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Review Article

Glucose sensors: a review of current and emerging technology

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Abstract

Glucose monitoring technology has been used in the management of diabetes for three decades. Traditional devices use enzymatic methods to measure glucose concentration and provide point sample information. More recently continuous glucose monitoring devices have become available providing more detailed data on glucose excursions. In future applications the continuous glucose sensor may become a critical component of the closed loop insulin delivery system and, as such, must be selective, rapid, predictable and acceptable for continuous patient use. Many potential sensing modalities are being pursued including optical and transdermal techniques. This review aims to summarize existing technology, the methods for assessing glucose sensing devices and provide an overview of emergent sensing modalities.

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Keywords Glucose sensors, technology, Continuous glucose monitoring, closed loop

Abbreviations CG-EGA, continuous glucose error grid analysis; CGMS, continuous glucose-monitoring system; ConA, concanavalin A; FRET, fluorescence resonance energy transfer; GOx, glucose oxidase; HbA_{1c}, glycated haemoglobin; ISF, interstitial fluid; MIR, mid-infrared; NIR, near infrared; OCT, optical coherence tomography

Introduction

Intensive treatment of glucose in Type 1 diabetes reduces the risk of complications, in particular the microvascular complications of retinopathy, nephropathy and neuropathy [1]. However, this reduction in complications was associated with an increased incidence of hypoglycaemia.

Maintaining euglycaemia while avoiding debilitating hypoglycaemia and intermittent hyperglycaemia requires treatment to be titrated to glucose levels measured by the patient and by healthcare professionals using glucose-sensing devices. Point-sample results for capillary blood glucose are predominantly used at present to adjust diabetes treatment.

Many technologies are being pursued to develop novel glucose sensors (Fig. 1), including non-invasive, continuous monitors. This paper reviews the history of glucose sensors and methods of sensor assessment and provides an overview of emerging glucose-sensing technology for clinicians.

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History of glucose sensors

The first patent for a blood glucose monitor for point-of-care use in diabetic patients was filed in 1971 in the USA by Anton Clemens. The Ames Reflectance Meter was a device which automatically assessed the colour change of enzyme-based reagent strips. Prior to this, blood glucose had been estimated by reading the colour change by eye from a chart. The Ames Reflectance Meter was succeeded by the Ames Eyetone, which became commonplace but was restricted to clinical areas such as physicians' offices and hospital wards.

One of the first patients to use the device at home was Richard Bernstein, a US physician with Type 1 diabetes. He wrote *Dr. Bernstein's Diabetes Solution: A Complete Guide to Achieving Normal Blood Sugars*. The first studies using the Ames Eyetone Meter for home blood-glucose monitoring were published in 1978. Peterson *et al.* [2] recruited 10 patients with Type 1 diabetes with a mean age of 25 years. At the time of entry to the study, their mean glycated haemoglobin (HbA_{1c}) was 10.3%. Following education to use the Ames Reflectance Meter at home and a follow-up of 4–6 months, the mean HbA_{1c} was 5.4% [2], showing dramatic improvement in glycaemic control. With larger numbers ($n = 64$), Sönksen *et al.* showed that 64% of patients were able to maintain 80% of blood glucose values < 10.0 mmol/l over a period of > 1 year [3]. In

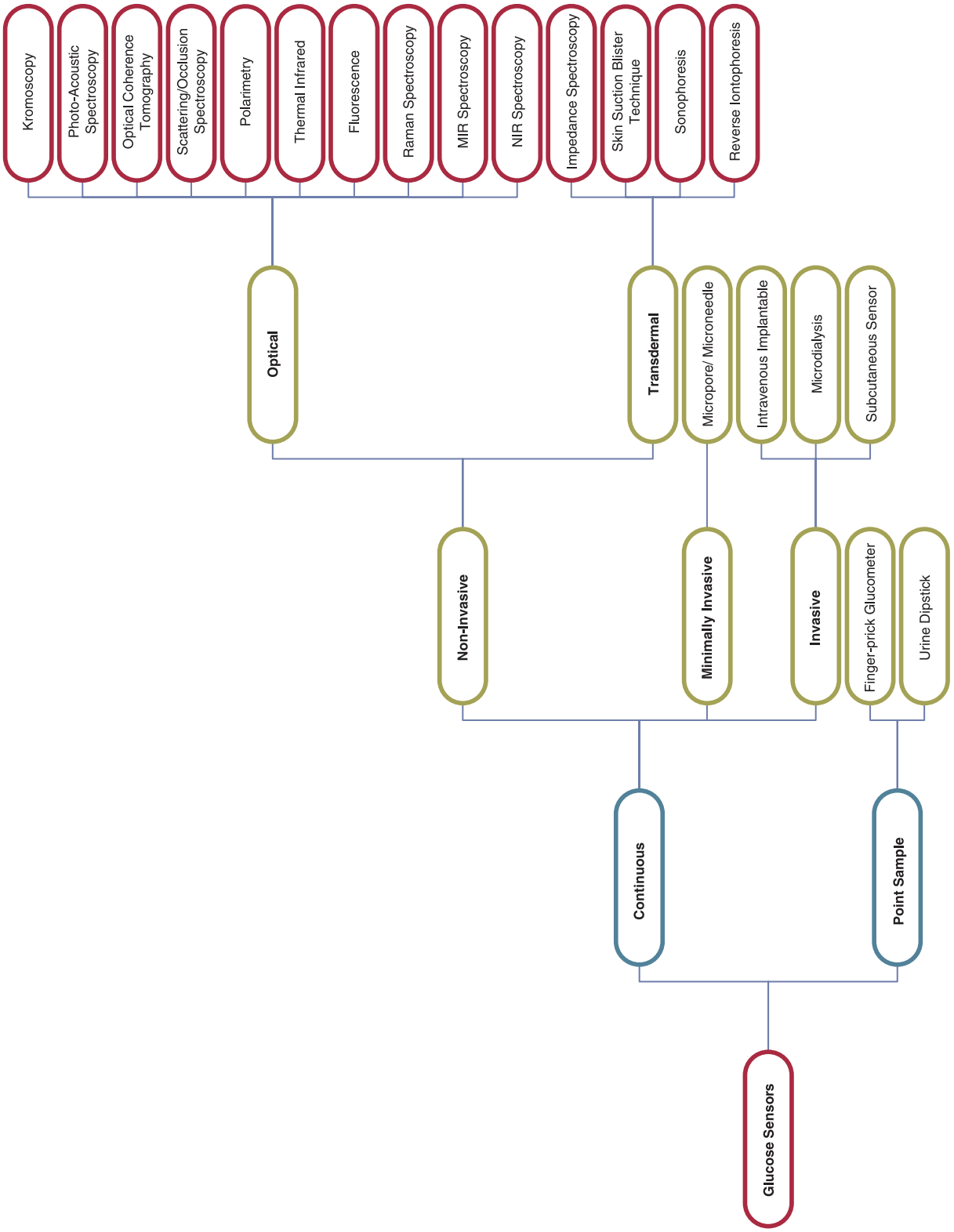

FIGURE 1 Technologies under development for glucose sensors. MIR, mid-infrared; NIR, near infrared.

a similar sized study cohort ($n = 69$), half of the patients had capillary blood glucose profiles in which no more than one value exceeded 10.0 mmol/l [4]. Other, albeit smaller, studies confirmed these results [5–7].

