Chapter 15
Nano- and Microelectrochemical Biosensors for Determining Blood Glucose

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Abstract Blood glucose analysis is currently an important issue both for ordinary people wishing to monitor their health and for patients suffering from various diseases, especially diabetes mellitus. This chapter considers nano- and microsized devices based on biological receptors for glucose level measurements. As attention is largely paid to non-invasive techniques, we also touch upon the existing glucose assays for other fluids of the organism that require no skin-cover integrity to be disturbed. We present data on the development of continuous blood glucose level monitoring to provide a true picture of glycemic changes over large periods of time. The advantages and limitations of using enzyme electrochemical biosensors for glucose detection are discussed. A part of the work deals with specific features of using carbon and metal nanoparticles in biosensors to improve their properties. The current fundamental research in the development of biosensors and available commercial devices is also discussed.

Keywords Blood glucose · Diabetes · Glucose biosensors · Microsensors · Nanosensors · Non-invasive glucose analysis · Continuous monitoring of glucose · Electrochemical biosensors · Nanomaterials

Nomenclature

AuNPs	gold nanoparticles
FAD	flavin adenine dinucleotide
GDH	glucose dehydrogenase

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GOx	glucose oxidase
LOD	limit of detection
MPtNT	one-dimensional platinum nanotubes
MWCNTs	multiwalled carbon nanotubes
PQQ	pyrroloquinoline quinone
PtNPs	platinum nanoparticles
TTF-TCNQ	tetrathiafulvalene tetracyanoquinodimethane

15.1 Introduction

According to the data by the World Health Organization, an estimated 422 million people in the world suffer from diabetes, and by 2030, the disease will become the cause of each seventh death on the planet (Global report on diabetes 2018). The global level of the disease has doubled over the last 35 years, from 4.7 up to 8.5%. In Russia, 4,498,955 patients with this diagnosis have been registered by 2018 (Dedov et al. 2018). The causes of the disease have not been yet fully understood, which requires the fast and efficient quantitative determination of glucose.

There are three different types of diabetes:

1. Type 1 diabetes usually affects the young and is a disease characterized by the loss of β-pancreatic cell ability to produce and secrete insulin. Approximately 10% of diabetics have Type 1 (Rodriguez et al. 2020).
2. Type 2 diabetes commonly affects older patients and occurs when the pancreas does not produce enough insulin or when the body does not use the produced insulin effectively. Type 2 diabetes makes up more than 90% of all cases of diabetes in adults, according to the Centers for Disease Control and Prevention (Kim et al. 2019).
3. Sometimes gestational diabetes is singled out as the third type. It is a temporary condition that occurs during pregnancy and affects 2–4% of all pregnancies with a further increased risk of developing diabetes (Stewart 2020).

Excess of glucose in blood plasma causes a hyperglycemic state that leads to numerous complications such as blindness, cardiovascular diseases, and renal failure (Lee et al. 2018). Due to severe medical consequences of diabetic complications, patients critically require continuous personal monitoring and control of blood glucose level.

Most personal sensors developed to date are based on electrochemical devices with an enzyme as a receptor element and are associated with invasive blood taking techniques. Biosensor models are developed in several major directions. As invasive techniques are inconvenient for the user and may cause a discomfort, patients may fail to observe the control of glucose level. For this reason, one of the directions of glucose biosensor research is associated with the development of non-invasive diagnostic monitoring methods (Sadana and Sadana 2015). There are numerous papers that are investigating a relation between the level of glucose in human blood and

other physiological fluids (sweat, tears, saliva) and the possibility of developing wearable non-invasive devices for glucose measurements. Another direction of research aims to improve the parameters of biosensors themselves—to enhance the accuracy of the assay, to preserve the activity of the receptor elements at a high level for a prolonged period of time. Yet another important direction is related to the research and development of totally autonomous self-powered devices that require no external power supply sources and are capable of transmitting information to the user wirelessly.

The chapter briefly reviews the general state of the art in electrochemical biosensors for blood glucose determination. A brief history of the development of glucose biosensors is given, and they are compared with other standard glucose assay techniques. Emphasis is made on the assessment of the possibility of continuous and non-invasive glucose measurements in blood and other biological fluids, as well as on the use of various nanomaterials as glucose biosensors' elements.

