State of the Art and New Perspectives in Non-Invasive Glucose Sensors

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ABSTRACT

Self-monitoring of blood glucose levels provides diabetic patients with a prompt method of measuring their blood glucose concentration, as opposed to conventional laboratory measurements. Frequent testing aids patients in the prevention and detection of hyper or hypoglycemia events. Only invasive and minimally invasive glucose sensors are currently commercially available. However, during the last couple of decades, work towards the development of a non-invasive glucose monitor has increased significantly among research groups with motivating results. Many techniques have been studied and implemented, each with their particular advantages and challenges. This paper presents a qualitative review of different technologies of non-invasive glucose sensors: spectroscopy-based methods, transdermal extraction-based methods, fluorescence, electromagnetic variations and polarimetry. This study identifies strengths and opportunities of currently available glucose monitoring techniques, as well as main characteristics and performance variables for an ideal non-invasive monitor. The most promising approaches towards the development of a truly non-invasive and clinically accurate glucose sensor are discussed.

Keywords: Diabetes mellitus, non-invasive glucose monitoring, spectroscopy, transdermal glucose extraction, fluorescence, polarimetry, electromagnetic variations.

RESUMEN

El auto-monitoreo de los niveles de glucosa en sangre proporciona a los pacientes diabéticos de un método rápido para medir sus niveles de glucosa en comparación con los métodos convencionales de laboratorio. Las mediciones frecuentes permiten a los pacientes prevenir y detectar episodios de hiper o hipoglucemia. Actualmente solo se encuentran disponibles comercialmente sensores invasivos o mínimamente invasivos. Sin embargo, a lo largo de las últimas décadas ha aumentado significativamente el trabajo de los grupos de investigación hacia el desarrollo de un sensor de glucosa no invasivo, obteniendo resultados motivadores. Una gran cantidad de técnicas han sido estudiadas e implementadas, cada una con ventajas y retos particulares. Este trabajo presenta una revisión cualitativa de los diferentes métodos no invasivos de sensado de glucosa: métodos basados en espectroscopía, métodos basados en la extracción transdérmica, fluorescencia, variaciones electromagnéticas y polarimetría. Mediante este estudio se identifican las principales ventajas y retos de cada técnica, así

como las principales características y variables de desempeño para un sensor no invasivo ideal. Se discuten las técnicas más prometedoras hacia el desarrollo de un sensor con precisión clínica y verdaderamente no invasivo.
Palabras clave: Diabetes mellitus, monitoreo no invasivo de glucosa, espectroscopía, extracción transdérmica de glucosa, fluorescencia, polarimetría, polarimetría, variaciones electromagnéticas.

INTRODUCTION

Self-monitoring of blood glucose levels provides diabetes patients with a continuous, robust and reliable procedure to determine blood glucose concentration, as opposed to conventional laboratory tests. Frequent verification of glucose levels is crucial in the treatment of diabetes, and it aids patients in preventing and detecting hyper or hypoglycemia events. Many commercial glucose monitors exist, most of them requiring a small drop of blood obtained through a puncture of the skin with a lancet, usually on the tip of the fingers (commonly referred to as finger-stick testing). These are invasive glucose monitors, and are highly uncomfortable for patients due to the frequent puncturing of up to 10 times a day. Additionally, measurements with these devices usually present an error of approximately 6-7% [1]. This percentage may increase depending on the size and quality of the sample, human error during sample extraction, faulty calibration, humidity, and lack of hygiene in the extraction area [2].

These problems have generated great interest in the exploration of new non-invasive monitoring techniques. The aforementioned exploration should lead to systems that will be able to detect and estimate glucose concentrations by meticulously eliminating different sources of interference. One of the main goals of these systems is to achieve high precision in their measurements, providing at least the same measurement errors as conventional, finger-stick methods. Unfortunately, none of the few non-invasive self-monitoring devices that were approved over the years by the US Food and Drug Administration (and consequently introduced to the market) offered low measurement error to replace lancet approaches, leading to their eventual disappearance.

Currently, only invasive and minimally invasive glucose sensors are commercially available. However, during the last couple of decades, work towards the development of a non-invasive glucose monitor has increased significantly among research groups with promising results. Many techniques have been studied and implemented, generating particular benefits and drawbacks.

This paper presents a qualitative review of non-invasive glucose sensors, which includes operating principles as well as the strengths and weaknesses of each sensor type. The overview of glucose monitoring presents three main categories: invasive, minimally invasive and non-invasive glucose monitoring. The most representative non-invasive glucose sensing techniques are described. Finally, the most important specifications of ideal non-invasive glucose sensors are summarized over a table which identifies the most promising approaches.