These early studies of self monitoring of blood glucose demonstrated that the technology was acceptable to patients and improved glycaemic control. Importantly, self monitoring also reduced the frequency of hypoglycaemia.

Following the initial studies, interest in home blood-glucose monitoring increased and in 1986 the American Diabetes Association, Food and Drugs Administration and the National Institutes of Health issued a joint statement recommending self-monitoring of blood glucose for the following uses:

- pregnancy complicated by diabetes mellitus;
- individuals with a propensity for severe ketosis or hypoglycaemia;
- individuals prone to hypoglycaemia who may not experience the usual warning symptoms;
- individuals on intensive treatment programmes, especially those using portable insulin infusion devices and multiple daily injections;
- individuals with abnormal renal glucose thresholds.

The Consensus Panel also indicated that, although controversial, home blood-glucose monitoring may be useful for patients with diabetes not treated with insulin [8].

Subsequent to this, self-monitoring of capillary blood glucose has become an important component of the management of Type 1 diabetes, providing patients with a tool to guide treatment and identify hypo- or hyperglycaemia. Self monitoring of blood glucose has also been investigated in Type 2 diabetes [9–11], but consistent glycaemic improvement has not been demonstrated. Present National Institute for Clinical Excellence (NICE) guidance [12] recommends: self monitoring of blood glucose for patients with Type 2 diabetes on insulin treatment; those on oral glucose-lowering medications to provide information on hypoglycaemia; assessing changes in glucose control resulting from medications and lifestyle changes; monitoring changes during intercurrent illness and to ensure safety during activities, including driving.

The ideal glucose sensor should be selective for glucose with a fast, predictable response to changing glucose concentrations. It should depend on a reversible and reproducible signal to provide results and sensor fabrication must be reproducible and cheap on a large scale. It should have a long operational lifetime under physiological conditions, but most of all must be acceptable to the patient. Therefore, it should be non-invasive, should not require user calibration and would ideally provide real-time continuous information regarding glucose. Continuous glucose monitoring provides data about the direction, magnitude, duration, frequency and potential causes of fluctuations in blood glucose levels, providing patients with real-time data and alarms at times of hypoglycaemia or rapid glucose change. Continuous glucose monitoring is also required to implement closed-loop control. Recent consensus guidelines for continuous glucose monitoring [13] have emphasized the importance of patient education and clinical understanding of the technology and the data it provides.

The present International Organization Standard (ISO DIS 15197) states, for reference glucose values ≤ 4.2mmol/l (75 mg/dl) the sensor should detect a result within 0.83 mmol/l (15 mg/dl) and, for reference glucose values ≥ 4.2 mmol/l, the sensor should be within 20%. Beyond regulatory standards, the accuracy (correctness) and precision (degree of reproducibility) of glucose sensors against reference methods can be measured in many ways. Linear regression and correlation co-efficient may be used to assess accuracy [14], but the clinical significance of an error in glucose sensing is dependent upon the absolute value of the reference and sensor glucose, as well as the direction and magnitude of the error. Regression analysis is dependent on a highly accurate reference method and correlation coefficients may be high when precision is poor. Regression measures deviation from the desired line $x = y$, but correlation coefficients may be high regardless of the y intercept or gradient. Both regression and correlation are dependent on the range of the measured values.

A frequently used method of assessing glucose monitors is the Clarke error grid which was developed in the 1980s. This assesses a monitor's performance on the y axis against reference glucose on the x axis, assigning a clinical risk to any glucose sensor error [15,16].

Results fall into one of five risk zones, labelled A, B, C, D and E (see Fig. 2). The areas are defined as follows: zone A, clinically accurate; zone B, benign; zone C, overcorrect; zone D, failure to detect; zone E, erroneous.

Zones A and B are clinically acceptable, but results falling in zone C may prompt overcorrection leading to hypo- or hyperglycaemia. Zone D represents a failure to detect hypo- or hyperglycaemia and Zone E represents erroneous results. In order to create risk boundaries, the Clarke error grid construction assumes (i) that target blood glucose is 3.9–10.0 mmol/l, (ii) that patients will only correct outside of that range, (iii) that correction is inappropriate if the blood glucose finishes outside of that range and (iv) that failure to treat blood glucose between 3.9 and 13.3 mmol/l is inappropriate. Boundaries between risk in the Clarke error grid are not sequential, meaning that a small change in glucose concentration reported by a sensor can move a result from the benign zone B to the clinically significant zone D, or vice versa. Despite the assumptions made in the construction of the Clarke error grid and its weaknesses in implementation, it has been extensively used in assessing glucose sensors.

In 2000, Parkes *et al.* proposed the Consensus error grid based on the responses of 100 diabetologists asked to assign risk to self-monitoring blood-glucose errors (Fig. 2) [17]. The consensus error grid has continuous risk groups, avoiding the skipping possible in the Clarke error grid and is based on the consensus opinion of more physicians. It also has separate error grids for Type 1 and Type 2 diabetes, allowing for separate assessment. However, the physicians' responses were based on

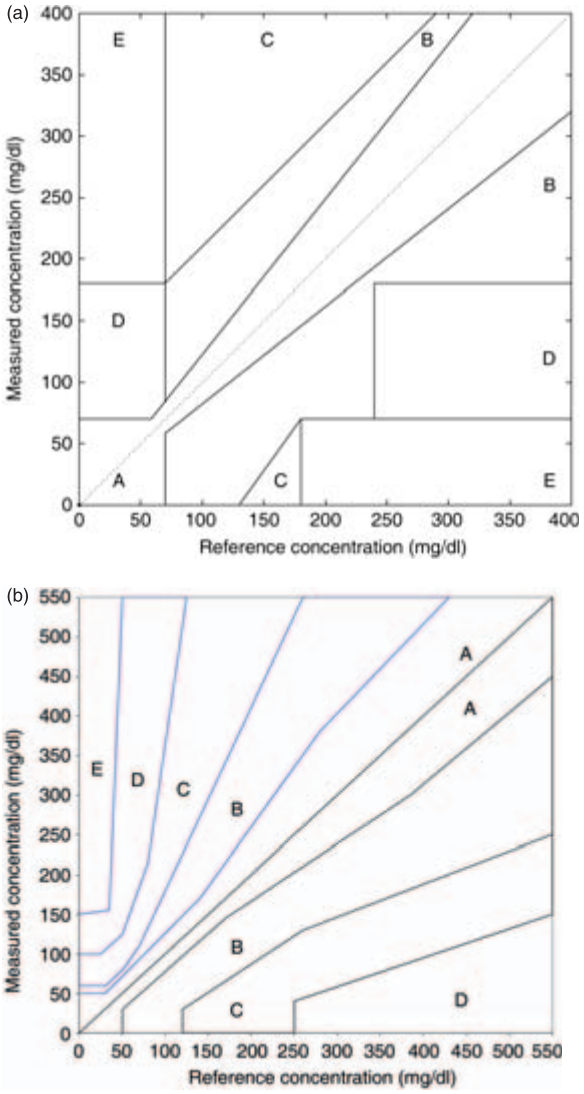

FIGURE 2 Glucose sensor error grids: (a) Clarke error grid; (b) consensus (Parkes) error grid.

specific patient examples and may not be transferable. In direct comparison using the same data set, the Consensus error grid rated 98.6% of the data points as clinically acceptable compared with 95% in the Clarke error grid.

