In vivo glucose sensing for diabetes management: progress towards non-invasive monitoring

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BMJ 1999;319;1289-

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BMJ 1999;319:1289

A device for continuous in vivo monitoring of glucose concentration in people with diabetes has been a clinical and research priority for many years but now has an urgency which is probably unquestioned in diabetes care. The purpose of this article is to explain recent advances in technology that are bringing glucose sensors closer to routine use and to highlight some of the remaining problems. Important new technologies include artificial receptors for glucose, tissue fluid sampling techniques, and new approaches to non-invasive sensing, such as fluorescence lifetime measurements.

Summary points

One of the main reasons for developing in vivo glucose sensors is the detection of hypoglycaemia in people with insulin dependent (type 1) diabetes

Until recently, research and development largely focused on needle-type glucose sensors (enzyme electrodes) implanted in the subcutaneous tissue. Problems of calibration and drift have delayed clinical application, but one device for trend monitoring is now being commercialised and is entering practice

Several new approaches will accelerate development of in vivo glucose sensors, including totally implanted sensors with more robust artificial glucose receptors. These might be interrogated from outside the body by measurement of changes in near infrared fluorescence intensity or decay lifetime

Tissue fluid sampling and extraction techniques—such as microdialysis and reverse iontophoresis—enable glucose to be measured outside the body under more controlled conditions but need further development

Non-invasive glucose sensing will maximise acceptance by patients and overcome biocompatibility problems of implants. Near infrared spectroscopy has been most investigated but the precision needs to be improved for eventual clinical application

This is a selective overview that concentrates on research of the past five years, which we have assembled largely through personal experience and research in the specialty and from recent international workshops and meetings.

The need for in vivo glucose monitoring

The main reason for developing in vivo glucose sensors is for the detection of hypoglycaemia in diabetes. Patients with insulin dependent (type 1) diabetes have always feared low blood glucose concentrations,[1] especially during the night, when self monitoring of blood glucose concentration with finger prick methods cannot be performed, and those without warning symptoms (hypoglycaemia unawareness) are especially vulnerable.[2] But automatic hypoglycaemia detection has become a major goal for glucose sensing research since it became clear that strict blood glucose control is usually accompanied by a clearly increased frequency of hypoglycaemia.[3] It is simply very difficult indeed to achieve and maintain near normoglycaemia in people with type 1 diabetes without incurring the penalty of potentially dangerous low blood glucose concentrations.

One example of the major clinical benefit of hypoglycaemia detection with an implantable glucose sensor can be seen by studies that have shown that falls in tissue concentrations of glucose (measured by a sensor) often precedes the fall in blood glucose and may act as an early warning to the patient of impending hypoglycaemia.[4]

The notion that an in vivo glucose sensor might be coupled via a computer to a portable insulin infusion pump to create an artificial endocrine pancreas controlled by feedback is appealing, of course. Indeed, a glucose sensor is a prerequisite for a totally implantable artificial pancreas[5] but such systems have been put to one side for the moment as an ambition for routine management. Safe delivery of insulin in this way will require glucose sensors that have proved totally reliable after many years of "open loop" testing. We are far from being at or near that stage.

Implanted sensors and their problems

At first sight the task seems simple enough. A well established laboratory chemistry technique for measuring glucose concentration can be immobilised on a probe that is implanted in the tissues (say, subcutaneously), with the signal relayed to a meter outside the body by wire, fibre optic, or telemetry. Reagentless probes or "biosensors" for measuring glucose in vitro were first described in the 1960s[6] and glucose sensors first tested in vivo in animals the early 1970s.[7] Biosensors were indeed among the most promoted technologies of the 1980s, with the expectation of numerous applications in clinical analysis.[8]

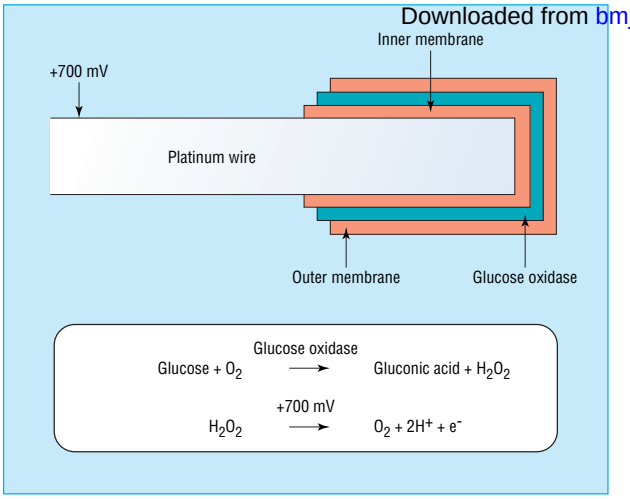

Fig 1 Typical construction of enzyme electrode for glucose sensing. Glucose oxidase is immobilised at platinum anode. Inner membrane—such as cellulose acetate—filters interfering substances, and outer membrane—such as polyurethane—controls diffusion of glucose and improves biocompatibility. Hydrogen peroxide produced by glucose oxidation is detected electrochemically