15.2 Standard Methods of Glucose Determination in Clinical Laboratories

There are numerous methods that enable quantitative determination of glucose in the model and the organism (Galant et al. 2015; Wang and Lee 2015). The following trends of assay are singled out: optical (spectrophotometric, spectrofluorimetric, diffraction), electrochemical (amperometric, potentiometric, conductometric), chromatographic, titrimetric, and calorimetric. Clinical diagnostic laboratories mainly use enzymatic spectrophotometric and amperometric glucose determination methods. Outside clinical laboratories, photometric techniques with test strips are additionally used. Chromato-mass spectrometric procedures are frequently used to certify standard blood serum samples. Spectrofluorimetric, diffraction, and chromatographic methods are widespread in biomedical research. Such methods as non-enzymatic spectrophotometry and electrochemistry, titrimetric and calorimetric procedures, as well as a number of enzymatic spectrophotometric procedures are not used in most cases in modern clinical and diagnostic practice (Buzanovskii 2015).

The earliest methods of glucose detection are titrimetric. They are based on the ability of glucose to reduce copper salts or potassium ferricyanide at one of the stages of its detection. The method is not applicable for assaying glucose in biological objects due to a high error of manual measurement (Simon et al. 1968).

More modern chromatographic methods include gas chromatography–mass spectrometry, which enables analyses of complex–composition mixtures (Gladilovich and Podolskaya 2010). This method is highly sensitive; however, the assays of glucose and other sugars require preliminary stages of sample preparation (Andreis et al. 2014) due to the extraction of initial samples and their transfer to sublimable forms mainly by ester formation.

A more widespread method is high-performance liquid chromatography using UV, fluorescence, or refraction-index detectors (Yuh et al. 1998). The main

advantage of this method is the simplicity of sample preparation, because the components to be determined are separated in an aqueous medium. For glucose detection processes, the method also requires preionization of sugars in solution by increasing pH of the medium.

A highly popular group of glucose detection techniques in aqueous media are colorimetric methods. They can be divided into non-enzymatic and enzymatic. Spectrophotometric enzymatic methods of glucose determination that possess an exceptional selectivity, fast assay rate, and sufficient accuracy are a good alternative for assays of manufactured foods but are insufficiently effective for blood assays (Moodley et al. 2015).

In recent years, most clinical measurements have been based on electrochemical biosensors. They contain a specific enzyme glucose oxidase (GOx) immobilized on the surfaces of various types of electrodes. Glucose breaks down as the result of the operation of the enzyme, and the amount of the formed hydrogen peroxide or consumed oxygen is registered. New-generation biosensors have also emerged, which register the direct transfer of electrons from the active site of the enzyme, which catalyzes the glucose breakdown reaction, to the electrode (Yu et al. 2014).

15.3 History of the Research and Development of Glucose Biosensors

Over 15,000 original articles, reviews, and monographs on the development of enzymatic glucose biosensors have been published since 1962 up to the present. Initially, 40 years ago, about one article per year was published on the subject of biosensors; to date, their number reaches 4500 articles (Turner 2013; Olson and Bae 2019), and the world market of (bio)sensors has grown from 5 million to 17 billion US dollars (Biosensors Market Report 2019–2026). These rapid growth rates are largely due to the colossal demand in personal analyzers, for example, glucometers. By the data of the World Health Organization, about 4.5 million diabetes patients in Russia and more than 200 thousand in Moscow need constant (no less than twice a day) blood glucose assays. On the other hand, interest in personified medicine as well as in various smart diagnostic personal devices based on biosensors is high today as never before. The milestones achieved in the development of commercially available glucose biosensors are presented in Table 15.1.

The idea of the glucose enzymatic electrode, the first-generation biosensor, was proposed in 1962 (Clark and Lyons 1962). The first device was based on a thin layer of GOx, trapped on the surface of an oxygen electrode (by means of a semi-transparent dialysis membrane), and monitoring the oxygen consumed in the reaction presented in Fig. 15.1.