Error grids, regression and correlation all provide static accuracy data but do not take into account the timing of paired results. For continuous glucose monitoring assessment, the time sequence of results is an important factor to consider. In 2004, Kovatchev *et al*. described the continuous glucose error grid analysis (CG-EGA) [18]. This takes into account the temporal characteristics of continuous monitors and the lag between measuring blood for reference values and interstitial fluid for continuous sensor values. The CG-EGA uses the sum of error analysis of point glucose values (i.e. accuracy) and the rate of glucose change. The performance of the sensor is then stratified for hypoglycaemia, euglycaemia and hyperglycaemia, taking into account the different performance characteristics of continuous sensors in these ranges. Results obtained using the CG-EGA depend on sampling frequency and assume a lag time of less than 7 min.

Paired data from continuous glucose monitors and single-point measurement may be used to assess accuracy. The mean difference (MD = mean of the sensor – reference values) and mean relative difference (MRD = MD/reference value × 100) provide an estimation of systematic over- or under-estimation by the sensor but balanced errors each side of the reference value are cancelled out. Mean absolute difference (MAD = mean of the absolute difference between sensor and reference value) and mean absolute relative difference (MRD, the absolute difference expressed as a percentage of the reference value) provide an estimation of overall accuracy but do not give any idea of direction of the error. Bland–Altman plots express the difference between the two methods against the mean of both methods at a paired data point [19]. This method may be used to assess accuracy or precision by plotting different measurement modalities or the same modality repeated and gives a graphical representation of agreement in addition to demonstrating any relationship between systematic bias and glucose concentration.

Combined curve fitting uses least-squares linear regression to fit the sensor values to a curve. Assuming the sensor curve is the same as the reference, these can be overlaid, allowing calculation of the horizontal shift (lag) and vertical shift (bias). This technique does not require simultaneous paired data points and has been used to calculate the lag between sensor types [20] and between blood and interstitial fluid [21].

Glucose sensors may be invasive, non-invasive or minimally invasive and may depend on transdermal technologies or sampling of blood or interstitial fluid (ISF). Techniques may measure glucose directly or may measure surrogates for changes in glucose concentration. Many of the technologies reviewed assess glucose within the ISF (see Table 1).

Transdermal technologies use physical energy to access ISF or blood and extract glucose. These may be viewed as non-invasive as they do not physically breach the skin barrier. However, they do alter the properties of skin and may cause erythema, local irritation and blistering. Optical technologies use the physical properties of light to assess glucose in the ISF or in the anterior chamber of the eye. These principles are truly non-invasive.

Interstitial fluid

Interstitial fluid is a small-volume compartment. Glucose enters the ISF from blood in a free and rapid exchange and is removed from the ISF by uptake into cells within the compartment. Glucose concentrations in the ISF are dependent on blood flow, metabolic rate and the rate of change of glucose concentration in blood. Peak glucose concentrations in interstitial fluid lag behind rises in blood glucose concentration by between 2 and 45 min. Studies have suggested that the mean lag time is 6.7 min [22]. However, when blood glucose is falling, interstitial fluid glucose concentrations may fall before blood concentrations suggesting that ISF glucose may act as an

Table 1 Summary of emergent glucose sensor technologies

Principle	Modality	Compartment	Invasive	Product
Kromoscopy	Optical	ISF	Non-invasive	—
Photoacoustic spectroscopy	Optical	ISF	Non-invasive	—
Optical coherence tomography	Optical	ISF	Non-invasive	—
Scattering/occlusion spectroscopy.	Optical	ISF	Non-invasive	—
Polarimetry	Optical	ISF/anterior chamber	Non-invasive	—
Thermal infrared	Optical	ISF	Non-invasive	—
Fluorescence	Optical	ISF	Non-invasive	—
Raman spectroscopy	Optical	ISF	Non-invasive	—
MIR spectroscopy	Optical	ISF	Non-invasive	—
NIR spectroscopy	Optical	ISF	Non-invasive	
Impedance spectroscopy	Transdermal	Capillary blood	Non-invasive	—
Skin suction blister	Transdermal	ISF	Non-invasive	—
Sonophoresis	Transdermal	ISF	Non-invasive	—
Reverse iontophoresis	Transdermal	ISF	Non-invasive	GlucoWatch
Micropore/microneedle	Subcutaneous	Capillary blood/ISF	Minimally invasive	—
Intravenous implantable	Intravenous	Venous blood	Invasive	—
Microdialysis	Subcutaneous	ISF	Invasive	GlucoDay
Subcutaneous sensor	Subcutaneous	ISF	Invasive	Medtronic CGMS System DexCom STS Freestyle Navigator

CGMS, continuous glucose-monitoring system; ISF, interstitial fluid; MIR, mid-infrared; NIR, near infrared.

early warning for hypoglycaemia [23]. The lag time between blood and ISF glucose is dependent upon species, sensor type, sensor size, applied experimental stimulus (meal, i.v. glucose tolerance test, oral glucose tolerance test), whether the subject has diabetes and depth of sensor in tissue. Lag means that ISF sensors need to be calibrated to a blood glucose value at regular intervals. If there is a large disparity at the point of calibration, this error may be 'fixed' for all results given by the ISF sensor until the next calibration. The disparity between blood and ISF glucose changes may suggest that ISF glucose sensors are not ideally suited to use in closed-loop insulin delivery systems as hyperglycaemic complications and hypoglycaemia (and consequent neuroglycopenia) are dependent on blood glucose concentrations. In subcutaneous–subcutaneous closed-loop systems, where ISF is used to sense glucose and insulin is delivered subcutaneously, further lag is encountered in the delivery and absorption of insulin [24,25].

