the home user, needs to be educated in the calibration of the device, limitations and interpretation of the data.

CONCLUSION

Great progress has been achieved in in vivo glucose monitoring with various techniques available, from electrochemical to optical transducers. However, stable and reliable clinical applications of in vivo glucose monitoring are limited. Up to now, only a few models have been approved by FDA. They are not widely used. Improvement in biocompatibility, elimination of interference and increase of signal to noise ratio are the main challenges to be resolved. The potential for the further development of techniques for in vivo glucose monitoring are estimated in Table I.

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