The Clark technology was subsequently passed on to Yellow Spring Instrument Company, which in 1975 launched the first special biosensor for glucose (the YSI 23 analyzer) for direct measurement of glucose in 25-ml blood samples. Though it

Table 15.1 History of commercially available electrochemical glucose biosensors

Year	Device name	Company	Sample volume, μL	Measurement time, s	Features
1973 (re-launched in 1975)	YSI 23 analyzer	Yellow spring instrument company, USA	25		Glucose enzyme electrode that monitors hydrogen peroxide, which occurs during the oxidation of glucose in the body GOx; Fast glucose assay in blood samples; High price of Pt electrode could be used exclusively in clinical laboratories
1987	ExacTech	MediSense, USA	10–50	30	Second-generation biosensor using mediator electron transfer to the electrode; The first pocket-sized home glucose meter
1991	Glucocard GT-1610	Arkray, Japan	5	60	Second-generation biosensor using mediator electron transfer to the electrode; Compact, small sample size; Ascorbic and uric acid are interfering with the measurements
1992	i-STAT portable clinical analyzer	i-STAT Corp., USA	65	120	Glucose is measured amperometrically via the product (H_2O_2) of the glucose oxidase reaction; Measures the level Na^+, K^+, cl^- и NH_4^+ ions and glucose simultaneously
1997	Precision QID	Abbott, USA	3,5	20	Based on ExacTech technology, has built-in memory, remembers the last 10 patient results

(continued)

Table 15.1 (continued)

Year	Device name	Company	Sample volume, μL	Measurement time, s	Features
1998	OneTouch FastTake	LifeScan, Switzerland	1,5	15	LifeScan's first electrochemical sensor; Automatically drags the blood drop into the tip of the test strip on contact; 150 results in memory, automatic average results from 14 days
2000	FreeStyle blood glucose monitoring system	Therasense, USA	0,3	15	Glucose dehydrogenase is used instead of glucose oxidase; The system allows samples to be taken from multiple sites including the fingertips, forearm, upper arm, thigh, calf, and fleshy part of the hand; Has PC connectivity
2001	Sof-tact	MediSense, USA	2–3	20	Based on ExacTech technology; First instrument with automated testing, ease of operation, 450 results in memory, an average of 7.14 or 28 days; High price ($ 200 for the device and $ 50 for a set of test strips); Limitations of this device include a poorer than predicted uptake of alternate site testing, the considerable size of the instrument, and the restriction to a single measurement
2001	Precision Xtra	Abbott, USA	0.6 (1.5 for ketones)	5 (10 for ketones)	First device for measuring glucose and ketones simultaneously

(continued)

Table 15.1 (continued)

Year	Device name	Company	Sample volume, μL	Measurement time, s	Features
2001	GlucoWatch	Cygnus Inc., USA	–	Up to 3 measurements per hour for 12 hours	The first wearable continuous glucose meter to measure extracellular fluid glucose; Takes up to 3 hours to warm up, false alarm, inaccuracy, skin irritation and sweating, discontinued in 2008
2002	MiniMed	Sylmar, USA	–	Measurements once a minute	First commercial in vivo glucose biosensor contains an automated insulin pump; 1–2 years of work until replacement
2002	Accu-Chek advantage	Roche, Switzerland	3–3.5	15	Glucose dehydrogenase is used instead of glucose oxidase; Features 17 test strips in a single drum that is fully integrated into the meter, which avoids the need for handling of individual strips; Automatic selection and removal of test strips at the touch of a button
2003	Ascensia contour	Bayer, Germany	0.6	5	Glucose dehydrogenase is used instead of glucose oxidase; Needs no coding; Device was calibrated by plasma, in which the concentration of sugar is 11% higher than in blood
2006	STS continuous glucose monitoring system	Dexcom, USA	–	–	Second FDA-approved continuous glucose monitoring system; Can be used within 3 days before replacement

(continued)

Table 15.1 (continued)

Year	Device name	Company	Sample volume, μL	Measurement time, s	Features
2009	Countour USB	Bayer, Germany	–	–	The first and only blood glucose monitor that plugs directly into a computer, providing instant access to software that can help optimize diabetes management by analyzing blood glucose readings
2009	DIDGET	Bayer, Germany	–	–	The first device for children with diabetes, integrated with a game console
2011	AgaMatrix nugget + iBGStar app	Sanofi Aventis, France	–	–	The first meter combined with a mobile application for the iPhone
2015	G4 platinum CGM system	Dexcom, USA	–	–	First FDA-approved glucose monitoring mobile app
2016 – For use in clinics (2017 – For personal use)	FreeStyle libre	Abbott, USA	–	–	First CGM that does not require calibration with fingerstick measurement; Can be worn up to 10 days without replacement (up to 14 days with the pro version); 12 hours of warm-up before measurements in the first version of the device (then reduced to 1 h)