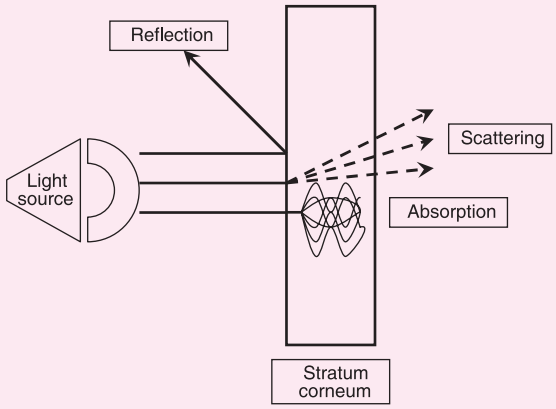

FIGURE 3 Optical properties of light utilized in glucose detection.

Non-invasive glucose sensors

Optical transducers

Optical transducers use light in variable frequencies to detect glucose, utilizing different properties of light to interact with glucose molecules in a concentration-dependent manner (Fig. 3). Spectroscopic measurement of reflected or transmitted light may also be well suited to measuring glucose across the anterior chamber of the eye as a result of the reduced interference from the components of skin and decreased biochemical artefact compared with ISF.

Infrared absorption spectroscopy

Mid-infrared (MIR) light has a wavelength of between 2.5 and 50 μm (Fig. 4). In absorption spectroscopy, the intensity of a beam of light of specific wavelength is measured before and after interaction with matter. Absorption bands seen in this wavelength are because of fundamental stretching and bending of molecules. MIR spectroscopy has been used to measure glucose concentrations in blood and water *in vitro*. However, glucose measurement is hampered by significant background absorption by other molecules, in particular water. In addition to this, MIR light has a low path length through tissue, meaning

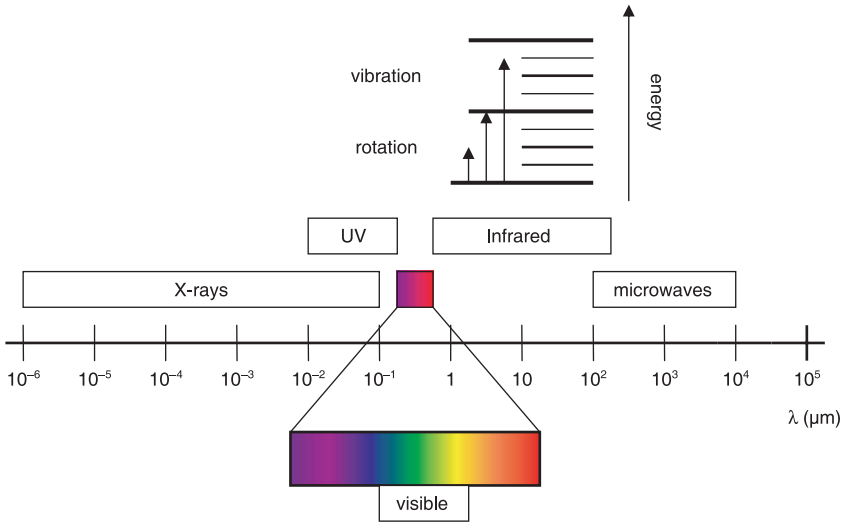

FIGURE 4 Wavelengths of radiation and region of light causing molecular rotation and vibration, as used in glucose measurement. UV, ultraviolet.

that it is only able to measure superficial concentrations. Despite this, glucose concentrations have been measured in whole blood *in vitro* with a standard prediction error of 0.95 mmol/l [26].

In contrast to MIR, near infrared (NIR) light (wavelength 0.7–1.4 μm) provides an optical window in which 90–95% of light passes through the stratum corneum and epidermis into the subcutaneous space independent of skin pigmentation. It is already successfully used to non-invasively monitor the concentration of oxygenated and deoxygenated haemoglobin in the preterm infant brain [27].

Specific absorption of NIR is dependent on molecular structure and glucose absorption peaks in this region are small. In order to calculate glucose concentrations from the absorption spectrum, transmission and reflection techniques have been used. To quantify a single solute in a complex solution, several wavelengths are used and multivariate analysis with calibration is used to derive glucose values.

All spectroscopic methods face multiple problems, including scattering of light. Scattering itself can be used to sense analytes including glucose and does not utilize specific absorption bands as it is independent of wavelength. However, in spectroscopic techniques, scattering reduces the signal : noise ratio. Scattering is also a variable effect, being dependent on hydration, blood flow, temperature and non-glucose metabolites. There is significant heterogeneity of light-absorbing and light-scattering structures (such as fat) between individuals and within individuals over time, necessitating frequent calibration. NIR spectroscopic methods also need to overcome the heterogeneous distribution of glucose between cells, interstitium and blood within skin. NIR spectroscopy is influenced by interferents and is temperature dependent.

Blood glucose has been measured across the oral mucosa using NIR [28]. Correlation was good ($r = 0.91$) but the glucose-specific absorption peaks significantly varied with the pressure applied by the device. The oral mucosa is an obvious candidate for sensing, as it is well vascularized and may be trans-illuminated. However, any oral measurement includes saliva, which may contain a different glucose concentration, and residual food may contain interferents. NIR spectroscopy has been measured across the tongue [29]. The standard error of prediction was 3.5 mmol/l, with significant variability caused by variability in tissue fat. NIR therefore ideally requires consistent tissue fat in addition to a path length in the order of 5 mm [30]. In order to solve some of the problems posed by transdermal optical techniques, NIR has been proposed in combination with techniques for sampling ISF, including sonophoresis [31] (see relevant section).

Despite these obstacles, NIR is an attractive technology for non-invasive glucose sensing. Clinical trials of an NIR device have shown a 15.1% mean absolute relative difference against laboratory blood glucose measurement and 99.3% of points in areas A and B of the Clarke error grid [32].

Kromoscopy

Kromoscopy assesses the relative intensities of overlapping spectroscopic responses from four detectors recording spectra over different wavelengths of NIR light. Complex vector analysis is used to enhance differentiation between the target analyte and interferents.

To date, urea and glucose have been successfully differentiated *in vitro* in binary solutions [33].

Thermal infrared

Thermal infrared is a surrogate measurement technique based on the principle that the cutaneous microcirculation is dependent on the local glucose concentration. This relationship is exploited by inducing controlled, periodic temperature variations in the skin and assessing MIR light scattering in the skin at different tissue depths. Following calibration to temperature, the degree of scattering can be correlated with glucose concentrations.

In preliminary studies in 20 subjects, thermal emission from the tympanic membrane allowed a calibration model to be

developed which was then used to calculate glucose concentrations in six further subjects. Good correlation was found (r = 0.87) and 100% of points were in areas A and B of the Clarke error grid [34].