1) GLUCOSE MONITORING

Blood glucose monitors can be categorized in three well-defined groups based on their interaction with a patient's body. The first group includes invasive monitors, most based on finger-stick testing; a procedure where a small blood sample is extracted by puncturing of the skin with a lancet. The extracted sample is deposited over a chemically active disposable test strip that is inserted to the monitor, which displays the glucose concentration. The device is based upon an electrochemical reaction between the blood and chemical analytes from the strip. Other invasive glucose measurement devices include hospital bedside monitors, which are attached to the patient's intravenous lines to obtain blood samples first, and provide glucose estimations afterwards by optical methods such as near-infrared spectrometry.

The second group consists of minimally invasive devices, which rely on the measurement of interstitial fluid (IF) by means of a small sub-dermal implant. This implant converts glucose concentration from the patient's IF into an electronic signal [3]. Other minimally invasive monitors involve microdialysis techniques applied in the extraction of IF samples for *a posteriori* analysis. Microdialysis is a very important tool for sampling free drug concentrations from any tissue to determine their pharmacokinetics. In combination with a suitable detection technique, microdialysis allows monitoring of time-dependent changes in tissue's chemistry. In microdialysis for glucose measurement, the extraction occurs by means of a fiber generally inserted in the adipose tissue of the abdominal region [4].

The third group represents all apparatuses using non-invasive sensors. Non-invasive glucose sensing research strives to provide an effective solution to the discomfort suffered by the perforation of the skin that many invasive devices require. Other advantages that make this approach attractive are the possibility of real-time and continuous monitoring as well as the elimination of infection risks. Figure 1 summarizes the three main non-invasive glucose monitor groups.