The most studied glucose sensors are of the type called "amperometric enzyme electrodes" (fig 1), in which the enzyme glucose oxidase is immobilised at a charged electrode and glucose concentrations monitored by the change in current flow caused by the enzyme catalysed production of hydrogen peroxide[9–11] or, less often, by the consumption of oxygen.[12] A modification of enzyme electrode technology is now well established in some commercial devices for self monitoring of finger prick samples for blood glucose concentration (for example, MediSense) and has been applied to in vivo sensing.[13]

Usually the sensor is configured as a fine needle or flexible wire, with the active sensing element at or near the tip, and implanted in the subcutaneous tissue. Such sensors are regarded as "minimally invasive" and their subcutaneous implantation avoids the problems of septicaemia, fouling with blood clot, and embolism, which are potentially associated with intravascular placement.

There were initially encouraging test results with needle type sensors over a few days in animals and humans,[9 13–16] but clinical development has been slow. A device based on this technology (MiniMed) has recently received approval as a trend monitor to supplement finger prick blood glucose measurements; there is as yet no direct readout of blood glucose by the patient but playback of data after 72 hours via a computer for review by a physician. Why has progress been difficult with this technology? The glucose concentration in the subcutaneous interstitial fluid recorded from implanted sensors[9–16] or measured by another technique such as microdialysis[17] is indeed proportional to the blood glucose concentration under most circumstances, and some sensors give excellent estimates of glycaemia for up to a week or so in both animals and humans. But the sensor output in vivo is suppressed by a variable amount compared with the in vitro signal at the same glucose concentration,[18 19] thus requiring careful calibration procedures, and, importantly, the output can drift unpredictably.[14 15] The basis of these erratic responses is unclear and has prevented rational modifications of sensors. The present evidence favours a reversible coating of the implanted sensor or the diffusion into the sensor of low molecular weight inhibitors of the sensing mechanism (which can be washed off after explantation), but the chemical nature of the interference is unknown.

New strategies for improving implanted glucose sensors

Industrial mass production techniques ensure reproducibility of sensor construction and functionality, and, in addition, other approaches are being used to improve the performance of subcutaneous glucose sensors.

Microperfusion

This aims to control the microenvironment at the sensing site by using slow, open flow perfusion of isotonic buffer over the tip of the electrode.[20] The thin, mobile aqueous film may provide a protective barrier, wash away inhibiting molecules or cells, or hydrate the tissues.

Totally implanted sensors

Part of the rationale behind totally implanted sensors is that short term, transcutaneous implantation of needle-type sensors induces a wound response with acute inflammation, changing protein, fluid, and cellular accumulation, and thus resulting in variable concentrations of glucose and oxygen. This may contribute to the instability of these sensors. In contrast, long term implantation induces an encapsulating foreign body response, and there is some evidence that this may be a more stable sensing environment. Glucose sensors totally implanted in the subcutaneous tissue of dogs were inactive for the first few days, unstable for the next 7-14 days, and then became relatively stable for the subsequent several weeks, though still not sufficiently so for clinical use.[21]

Abiotic glucose receptors

Artificial alternatives to enzymes as glucose recognition molecules are being sought because they may not be affected by in vivo interferences and may be more robust for long term implantation. One of the most promising techniques for creating artificial receptors is called "molecular imprinting" or "plastic antibodies" (fig 2).[22] Here, monomers that have chemical groups that interact with a template molecule related to the analyte are polymerised around the template, the template is then removed, leaving a polymer that is specific in shape and binding capacity for the analyte. An example for glucose sensing uses the interaction at alkaline pH between a metal ion complex and glucose, which releases hydrogen ions on glucose binding.[23] A porous polymer specific for glucose has been made whereby glucose concentration can be measured by titratable release of protons.