Raman spectroscopy

Raman spectroscopy assesses scattering of single wavelength light. This is dependent on rotational or vibrational energy states within a molecule and highly specific absorption bands are seen with Raman spectroscopy which can be used to identify and quantify molecules. It has the benefit of reduced interference from water compared with MIR or NIR spectroscopy. However, the Raman signal is weaker than with other technologies, requiring powerful detectors for physiological concentrations of glucose. The Raman signal is also susceptible to turbidity, haematocrit, skin thickness and melanin. As with other optical techniques, Raman spectroscopy requires multivariate analysis. Surface-enhanced Raman spectroscopy has also been used *in vivo* in rats [35]. This requires metal nanoparticles to be implanted. Surface enhancement has the advantage of greatly enhancing the signal strength.

In vitro trials of Raman spectroscopy have shown clinically acceptable detection of glucose in solution in the presence of urea, ascorbate and lactate [36]. In small clinical studies, good correlation was found (r = 0.91) [37].

Polarimetry

Polarimetry measures the optical rotary dispersal of polarized light. Polarized light is light with all waves oscillating in the same plane. An optically active substance is one which can rotate the plane of polarized light. When polarized light is shone through a solution of an optically active substance, and when the light emerges, its plane of polarization is found to have rotated when measured by a polarimeter.

Rotation of light is dependent on many factors including pH, temperature, wavelength of light, path length of light and concentration of the target molecule. Maintaining polarization of light through skin is not possible and the technology needs to measure milidegree rotation under conditions of > 95% retained polarization through tissue less than 4 mm thick. This means that ISF glucose and blood glucose cannot be measured with this technology. However, it is possible to implement it across the anterior chamber of the eye. One obstacle to this is the cornea which will cause some intrinsic rotation. This can be compensated for using hardware [38]. In addition to this, as with ISF glucose, there is a physiological lag time between peak blood glucose concentrations and peak glucose concentrations in the aqueous humour. Experimental data using the rabbit aqueous humour suggest that mean lag time to peak glucose concentration is 5 min [39]. *In vitro* measurement of glucose concentration across excised rabbit cornea showed good correlation (r = 0.99) [38].

Scattering

The optical scattering properties of a tissue depend on the relative refractive indices of a particle (e.g. cell membranes, collagen fibres) and its medium (e.g. blood, ISF). As glucose concentrations rise, the refractive index of blood or ISF falls, while that of the surrounding matrix remains constant, changing the degree of scattering of NIR light. Scattering is, therefore, a surrogate measure for glucose. *In vivo* studies have shown that scattering reduces by around 1% per 5.5 mmol/l increase in glucose concentration [40]. Scattering was affected by large inter-individual differences and sensor drift was 2% per 4 h. It was also subject to motion artefact, temperature changes and changes in other physiological parameters such as water and protein. However, no difference in scattering was noted between diabetic and non-diabetic volunteers. In 19 of 26 measurements *in vivo*, the r value was greater than 0.75 [40].

Optical coherence tomography

Optical coherence tomography (OCT) was first developed to perform tomographic (i.e. plane sectional) imaging of the eye. An OCT system uses a low-power laser source, an in-depth scanning system, a sampling device and a light detector.

The skin is irradiated with coherent light (light in which the emitted photons are synchronized in time and space) and backscattered radiation is recorded. Using this technique, glucose concentrations in the dermis can be determined [41]. OCT is a surrogate measure for glucose. Sources of error include motion, tissue heterogeneity and interfering analytes [42]. However, OCT is not affected by urea, sodium chloride (NaCl), potassium chloride (KCl), temperature (±1°C), blood pressure, heart rate or haematocrit [43].

OCT has been tested *in vivo* in 15 healthy volunteers undergoing an oral glucose tolerance test and using OCT over the forearm. The OCT signal correlated well with laboratory glucose values (r = 0.8–0.95) in individual subjects suggesting future potential [44].

Occlusion spectroscopy

Occlusion spectroscopy uses scattering technique and exploits pulsatile arterial flow. As with scattering and OCT, it is a surrogate measure for glucose. Erythrocyte aggregation results in enhanced transmission of light and can be reproduced *in vivo* by applying greater than systolic pressure to the fingertip for 2–3 s. Measuring the scatter pattern at the site of erythrocyte aggregation allows calculation of glucose concentration [45].

The advantage of this technique is that it measures arterial glucose but it is vulnerable to many intravascular variables such as drug treatment, intrinsic erythrocyte aggregation, free fatty acid concentration and chylomicrons. A clinical study showed a mean absolute relative difference of 17.2 and 95.5% of values in zones A and B of the Clarke error grid [46].

Photoacoustic spectroscopy

Photoacoustic spectroscopy uses the principle that absorption of light causes ultrasonic waves. Tissue is illuminated by a light source at a specific wavelength and the absorbed radiation causes localized heating. The small temperature increase is dependent on the specific heat capacity of the tissue irradiated. Volumetric

expansion from heating causes an ultrasound pulse to be generated which can be detected. Increasing tissue glucose concentrations reduce the specific heat capacity of tissue and thus increase the velocity of the generated pulse, making photoacoustic spectroscopy a surrogate technique for glucose estimation.

This technology has been tested in aqueous solution, in gelatine-based tissue simulations, whole blood and *in vivo* [47,48]. No interference was noted from NaCl, cholesterol or albumin.

A prototype wristwatch using photoacoustic spectroscopy has been developed. Initial studies on 10 normal individuals showed good glucose prediction ($r = 0.71$) and all points falling in regions A and B of a Clarke error grid [49].

A recent study in 62 patients showed a relative absolute difference of 19.9% against venous blood samples in oral glucose tolerance test, meal test and intravenous glucose tolerance test. Between 89 and 95% of values were in areas A and B of a Clarke error grid [50].

Fluorescence

Fluorescence uses the principle of varying light emission from molecules in different states. It is fast, reagentless and extremely sensitive. Many fluorescence-based glucose sensors are based upon the affinity sensor principle where glucose and a fluorescein-labelled analogue bind competitively with a receptor site specific for both ligands. Concanavalin A (ConA), a plant lectin from the jack bean, is frequently used as the receptor molecule as it has four glucose binding sites [51]. Commonly used competitive binders are dextran, α-methyl mannoside and glycated protein.

Fluorescence was first used to detect glucose in 1984 [52]. ConA was immobilized on the inner wall of a microdialysis fibre connected to a single optical fibre. Fluoroscein-labelled dextran within the fibre was displaced from binding sites by glucose, causing increased fluorescence proportional to glucose concentration.

In 1988, this concept was developed further with a fluorescence resonance energy transfer (FRET) system [53]. In FRET, energy is transferred from a fluorescent donor molecule to an acceptor molecule by dipole–dipole interaction, leading to decreased fluorescence and a decrease in the lifespan of the donor molecule (Fig. 5). Dipole–dipole interactions take place at the Angstrom level, making FRET extremely sensitive to changes in separation between donor and acceptor. FITC was used as the fluorescent donor molecule and rhodamine as the acceptor molecule. Rhodamine was bound to ConA and dextran was labelled with FITC. As glucose displaced labelled dextran, the distance between the donor and acceptor increased, leading to a decrease in the FRET signal. Glucose detection was linear up to a concentration of 11.1 mmol/l. Under normal conditions, ConA irreversibly aggregates in a period of hours, making it unsuitable as part of a glucose sensor. However, recent work has included immobilizing it within a hydrogel or membrane capsule [54,55].