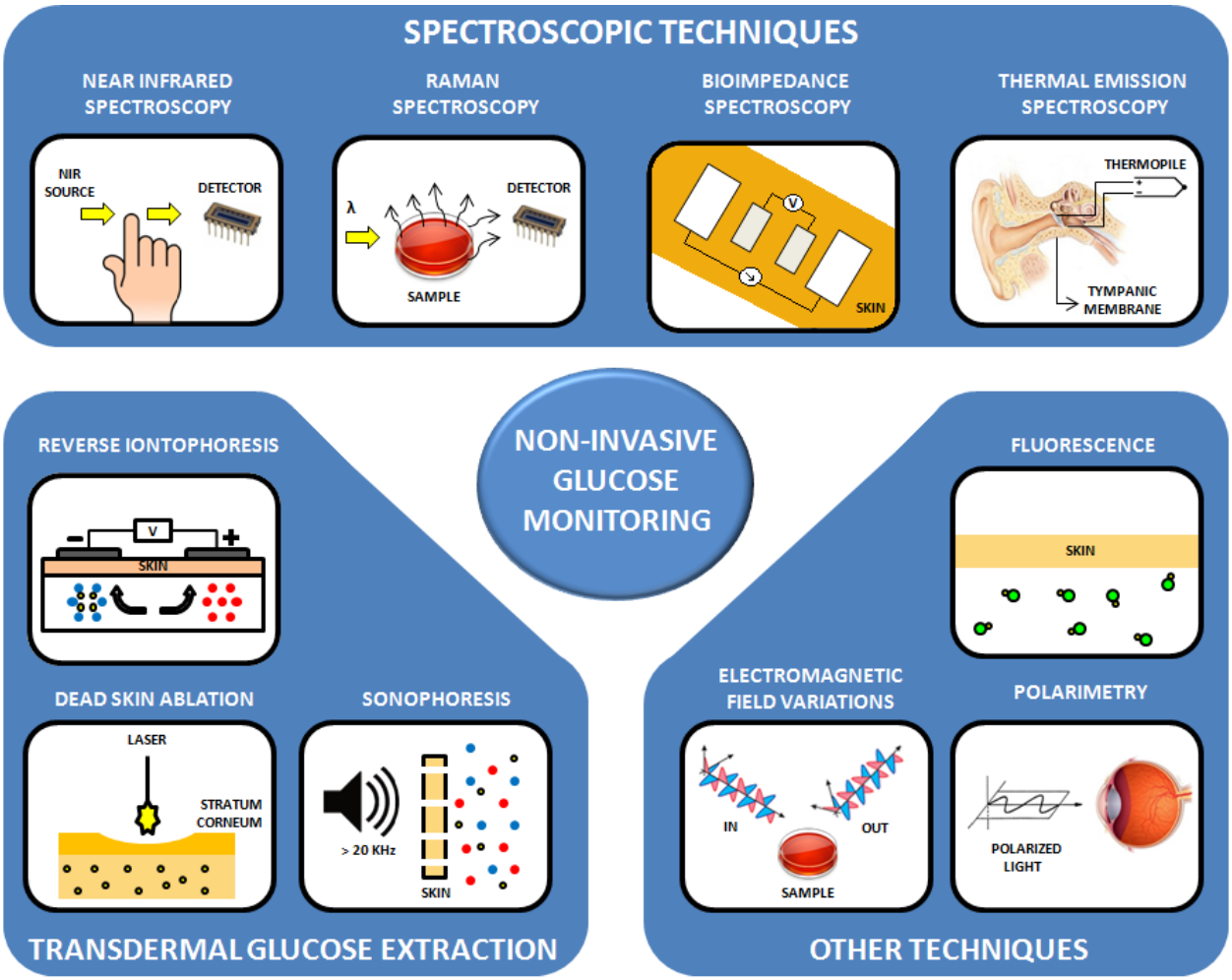

Figure 1: Overview of non-invasive glucose monitoring techniques

2) NON-INVASIVE GLUCOSE SENSORS

Apparatuses and devices to measure glucose using non-invasive techniques are classified in three groups: spectroscopic techniques, transdermal glucose extraction and other techniques. Non-invasive glucose sensors have been studied and developed by research teams around the world. The following sections discuss in some detail the different techniques explored.

Spectroscopic techniques

Spectroscopy is the study of objects based on their wavelength spectrum, both when they emit or absorb light. Furthermore, through the spectroscopic technique of spectrophotometry, the concentration of a given chemical species can be assessed. Figure 2 shows the basic components of a spectrophotometry system. White light from a source (containing a wide range of wavelengths) is directed towards an entrance slit and the collimated light passes through a diffraction system (e.g. a prism or a diffraction grating). The diffractive element divides the incoming light into its wavelength components, are

then collimated by an exit slit selecting a particular wavelength range. The selected range is directed towards a sample and the transmitted light is finally directed towards a detector system.

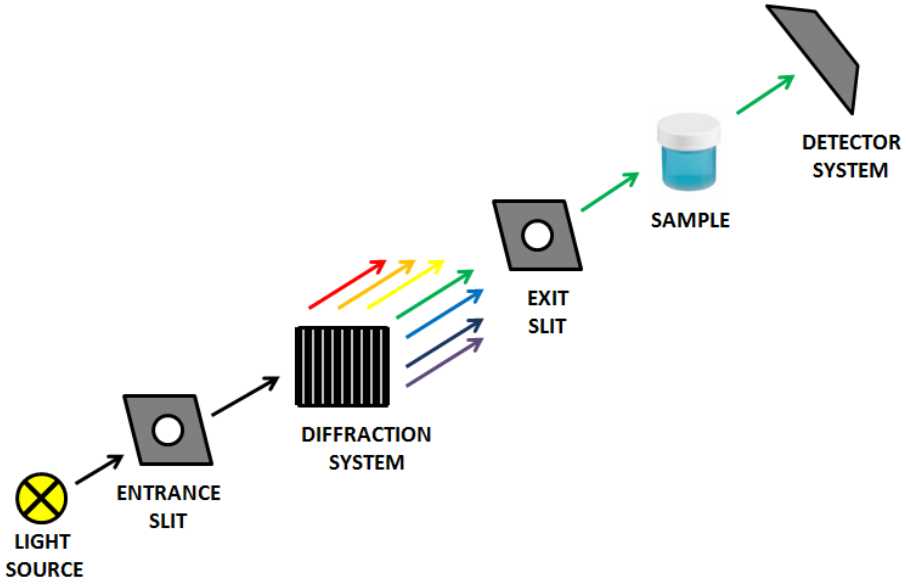

Figure 2: Basic Components of a Spectrophotometer.

Four spectroscopic techniques that have been applied to non-invasive glucose monitoring are described next: Near-infrared absorption spectroscopy, Raman spectroscopy, bioimpedance spectroscopy and thermal emission spectroscopy.