Fluorescence technologies

Another approach to artificial glucose receptors uses fluorescent molecules—such as the compound produced by the coupling of the fluorescent dye, anthracene, to boronic acid, which covalently but reversibly binds to two of the hydoxyl groups

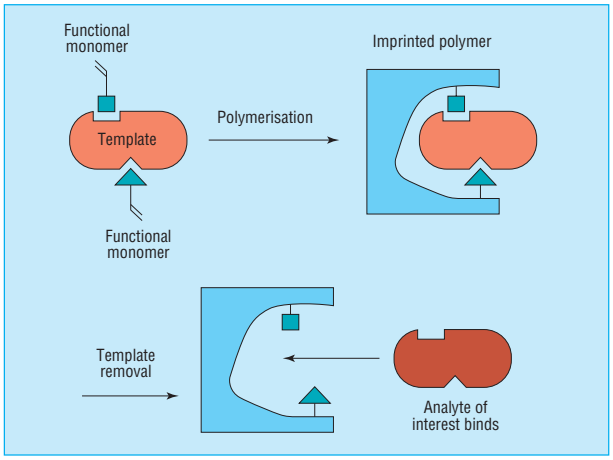

Fig 2 Principle of molecular imprinting for generating artificial glucose receptors. Monomers have groups that interact with analogue of analyte (glucose). This acts as template during polymerisation. Removal of template leaves robust polymer that binds analyte of interest

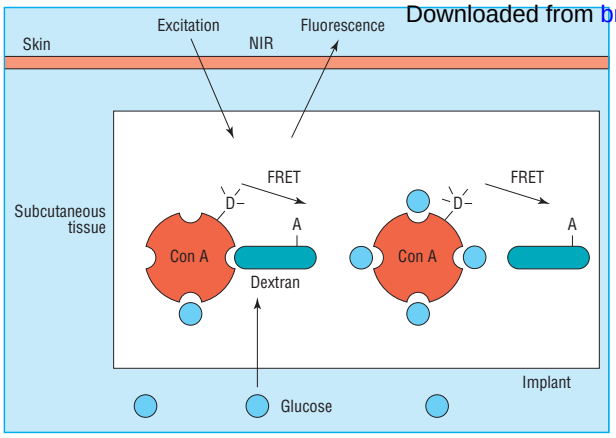

Fig 3 Glucose assay with fluorescence resonance energy transfer (FRET) technology. Concanavalin A (Con A) competitively binds glucose and sugar polymer, dextran. FRET occurs between Con A labelled with fluorophore donor (D) and dextran labelled with an acceptor (A). Displacement of dextran by glucose reduces FRET and increases fluorescence intensity and lifetime. Use of near infrared dyes allows excitation and fluorescence recording from outside body (NIR=near infrared fluorescence)

on glucose.[24] With this receptor, a change in fluorescence intensity occurs on glucose binding.

Fluorescence measurements are particularly well suited to totally implanted sensors because dyes might be used that can be excited and emit light in the near infrared region of the light spectrum, and as infrared light passes through several centimetres of tissue, such probes can be inserted in the subcutaneous tissue and both activated and interrogated from outside the body. Techniques for measurement of fluorescence decay lifetimes with pulsed light sources have several advantages for in vivo sensing over the more usual measurements of fluorescence intensity, including easier calibration because the lifetime is not notably affected by changing concentrations of the fluorescent label or by photobleaching or light scattering in the tissues. Extremely sensitive techniques (such as time correlated, single photon counting) exist for monitoring fluorescence lifetime changes.[25]

An example of a useful fluorescence technique is "fluorescence resonance energy transfer" (FRET), which relies on the transfer of excitation energy from one fluorescent molecule (the donor) to another nearby molecule (the acceptor) that has overlapping spectral properties. Changes in fluorescence intensity or lifetime are reporters of the changing distance between the donor and acceptor (for example, receptor and ligand; fig 3). Model FRET schemes have been described for glucose sensing in vitro with the glucose binding lectin concanavalin A coupled to near infrared fluorescent molecules.[26 27]

Conformation change in a protein on binding substances can also be sensed when the label is an environmentally sensitive fluorophore (fig 4). Molecular engineering techniques are being used in this respect for the rational adaptation of proteins to produce new molecules with modified functions more suited to sensing. For example, environmentally sensitive fluorescent groups have been incorporated into allosteric proteins such as the glucose binding protein from *Escherichia coli*.[28] This protein undergoes a large conformational change on glucose binding that can be transduced into a change in fluorescence in the engineered protein.

Tissue sampling

A possible way of overcoming the biocompatibility problems of implanted sensors entails transport of tissue fluid outside the body for more controlled assay. Microdialysis is the most popular and has undergone considerable testing over a few days in humans.[29–31] A probe containing a hollow dialysis fibre is inserted in the subcutaneous tissue and perfused at a slow rate with isotonic fluid so that glucose diffuses into the fibre and is pumped outside the body for online assay—for example, by an enzyme electrode. By perfusing the fibre at a high and low rate with glucose solution and comparing the glucose concentration in the emerging dialysate, the absolute tissue glucose concentration can be calculated, compensated for system drift.[32]

"Reverse iontophoresis" is another sampling technology in which transdermal extraction of interstitial fluid is achieved by applying current to two electrodes mounted on the skin surface.[33 34] Glucose is carried to the surface by electro-osmotic flow of water. Possible problems are the time it takes to collect sufficient fluid for analysis (15-20 minutes), the low glucose concentrations in the extracted fluid (about 1/1000 of those in blood), variable flux of glucose across the skin, and the effects of prolonged use at one skin site; but considerable research and development is under way aimed at commercialisation as a watch-type device with integral electrochemical determination of extracted glucose (Cygnus Inc).