Other fluorescence techniques use enzyme catalysed reactions to change fluorescent status.

Glucose oxidase (GOx EC 1.1.3.4) catalyses the reaction:

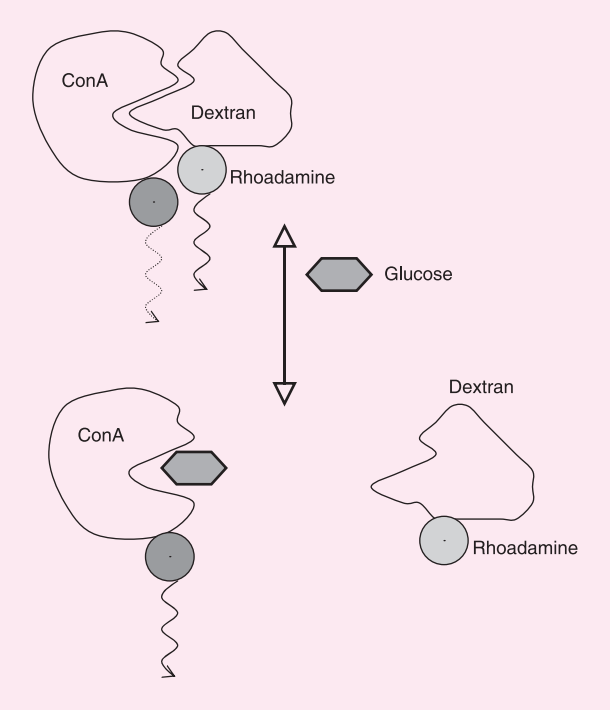

FIGURE 5 Fluorescence resonance energy transfer (FRET) fluorescence. ConA, concanavalin A.

$$\text{Glucose} + \text{Oxygen} \xrightarrow{\text{Gox}} \text{Gluconolactone} + H_2O_2$$

Fluorescein may be bound to glucose oxidase [56], allowing energy transfer between the flavin group of GOx and the fluorescein. The GOx enzyme may be further utilized by measuring oxygen consumption or hydrogen peroxide production by fluorescence [57]. GOx can also be used as glucose-binding protein, utilizing its intrinsic fluorescent properties and may be used without its flavin group with a fluorescent label [58].

Hexokinase (EC 2.7.1.1)/glucokinase (EC 2.7.1.2) enzymes catalyse the following process:

$$\text{ATP} + \text{Glucose} \rightarrow \text{ADP} + \text{Glucose-6-phosphate}$$

Hexokinase from yeast has intrinsic fluorescence which is quenched by glucose binding, making it an ideal candidate for a glucose sensor [59]. However, it is an unstable enzyme at physiological temperature. It can be immobilized in a sol-gel, improving stability, biocompatibility and the kinetic range of the enzyme [60]. Immobilization also avoids quenching of the fluorescence by interferents. Other glucose-binding proteins used in fluorescence techniques to sense glucose are derived from bacteria [61].

Synthetic boronic acid derivatives have been extensively studied for their extremely specific glucose binding and ability to be coupled to fluorescent moieties. However, they have limited solubility *in vivo*. A glucose-sensing contact lens has been

developed using boronic acids to measure lachrymal glucose concentrations [62]. The mechanism requires a hand-held external light source/detector and thus does not provide continuous sensing information. Boronic acid derivatives have also been used in holographic contact lens glucose sensors. Boronic acid within a polymer binds glucose, swelling the polymer and altering the colour of light reflected by the holographic element of the contact lens [63].

For potential analyte detection methods, human tissue has intrinsic fluorescence at specific wavelengths [64]. NAD(P)H is a fluorescent species but NAD is not, allowing NAD(P)H production to be assessed by fluorescence. NAD(P)H is produced in a variety of glucose-dependent pathways, including the tricyclic acid cycle, glycolysis and the hexose monophosphate pathway. Adipocytes and fibroblasts demonstrate intrinsic fluorescence very similar to that of NAD(P)H and this increases with increasing glucose concentrations *in vitro* to a maximum at 10–15 mmol/l [64]. The addition of insulin had no effect on the fluorescence, suggesting that diabetes treatment would not alter the signal. Free fatty acids and β-hydroxybutyrate similarly had no effect on the signal.

Transdermal sensors

Reverse iontophoresis

Reverse iontophoresis is a transdermal technique using the application of physical energy to access the ISF. A low electric current is applied across the skin between two electrodes, causing charged and uncharged species to pass across the dermis at rates significantly greater than passive permeability, with ions moving across the skin to maintain neutrality. Skin is negatively charged at physiological pH and so is selectively permeable to cations, mainly sodium. The flow induced by reverse iontophoresis carries neutral molecules, including glucose, from anode to cathode [65] (Fig. 6). The glucose concentration in the fluid collected by reverse iontophoresis is 1000 times lower than that of ISF. Glucose may be sensed within the collected fluid using a glucose oxidase-based platinum electrode, sensing the production of H_2O_2.

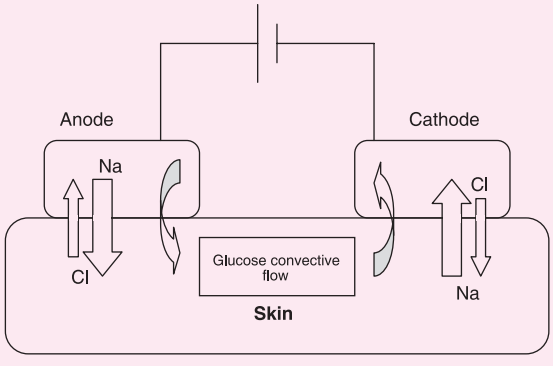

FIGURE 6 Reverse iontophoresis principle. Cl, chloride; Na, sodium.

Transdermal iontophoresis has several potential advantages over other enzyme-based electrode sensor systems. The concentration of glucose is low so oxygen supply is not a limiting factor to glucose oxidase. In addition, the skin filters large molecules, reducing electrode fouling and, in the iontophoresis system, electrochemically active species which may be interferents, such as urate and ascorbate, are taken away from the sensor electrode to the anode. However, the system is complicated to use and requires a long warm-up time along with calibration. The low power current causes mild to moderate erythema and the system does not operate if there is sweat present on the skin.

In vivo, results of trials using reverse iontophoresis showed 96.0–98.9% of points in areas A and B of the Clarke error grid and an *r* value between 0.78 and 0.9 [66,67].

The Cygnus GlucoWatch reverse iontophoresis sensor was the first transdermal glucose sensor licensed by the US Food and Drugs Administration (FDA). In clinical trials it showed adequate precision for home blood-glucose monitoring [68]. However, it was not useful for detection of hypoglycaemia with a sensitivity of 23% for glucose concentrations of ≤ 3.3 mmol/l and a 51% false alarm rate [68]. The device was withdrawn in 2008.