Near-infrared absorption spectroscopy

Infrared spectroscopy is a well established and constantly developing analytical technique which allows for the rapid, high-throughput, non-destructive analysis of a wide range of sample types [5]. This technique is based upon the principle that every molecule has resonance absorption peaks which are directly related to the molecule's concentration in a sample [6]. Because of this, radiation may be directed through body tissue and the exiting light can be analyzed to determine the content of glucose. Near-Infrared (NIR) absorption spectroscopy techniques have long been mainstays of nondestructive chemometric analysis and therefore, they hold great potential for the development of non-invasive blood glucose measurement techniques [7].

The main obstacles for *in vivo* blood glucose measurement by NIR absorption spectroscopy are the interference from some absorbing species; multiple scattering in skin, muscle and bone; and the strong absorption bands of water [8].

Different wavelength ranges have been utilized by research groups based on the target body area (e.g. eye and finger or forearm skin), most of them within the therapeutics' wavelength window of 600-2500 nm. Wavelengths within this range are selected due to the weak absorbance by water, the high energy of the signal source, as well as the availability of commercial light transducers [1].

A novel technique, named pulse glucometry [8], aims to remove the aforementioned interferences by obtaining data from a blood-only sample using instantaneous differential NIR spectrometry (at the wavelength range from 900 to 1700 nm.). This technique is based on two almost instantaneous measurements over a tissue sample which is usually the tip of a finger. Thus, changes in optical absorption between both measurements depend of a blood volume change produced by cardiac pulses. By a subtraction process, the interference of basal elements is then removed. Pulse glucometry has been tested in humans obtaining promising results [8].

Another technique used to remove interference caused by strong absorption bands of water is called attenuated total reflectance (ATR), in which a diamond prism is placed in contact with the finger tip. When an infrared light beam travels through a prism, it reflects several times, forming an evanescent wave which extends into the sample. The beam is then collected by a detector as it exits the crystal. Changes in the light absorption are related to the concentration of blood glucose [9].

NIR spectroscopy data relates to many overlapping bands and thus requires multivariate analysis [5]. Classical least squares method [7], partial least squares [10], support vector machines [11] and artificial neural networks [12], have been utilized as multivariate calibration models for *in vivo* spectroscopic analysis.

A potential non-invasive glucose sensor based on NIR spectroscopy requires the miniaturization of a spectrophotometer's components, so as to provide a portable device which satisfies patient's need of frequent testing. Work towards the miniaturization of a spectrophotometer's components for non-invasive sensing of glucose has been presented [13, 14].

Raman spectroscopy

When radiation impacts upon a sample, most of the incident light suffers Rayleigh scattering and a small portion of light undergoes frequency shifts [7]. The measurement of those shifts is known as Raman spectroscopy [5]. Water has a poor scattering response, thus, Raman spectroscopy provides a spectral signature that is less influenced by water than NIR spectroscopy [7]. Therefore, measurements can be made directly from bio-fluids, although, the effect is weak, making the collection times relatively long [5].

To avoid long collection times, high power lasers are an alternative; however, these are harmful for human tissues. This has led to efforts in developing crystal structures so that the Raman scattering may be initiated and enhanced for a low power incident beam. The use of noble metal clusters inside a photonic crystal structure was proposed. This approach would result in surface-enhanced Raman scattering, providing enhancements of up to one million times [15]. Monochromatic lasers are the tendency regarding the sources utilized when applying this technique. The most common wavelengths ranges are from 785 nm [16, 17] to 830 nm [16, 18, 19].

Studies show that Raman spectroscopy is feasible in *ex vivo* porcine eyes [16] and transcutaneously in human forearms [18]. A portable device has also been described [19], but proof of its functionality as a non-invasive glucose monitor in humans has not been presented so far.

Bioimpedance spectroscopy

Bioelectrical impedance analysis (BIA) is a diagnostic technique based on the study of the passive electrical properties of biological tissues. The practical use of these electrical passive properties initiated near the middle of the twentieth century. Different techniques resulted in a collection of methods that are now used for multiple applications: determination of total body water, fat free mass, tissue characterization, apnea monitoring, venous thrombus detection, tomography, cardiography, pneumography and blood compounds analysis [20]. Usually, these methods share three common advantages: use of low-cost instrumentation, easy application in practice, and on-line monitoring availability.

BIA uses electrodes to apply low intensity currents in physiological fluids or tissues. The resulting voltage reflects changes in dielectric or dimensions of the target, therefore, enabling the monitoring of chemical compositions or even physiological events in animal and human bodies. Important features of BIA include low cost, fast response, simple implementation and safety. Such characteristics, mostly associated to the development of microelectronics, make BIA a promising tool for tissue characterization and compound quantification, especially by dielectric spectroscopy at high frequencies. Dielectric spectroscopy is a technique in which the dielectric properties of a medium are measured as a function of frequency [21].