was not the first commercial device for glucose measurement. Slightly earlier, in the late 1960s, the Ames Reflectance Meter (ARM) was developed, which combined Dextrostix dry test strips with photometry for measuring glucose in blood. Several more devices based on reflectance measurements and standard test strips, such as Reflomat (Boehringer Mannheim, Germany) and Eyetone (Arkray, Japan), were launched on the market in the early 1970s (Clarke and Foster 2012). These devices were successfully used in hospitals for a decade; still, most commercial devices for personified measurements of blood glucose levels are based on the ideas of Clark and Lyons. In 1973, Guilbault and Lubrano (1973) described an enzyme electrode

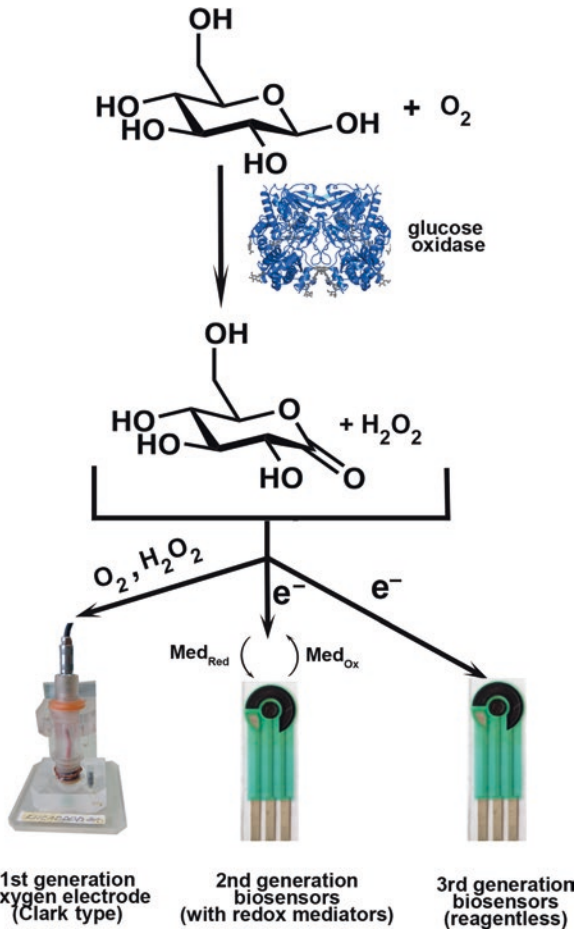

Fig. 15.1 The biochemical reaction underlying the first-, second-, and third-generation electrochemical glucose biosensors

for determination of glucose in blood based on the amperometric monitoring of oxygen peroxide released in the glucose oxidase-catalyzed reaction:

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

Updike and Higgs, in 1967, first successfully immobilized GOx in polyacrylamide gel, which enabled stabilizing the enzyme and simplifying measurements by the biosensor in biological fluids (Updike and Hicks 1967a, b). Since then, numerous variants of enzyme-based electrochemical sensors, differing by the design of the electrode, material of the membrane, or method of immobilization, have been described in the literature. It is also worth noting that not only GOx but also other

enzymes, for example, glucose dehydrogenase (GDH), can be used as a biological element:

$$\text{glucose} + \text{NAD}(\text{P})^{+} \xrightarrow{\text{GDH}} \text{glucono} - 1,5 - \text{lactone} + \text{NAD}(\text{P})\text{H} + \text{H}^{+}$$

Further improvements were related to the replacement of oxygen as a natural electron acceptor for a synthetic mediator. They laid the foundation of second-generation biosensors (Liu and Wang 2001). GOx is not capable of directly transferring electrons to the surface of traditional electrodes, because the FAD redox center is enclosed by a thick protein layer, and this blocks the direct transfer of electrons. Therefore, the use of a non-physiological electron acceptor for electron transfer and for solving the problem of oxygen deficit is the main approach in this generation of sensors. Mediators in these biosensors should oxidize the enzyme complex faster than oxygen, be insoluble and non-toxic. First- and second-generation biosensors have a number of drawbacks. Their commercial implementations are invasive or semi-invasive and require reference calibration; also, they are not able to serve as a part of an autonomous glucose-concentration regulation system (Hovorka 2006).