Sonophoresis

Sonophoresis uses low-frequency ultrasound to increase skin permeability. Ultrasound causes expansion and contraction of gaseous inclusions within the stratum corneum which opens pathways for ISF. In clinical trials, 2 min of ultrasound application have been followed by a 5-min period of vacuum to extract ISF at a rate of 25 μl/cm^2/h (greater than reverse iontophoresis). Analysis of extracted ISF against reference glucose measurement showed a mean relative error of 23, 92% of results in areas A and B of the Clarke error grid and an *r* value of > 0.7 [69]. The degree of ISF collection varies 10-fold between patients and between sites within patients. In later trials, the technique has been refined and vacuum is not required. Use of a shorter ultrasound period and hyperosmolar lactic acid improve ISF yield [70].

Skin suction blister technique

When a vacuum is applied over a small area of skin, a blister a few millimetres in diameter forms at the dermal–epidermal junction [71]. Fluid can be collected from the blister and analysed. The blister fluid is similar in composition to serum but with a lower protein concentration. Analysis of the fluid shows an increased concentration of lysosomal and cytoplasmic enzymes, suggesting cell damage [72].

Skin suction blister technique is a well-tolerated, painless procedure with a low infection risk, and glucose concentration in the fluid is lower than that seen in plasma, but correlates well [73]. It is likely to be a lower concentration as a result of consumption by cells within the blister. The time taken to form a blister was also recorded. Interestingly, this correlates well with HbA_{1c}.

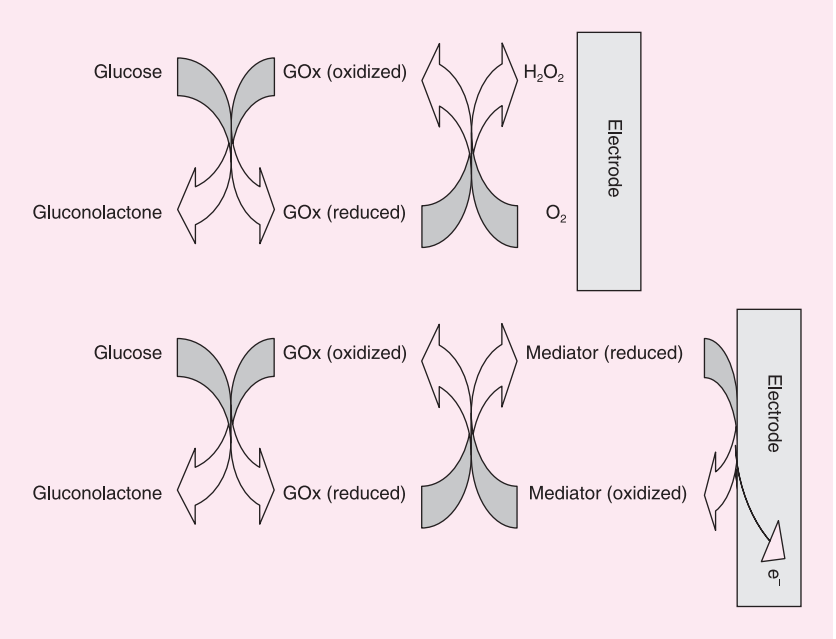

FIGURE 7 Enzyme electrode schematic and mediated electrode schematic. GOx, glucose oxidase.

Micropores and microneedles

Micropore techniques perforate the stratum corneum without penetrating the full thickness of skin. Micropores may be formed by pulsed laser or by the local application of heat. ISF can then be collected by vacuum. Clinical trials of pulsed laser in 56 diabetic subjects showed good correlation of glucose concentrations with reference measurements over 2 days (93% of results were within areas A and B of the Clarke error grid and $r = 0.95$ [74]) and the procedure was well tolerated although blisters formed over the vacuum sites. These resolved within 1 week [74].

Hollow microneedles (175 μm thickness) have been used to sample capillary blood. They are able to sample 200 nl by capillary forces. The needles are almost sensation-less and blood can be analysed using an enzyme-based electrode system [75].

Glass microneedles for sampling of ISF have also been developed. These sample 1–10 μl of ISF using a multineedle array under negative pressure [76]. Good correlation with capillary blood glucose was reported in six subjects using microneedles.

Impedance spectroscopy

Impedance spectroscopy (also known as dielectric or radio-wave spectroscopy) measures the dielectric properties of a tissue. A small alternating current is passed across a tissue and the impedance is recorded as a function of frequency. Glucose is indirectly measured by its concentration-dependent interaction with red blood cells. This has the advantage of providing information about glucose in the vascular compartment. However, temperature variation, sweating and motion are all sources of error and the technique requires calibration [77].

In 2003, the Pendra device (Pendragon Medical) was granted a CE mark but the manufacturer filed for bankruptcy in 2004 [78]. The mean relative error of the device was 52%; 78.4% of results were within areas A and B of the Clarke error grid (with 4.3% in the potentially dangerous area E) and $r = 0.59$ [79].

Invasive glucose sensors

Subcutaneous needle-type sensors

Glucose may be sensed using enzyme electrodes. Enzyme electrodes use enzymes which catalyse reduction–oxidation (redox) reactions and in doing so accept or donate electrons. This movement of electrons may be exploited to produce a concentration-dependent current or voltage which may be measured using electrodes. Glucose oxidase (GOx) produces hydrogen peroxide which is directly proportional to the concentration of glucose. Peroxide may be measured amperometrically at a potential of approximately 0.7 volts using a platinum electrode (Fig. 7).

GOx is specific for glucose but is able to use alternative oxidizing sources to molecular oxygen. Using alternative electron donors in the reaction allows it to be independent of local oxygen concentrations and these alternatives are known as mediators (Fig. 7). This was first described using ferrocene [80]. A further advantage of mediators is that they may be regenerated at potentials where interference from other analytes such as ascorbate, urate and paracetamol is avoided.

The Medtronic (Medtronic Diabetes, Northridge, California) continuous glucose-monitoring system (CGMS) uses a subcutaneous needle-type amperometric enzyme electrode based on GOx. It has a 63-h average recording time and a 4-min response time. As a result of significant sensor drift, it requires calibration

four times per day. Sensor drift is postulated to be partly because of protein and cell coating of the sensor, variable tissue oxygen tension (pO_2), tissue interferents and wound response to the sensor which alters local blood flow. Sensor sensitivity remains low once it is explanted but improves after repeated washing and is therefore reversible [81,82].