A wrist glucose monitor based on impedance spectroscopy has been presented [22]. This monitor samples information from an LC resonance circuit using the skin as a dielectric. An important disadvantage is that it requires a calibration process in which the patient must rest for at least 60 minutes before starting measurements. Even though the frequency band was chosen in order to decrease the sensitivity to side effects, other environmental variables also produce measurement errors. For example, sweat and relocation of the sensor produce inaccuracies. Moreover, variations in temperature affect the sensor's signal since they have an impact on the permittivity of water and other molecules.

Thermal emission spectroscopy

Thermal emission spectroscopy (TES) measures IR signals generated in the human body as a result of glucose concentration changes. This technique is based on the principle that natural mid-IR emission from the human body, especially from the tympanic membrane, is modulated by the state of the emitting tissue. Its selectivity is based on the same principle as the selectivity of the absorption spectroscopy technique for analyte measurements [23].

Planck's law describes a relationship between the radiant intensity, spectral distribution, and temperature of a black body. The human body is an excellent black body emitter of mid-IR light. The

spectral characteristic of thermal emission is influenced by the individual's tissue composition and analyte concentrations. On the other hand, Kirchhoff's law states that, for the same wavelength, absorbance equals monochromatic emissivity when the entire body is at the same temperature. Since the tympanic membrane shares its blood supply with the hypothalamus, which is the body's temperature regulation center, it is an ideal site to measure body temperature.

A tympanic thermometer measures the integral intensity of IR thermal radiation. A sensor inserted in the ear canal can obtain a clear view of the membrane and its blood vessels to measure the emitted IR radiation. When compared with the theoretical black body radiation described by Planck's and Kirchhoff's laws, the membrane's IR radiation is spectrally modified by tissue composition. The instrument optically receives IR radiation from the target object, such as the tympanic membrane. The detecting system consists of an optical IR filter set and a thermopile detector sensitive to an IR region. One of the sensing elements is covered by an IR filter sensitive to the IR glucose signature, while an appropriate filter that does not have spectral bands characteristic to the measured analyte covers the other sensing area. Spectrally modified IR radiation from the tympanic membrane illuminates both windows. The difference between the intensities of the two radiation paths provides a measure proportional to the analyte concentration [24].

A non-invasive prototype based on thermal emission in the mid-IR spectral region has shown glucose measurements with acceptable accuracy. One of the advantages of this system is that individual daily calibrations are not required [23]. One of the drawbacks is that the intensity of the infrared radiation emitted by an eardrum is affected not only by its temperature but also by its thickness. Although the clinical results obtained using TES are promising, they are not yet considered acceptable under clinical accuracy standards.

Transdermal glucose extraction

Measuring glucose is also possible if the substance is extracted from interstitial fluid and the analysis is performed on an *ex post facto* basis. Three different techniques are presented in technical literature: reverse iontophoresis, sonophoresis and dead skin thermal ablation.

Reverse iontophoresis

Iontophoresis is a well known and studied technique in which charged and uncharged polar compounds are driven across the skin by the application of low DC currents [25]. Iontophoresis has been widely used for drug delivery purposes in dermatology, ophthalmology, otolaryngology among other disciplines [26]. Its counterpart, reverse iontophoresis, is the extraction of these compounds through the skin by the same concept of low electric current application.

In reverse iontophoresis a low level DC current is conducted through the skin between two electrodes. The migration of ions creates an electro-osmotic solvent flow toward the negative electrode, which makes the transport of polar uncharged species such as glucose possible. Skin has a negative

charge, thus, it is permselective to cations. The aforementioned flow is consequently used for the extraction of the compounds via the skin [27].

The monitoring of two interacting biomolecules, glucose and lactate, has been demonstrated to have particular success with this technique [28]. In the case of blood glucose monitoring, one device by Cygnus Inc. gained FDA approval in 2001 and was made available commercially. The GlucoWatch Biographer made non-invasive glucose measurements through the skin every 10 minutes for a period of 13 hours [29]. However, the GlucoWatch presented various limitations. First of all, the FDA suggested that the device should not be used as a substitute or replacement of finger-stick measurements, but as a support of standard home glucose monitoring devices. For the determination of insulin doses, the FDA also suggested confirmation of the GlucoWatch's measurement with a traditional finger-stick measurement. These suggestions automatically compromised its purpose. Other drawbacks were that device presented a 15 minute lag compared with finger-stick readings, and required 2 hours of warm-up and calibration, which could result in erroneous readings if not done correctly. GlucoWatch failed to satisfy patients' needs and expectations due to these and other inconveniences such as inconsistency in measurements, frequent skin irritation, itching and blistering, intermittent shutting off, and skipping of readings due to movements. Consequently, this device failed to offer reliable measurements and is no longer commercially available.