Completely non-invasive glucose sensing

There is no doubt that acceptance by patients will be at an optimum and bioincompatibility problems overcome by completely non-invasive approaches to glucose sensing.[35]

Near infrared spectroscopy

This is the most studied non-invasive technology.[36 37] Between about 600 and 1300 nm there is a so called "optical window" in tissues that are transparent to light in this spectral region. Absorption readings can be made by transmission or reflectance through or at tissues such as the finger tip[37] or oral mucosal surface.[38 39] The glucose absorption peaks are small but with readings at several wavelengths for many known glucose concentrations, complex multivariate techniques produce calibration models with good correlations between reference blood glucose and predicted glucose concentrations, both in vitro and in vivo. The precision, however, is presently not good enough to use this technology clinically. There may be many reasons for this, including unpredictable spectral variations that are not related to glucose but to factors such as tissue hydration, blood flow, temperature, light scattering (and thus the optical pathlength), overlapping absorption by non-glucose metabolites and a particularly strong absorption by water in the near infrared region, and movement artefact caused by changes in the alignment of the instrumentation. Research is now focused on understanding these variables in more detail.

Light scattering

An alternative non-invasive glucose sensing technology uses changes in light scattering in the tissues[40 41] and is based on the dependence of scattering in turbid suspensions on the ratio of the refractive indices of the particles (cells, membranes, fibrils, etc, in the case of tissue) to the solution (plasma, inter-

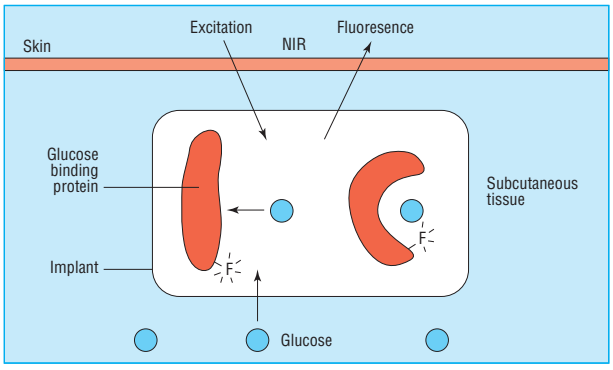

Fig 4 Glucose assay with protein labelled with environmentally sensitive fluorophore (F). Glucose binding alters protein conformation and changes fluorescence (increase or decrease, depending on specific fluorophore)

Some minimally invasive and non-invasive sensors for in vivo glucose monitoring in diabetes

Transcutaneous needle-type enzyme electrodes

Totally implanted sensors

- Enzyme electrodes
- Near infrared fluorescence based

Sampling technologies

- Microdialysis
- Reverse iontophoresis

Non-invasive technologies

- Near infrared spectroscopy
- Light scattering
- Photoacoustic spectroscopy

stitial fluid) in which they are suspended. The increase in refractive index of the plasma and interstitial fluid as glucose concentration increases lowers the scattering coefficient by about 1% for each 5 mmol/l change—that is, the tissue becomes slightly more transparent. Substantial variations, however, are observed in the magnitude of the scattering change for a given glucose change within and between individuals.[41] Much the same influences of temperature, tissue hydration, and probe alignment may apply here as do for near infrared spectroscopy, making immediate clinical application difficult.

Photoacoustic spectroscopy

This is less investigated. Pulsed infrared light is absorbed by molecules such as glucose and leads to thermal expansion and the generation of an ultrasound wave that is detectable at the skin surface by a piezoelectric microphone.[42] Encouraging results have been obtained in blood samples and in vivo in a small number of diabetic and non-diabetic people,[43] but results of extended clinical studies are awaited.

Conclusion

Problems with minimally invasive transcutaneous sensors have encouraged research on methods for tissue sampling and non-invasive technologies. These last range from totally implanted sensors that can be interrogated from outside the body—for example, by measuring fluorescence changes—to optical techniques for complete non-invasive monitoring. It is unlikely that only one technology for glucose sensing will be in use by the next generation of diabetic patients; more probably a range of devices will find clinical application, including transcutaneous implants used for a day or so at a time—the first in vivo glucose sensors to be commercialised, totally implanted sensors for long term use in selected people, and, perhaps the last to enter clinical practice but the most sought after, completely non-invasive glucose monitors.

Funding: Engineering and Physical Sciences Research Council.

Competing interests: None declared.

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