Clinical trials of the CGMS device show good correlation with reference glucose [83] with 96.6% of values in areas A and B of the Clarke error grid and an *r* value of 0.85 [84]. The CGMS provides retrospective data which can be assessed and interpreted to allow alterations in diabetes control. Evidence is conflicting as to whether CGMS improves glycaemic control. However, a recent study has suggested that HbA_{1c} improves by 0.5% in adults using real-time monitors on a daily basis. The same effect was not noted in children or adolescents [85]. Hypoglycaemia detection increases with CGMS and may be useful in eliminating severe or nocturnal hypoglycaemia.

The CGMS principle of a subcutaneous needle-based electrode has been used in the Paradigm closed loop insulin delivery system.

The DexCom STS (Dexcom, San Diego, California) and Abbott FreeStyle Navigator (Abbott Diabetes, Alameda, California) are newer devices which also use a subcutaneous enzyme-based electrode. The Navigator device uses an Osmium mediator. The DexCom STS (95.4% points in areas A and B of Clarke error grid, $r = 0.88$ [86]) is a wireless system using low-power radio and has a recording time of 72 h. The FreeStyle Navigator is also a wireless device and has a recording time of 5 days (98.4% points in areas A and B [87], $r = 0.84$ [88]).

Microdialysis

Microdialysis systems use a fine, hollow microdialysis fibre placed subcutaneously. The fibre is perfused with isotonic fluid from an *ex vivo* reservoir and ISF glucose freely diffuses into the fibre, where it is pumped to an electrochemical GOx-based electrochemical sensor. The sensor is *ex vivo* and so avoids the problems of fouling by proteins and cells. Consequently, it requires only one calibration per day and has less sensor drift than the CGMS system. In clinical trials, sensor accuracy is acceptable [89]. Using the Menarini GlucoDay system (Menarini Diagnostics, Florence, Italy), 95.5% of values are within areas A and B of the Clarke error grid with $r = 0.9$ [90]. Microdialysis systems have an inherent physical lag time for the dialysate to be pumped to the sensor and use more power. They also require perfusate solution, making the devices larger.

The concentration of glucose sensed in the dialysate is dependent on the degree of equilibrium with ISF. This equilibrium is variable and may be affected by factors relating to the membrane, the analyte and the surrounding tissue [91]. Over time, the physical and chemical properties of the membrane may change, as will the tissue characteristics such as pressure, volume, temperature and hydration. The flow rate and composition of the perfusate may influence glucose concentration and are tightly regulated by the device. Consequently, while the sensor is relatively protected from fouling by cellular and protein components, dialysate fluid and glucose recovery are significantly affected, making regular calibration necessary in microdialysis systems.

A novel microdialysis approach to glucose monitoring uses viscometric analysis of interstitial fluid through a microdialysis system [92]. Glucose freely diffuses into a subcutaneous microdialysis fibre from the ISF and displaces dextran from ConA. This leads to a concentration-dependent reduction in viscosity within the microdialysis fibre.

To assess clinical performance, microdialysis and subcutaneous needle-type sensors have been compared in patients with Type 1 diabetes [20]. The microdialysis probe displayed greater accuracy than the needle-type sensor but had an additional lag of 7 min as measured by combined curve fitting.

Summary

Table 2 summarizes the available accuracy data in human subjects for the sensing modalities discussed.

At present, not all of the modalities discussed have reached the stage of testing in human subjects and experimental method varies so direct comparison is difficult to make between modalities. However, most of the techniques tested to date show reasonable accuracy.

Of the sensing modalities discussed, NIR spectroscopy, optical coherence tomography, photoacoustic spectroscopy and fluorescence show the greatest promise in achieving the goal of an ideal glucose sensor. However, none of the devices in production at present meet the criteria for the ideal sensor and the sensing component of a closed-loop system remains a target for diabetes technology research.

Future work

Many technologies are under development to sense glucose with the final goal of implementing a closed-loop insulin delivery system, affording glycaemic control in the non-diabetic range to patients with Type 1 diabetes. Whilst some of the technologies are commercially available, none are suitable for autonomous closed-loop system implementation. The Medtronic Paradigm system transmits glucose sensor data to a compatible insulin pump. Despite linking components, it remains reliant upon external input for basal and postprandial glucose regulation.

An essential component of a closed-loop system is the control algorithm. This needs to replicate the basal and postprandial responses of the pancreatic B-cell to ensure delivery of appropriate insulin. A silicon B-cell has recently been developed with behaviour modelled on the electrical behaviour observed in the pancreatic B-cell [93]. The silicon B-cell output shows bursting behaviour similar to that observed in B-cells, as well as demonstrating low power consumption, making it ideal as an implantable or wearable device. Bursting behaviour may be coupled to insulin infusion and replicates physiological insulin release at differing glucose concentrations.

Table 2 Summary of accuracy data in humans for sensing modalities

Principle	Points in areas A + B of CEG (%)	*r* value
Kromoscopy	—	—
Photoacoustic spectroscopy	89.7 (OGTT) 94.5 (Meal test) 90.1 (IVGTT) [19]	0.71 [19]
Optical coherence tomography	—	0.8–0.95 [16]
Scattering/occlusion spectroscopy	—	> 0.75 in 19/26 measurements [15]
Polarimetry	—	0.99 [13]
Thermal infrared	100 [9]	0.87 [9]
Fluorescence	—	—
Raman spectroscopy	—	0.91 [12]
MIR spectroscopy	—	—
NIR spectroscopy	99.3 [7]	—
Impedance spectroscopy	78.4 [33]	0.59 [33]
Skin suction blister	—	—
Sonophoresis	92 [28]	> 0.7 [28]
Reverse iontophoresis	96–98.9 [25,26]	0.784–0.9 [25,26]
Micropore/microneedle	93 [30]	0.95 [30]
Intravenous implantable	95.8 [94]	0.83–0.93 [95]
Microdialysis	95.5 [41]	0.9 [42]
Subcutaneous sensor		
Medtronic CGMS	96.6 [35]	0.85 [35]
Abbott FreeStyle Navigator	98.4 [38]	0.84 [39]
Dexcom STS	95.4 [37]	0.88 [37]

CEG, Clarke error grid; CGMS, continuous glucose-monitoring system; IVGTT, intravenous glucose tolerance test; MIR, mid-infrared; NIR, near infrared; OGTT, oral glucose tolerance test.

For glucose sensors to operate under physiological conditions, biocompatibility must be considered. Fouling from protein and cell components in tissue, along with the inflammatory response to foreign bodies, dramatically reduce the lifespan of implantable sensors and there is ongoing work to solve this problem. Angiogenic layers on sensors encourage new capillary formation around a sensor, improving blood flow over the sensor window and reducing macrophage fouling. This approach has been shown to improve the lifespan of subcutaneous implantable sensors to 160 days, with calibration required every 20 days [94].

The ideal sensor for glucose remains elusive, but there is significant progress. As diabetes continues to become ever more prevalent, the need for improved technology is clear and a closed-loop insulin delivery system remains the goal, implementing glucose sensing in real time in a patient-acceptable design.

Competing interests

Nothing to declare.

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