Areas of further investigation regarding reverse iontophoresis are the effects of environmental variables in measurements (e.g. temperature, pH), adverse effects due to skin polarization (e.g. skin burning and blistering) and other factors such as perspiration and skin resistance [30]. Additionally, the use of the sodium ion as an internal standard has been reported to be of assistance in the determination of glycemic events by reverse iontophoresis without the need of calibration with a blood sample.

Sonophoresis

Sonophoresis is a technique that increases cutaneous permittivity to interstitial fluid by micro-vibrations produced when ultrasound waves are directed to the tissue with a piezoelectric transducer. This technique is similar to reverse iontophoresis and it usually enhances transdermal delivery of drugs.

A technique that implements a combination of a low-profile cymbal array and an electrochemical glucose sensor has been developed [31]. The sensor consists of amperometric electrodes combined with a novel glucose oxidase hydrogel. Glucose concentrations can be determined by increasing cutaneous permittivity to interstitial fluid, enabling the transportation of glucose to the epidermis surface. Some experiments show that through the placement of the aforementioned array in the zone of exposure to the ultrasound waves, glucose concentration may be determined, but error ranges during testing were unacceptably high. After comparing measurements with commercial glucose meters' readings, the difference between values of each respective technique was around 90mg/dl. Results for testing in humans have not been presented yet.

Dead skin thermal ablation

Glucose molecules are unable to reach the skin surface because of their inability to diffuse across the layers of dead skin cells. However, by opening a microscopic region of this layer through thermal ablation, glucose is allowed to diffuse naturally to the skin surface [32]. Thermal ablation has successfully been used for transdermal drug delivery and the potential of this technique for vaccination processes has been recognized [33].

A dermal patch with micro-heater elements has been fabricated and tested on surrogate human skin with promising results [32]. This patch is able to sample glucose molecules from interstitial fluid in a non- intrusive manner by applying a highly localized heat pulse to the dead-skin layer until ablation is reached. Due to the strict control of the applied heat pulse, only the dead-skin layer is ablated, leaving the underlying tissue and nerves unaffected. Therefore, this technique is considered as non-invasive.

Other techniques

In addition to Spectroscopy and Transdermal Glucose Extraction, three other techniques which can perform the measurement of glucose concentration are reported in technical literature: Fluorescence, Electromagnetic Field Variations and Polarimetry.

Fluorescence

Fluorescence-based sensors constitute a growing class of glucose sensors with significant contributions to the field over the last few years. This technique has been encouraged by special opportunities presented by the fluorescence principle for biological analysis such as fast, noninvasive, very highly sensitive and in some cases, reagent-less and non-consumable sensing [34, 35]. Different approaches have been explored in fluorescence-based sensors, including: auto-fluorescence, tattoo-based and tear-monitoring sensing.

One of the techniques for fluorescence-based non-invasive glucose monitoring is based on the measurement of cell auto-fluorescence due to NAD(P)H and signaling of changes in extracellular glucose concentration by fluorescent markers of mitochondrial metabolism [36]. However, these experiments have been performed using *in vitro* cell culture models, thus requiring further investigation for monitoring in humans.

A nanosensor similar to a tattoo dye consisting of 120nm-diameter hydrophobic spheres is under development [37]. The spheres consist of a glucose detecting molecule, a color-changing dye and a glucose-mimicking molecule. The detecting molecule will bind with either the glucose molecules or the mimicking molecule, depending on the concentrations of glucose in the medium. Consequently, the “tattoo” will fluoresce differently under an IR source according to the concentration of glucose. The sampling process repeats itself every few milliseconds with a 20 minute lag respect to actual blood glucose levels. Results have been positive on mice but the technique is yet to be tested in humans.

Another approach is based on the determination and monitoring of tear glucose levels, which are known to track glucose concentrations in blood [38]. The technique relies on disposable and colorless contact lenses embedded with boronic acid probes (containing fluorophores), which are known to chelate monosaccharides and thus provide a glucose-sensing mechanism through the lens' color variations [39]. These probes are compatible with the internal pH and polarity of the lenses. The extent of chelation can be monitored using different techniques, for example: fluorescence, colorimetry and polarization [40]. The reversible nature of this technique, as well as its sensitivity, suggests its feasibility as a continuous non-invasive monitor of physiological glucose.

Electromagnetic field variations

A change in blood glucose concentration causes a variation in the blood's dielectric parameters such as its conductivity. An electromagnetic sensor based on Eddy currents due to changes in the electromagnetic field can determine the concentration of glucose through the aforementioned variations [41]. The principle of the sensor is based on two coils, with a separation of 40mm between them. The conductivity fluctuations as a result of the change in glucose concentration involve the variation of the coils' output signal. The technique consists on applying a signal with a frequency of approximately 4 MHz to the primary coil, while the output signal is measured on the secondary coil [42]. The minimum change in glucose concentration that has been measured by this technique is ±2g/l [41].

Polarimetry

The use of polarimetry in the detection of analyte concentrations has existed for several years. The technique relies on the ability of optically active analytes to rotate the plane of polarization of incident linearly polarized light [43]. One possible analyte for this application is glucose, which in the aqueous humor of the eye closely mimics glucose levels in blood [44]. Aqueous humor is preferred as a target for this technique, as it does not present the high scattering coefficients that skin does. Additionally, this target is convenient due to the short delay time of only a few minutes with respect to blood glucose levels [45].

Parameters involved in the determination of an analyte's concentration by polarimetry are the specific rotation of the light source, the sample pathlength and the observed rotation of linearly polarized light caused by the analyte [46]. Glucose concentration will be directly proportional to the observed rotation in polarization for a known pathlength inside the sample.

Although polarimeters have existed for several years, it has not been until recently that their sensitivity has increased enough to be applied in the measurement of the low glucose concentrations seen in physiological conditions. The technique has presented obstacles and drawbacks, such as the inability to provide enough system stability to accurately measure the detected signal consistently over large periods of time, as well as the limits imposed by the complicated shape of the cornea [43, 44].

Furthermore, humor glucose levels are lower than blood levels and there are many biomolecules in it that contribute with confounding optical rotations [46].

Several research groups have studied different approaches to overcome polarimetry's drawbacks in glucose measurements. Two different approaches have been presented in literature [44], as well as the respective geometrical analysis for optically accessing the aqueous humor of the human eye. A digital closed-loop control system that improves the system's repeatability and stability without sacrificing accuracy has been utilized as well [43]. Another approach using a "true phase" measurement technique has been shown to be theoretically more immune than others to potentially confounding effects such as corneal birefringence and rotation, reducing additive amplitude noise effects [46]. However, a full blown version of a polarimetric device applied to glucose monitoring in humans has not been presented so far.

Although many ongoing research projects have reported developments of truly non-invasive blood glucose measurement techniques, more work and innovation efforts are still necessary towards the design of a portable and clinically accurate device.

3) SUMMARY OF STRENGHTS AND OPPORTUNITIES IN GLUCOSE MONITORING

Table 1-3 summarize the most important advantages and challenges of each of the techniques discussed in this paper, divided by their respective groups: spectroscopic techniques, transdermal glucose extraction and other techniques.

Table 1: Advantages and challenges in non-invasive glucose monitoring for spectroscopic techniques.

	Advantages	Challenges	Ref.
NIR Spectroscopy	• Well established analytical technique. • Non-destructive analysis. • Efficient removal of most interferents. • Successfully tested in humans.	• Requires multivariate analysis. • Device exists in the macroscale only, requires miniaturization. • Scattering in skin.	[5][6] [7][8] [9][10] [11][12]
Raman Spectroscopy	• Spectral signature less influenced by water compared to NIR spectroscopy. • Measurements directly from biofluids. • Feasible transcutaneously in forearms.	• Long collection times. • Collection times reducible by with lasers, but not without harm. • Proof of functionality in humans not presented.	[5][15] [16][17] [18][19]

Bioimpedance Spectroscopy	• Low cost instrumentation. • Simple application in practice. • On-line monitoring available. • Safe and with fast response.	• Very long calibration process with current devices • Susceptible to interference by different phenomena such as movement, sweat and temperature.	[20][21] [22]
Thermal Emission Spectroscopy	• Prototype has shown acceptable accuracy. • No daily calibrations needed.	• Intensity of radiation by eardrum is affected by its thickness, which is variable in humans. • Results of measurements not acceptable under clinical standards.	[23][24]

Table 2: Advantages and challenges in non-invasive glucose monitoring for transdermal glucose extraction techniques.

	Advantages	Challenges	Ref.
Reverse Iontophoresis	• Work is in advanced stages as devices have been developed and tested in the market.	• Devices have functioned only as auxiliary to invasive measurements. • There is lag and inconsistency in comparison with finger-stick method. • High calibration times. • Not unharmful, as devices produce irritation and blistering in patients. • Affected by environmental variables	[25][26] [27][28] [29][30]
Sonophoresis	• Preliminary in-vivo results have been described predicting glucose levels in rats.	• Experimental error ranges unacceptably high. • Results of testing in humans not reported.	[31][32]
Dead Skin Ablation	• Tested in humans with favorable results. • Not painful for patients as only	• Fabrication process presents complications that compromise the quality of the sensors.	[32][33]

	dead skin is affected.	• Still in prototypical stages.	

Table 3: Advantages and challenges in non-invasive glucose monitoring for other techniques.

	Advantages	Challenges	Ref.
Fluorescence	• Different approaches have proven the viability of the technique. • Painless and reversible.	• Has not been tested in humans, only on in-vitro cell culture models.	[34][35] [36][37] [38][39]
Electromagnetic Field Variations	• Even under unfavorable conditions, a change of +/- 2 g/l of glucose can be detected. • Linear relationship between the modulus of output/input signal and glucose concentrations. • Reproducibility in measurements	• Results are based on in-vitro experiments. There is still no testing in humans. • Work is still in preliminary stages and no integrated device has been presented. • Interference by diverse phenomena has not been addressed.	[41][42]
Polarimetry	• Aqueous humor does not present the high scattering skin does. • Aqueous humor presents a short time delay of only a few minutes respect to blood glucose levels. • There are advances in the reduction of confounding effects and in the stability and repeatability of measurements.	• Low system stability and consistency in measurements over large periods of time. • Shape of the cornea imposes limits. • Humor glucose levels are lower, and there are more confounding biomolecules involved. • No successful device has been presented.	[43][44] [45][46]

4) CONCLUSIONS

By means of this review, many desirable characteristics and performance variables were identified as key elements in the development of a truly non-invasive and clinically accurate glucose sensor. These characteristics include:

- Clinical accuracy for reliable results comparable to invasive and minimally-invasive techniques.
- Portability for fast transportation and availability.

- Short measurement time in order to reduce artifacts due to physical manipulation of the device (e.g. patient movement during the measurement, etc.) as well as for comfortableness of the patient.
- Simplicity in its calibration, in order to avoid human errors in this task that may lead to faulty results.
- Short or no lag at all regarding glucose levels compared with finger-stick or other invasive and minimally invasive techniques, in order to obtain real-time glucose concentrations.
- Robustness in its design to minimize the effects of possible interference sources in order to provide consistency in measurements. These interference sources include transpiration, movement, temperature, pH, scattering by body tissues and physiological fluids.
- High sensitivity and selectiveness in comparison with invasive and minimally-invasive techniques to provide significant results for the patients' glucose control schemes. Sensitivity improvements may be achieved by the parallel monitoring of more than one parameter. Selectiveness is important (e.g in spectroscopic techniques) given that the collected spectroscopic information must contain a selective signature for glucose relative to all the other components that can impact the signal.
- No side-effects to the patient such as irritation, burning or blistering to provide a truly painless measurement, thus improving the quality of life of diabetes patients.
- Reasonable instrumentation cost to provide a cost-effective and marketable solution.

The spectroscopic techniques described seem highly attractive due to the non-requirement of sensor implantation or *ex-vivo* blood sampling. The main obstacles of near-infrared absorption spectroscopy include confounding by other optical absorbers and strong absorption bands of water. The Pulse Glucometry technique aims to remove such interferences and has proven to be clinically accurate in the macro-scale; therefore, it represents one of the most promising approaches to truly non-invasive monitoring.

Other techniques described in this review also possess distinct technical advantages and favorable conditions for their successful implementation, and are dependent on their further refinement. Bioimpedance spectroscopy has also shown viability, simplicity, low cost and favorable results, and only requires work towards a more robust device regarding sources of inaccuracies. Additionally, glucose levels measurement by transdermal extraction of the interstitial fluid has previously reached a commercial level and thus only requires refinement of specific issues such as calibration. It has been reported that use of the sodium ion as an internal standard could refine the determination of glycemia by reverse iontophoresis without requiring calibration with a blood sample.

Evolution in sensor technology and microelectronics is bound to open the door to new approaches and methods that should aid the refinement of the discussed techniques, leading to truly non-invasive glucose sensors.

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