Third-generation biosensors are reagentless and are based on the direct transfer between the enzyme and the electrode without mediators. To provide for the direct transfer of electrons to the electrode, organic conducting materials can be used instead of mediators with high toxicity (Guo and Li 2010). Nevertheless, only a few enzymes, including peroxidases, demonstrate the direct transfer of electrons on the surface of standard electrodes (Teng et al. 2009). Providing for the direct transfer from other enzymes requires additional modification of electrodes (e.g., by nanomaterials, conducting gels). In particular, conducting organic compounds, such as tetrathiafulvalene tetracyanoquinodimethane (TTF-TCNQ), are capable of being carriers both for PQQ-dependent GDH and for GOx (Yoo and Lee 2010). For this reason, third-generation glucose biosensors possess a greater potential for creating needle-type implantable devices for continuous monitoring of blood glucose level.

Today, biosensor devices successfully compete with traditional methods routinely used in clinical diagnostics and assuming the use of expensive equipment, special premises, and qualified personnel. The development strategy of bioanalytical assay methods is shifted toward conducting the initial examination outside the healthcare facility within the framework of personified medicine approach. Research is aimed at the development and fabrication of inexpensive, sensitive, selective, and handy devices, convenient for an unsophisticated user.

15.4 Application of nanomaterials in Glucose Biosensors

The characteristics of glucose biosensors are often improved by nanomaterials (Fig. 15.2). They can be used to enhance the catalytic properties of electrodes by increasing the sensor surface area, to change the physical parameters of the

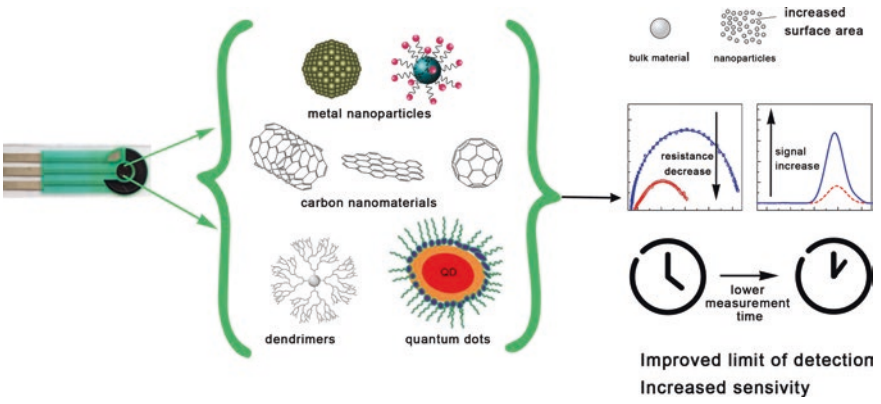

Fig. 15.2 Changes in the characteristics of electrochemical glucose biosensors when using nanomaterials

electrode (flexibility, elasticity, strength, etc.), and to enable the development of nanosized sensors (Cash and Clark 2010). Such nanomaterials as carbon nanotubes (Cosnier et al. 2014; Reshetilov et al. 2019), graphene (Karimi et al. 2015), electroformed nanofibers (Sapountzi et al. 2017), gold nanoparticles (Zhou et al. 2020), and quantum dots (Wang et al. 2018) are included into biosensors to increase their sensitivity, response time, and limit of detection (LOD) (Noah and Ndangili 2019). Such biosensors can be used to measure glucose both in vitro and in vivo (Taguchi et al. 2014).

Graphene and carbon nanotubes are used the most often among carbon nanomaterials (Zhu et al. 2012). Application of carbon nanomaterials enables reducing the resistance of charge transfer from enzyme's active site to the electrode surface, owing to which the biosensor signal, its sensitivity, and operation speed increase.

Valentini et al. (2013) used the one-stage application of polypyrrole film with GOx by electrophoresis to a gold microelectrode coated with single-walled carbon nanotubes. The linear range of concentrations was 4 up to 100 M, which covers the range of hypo- and hyperglycemia.

Tang et al. (2014) applied multiwalled carbon nanotubes (MWCNTs) in a composite with chitosan and polythionine. Owing to the use of conductive wires from MWCNTs, the authors achieved a higher amperometric signal and an LOD of 5 μM. The biosensor response time was a mere 15 s; the linear detection range, 0.04 to 2.5 mM.

Kang et al. (2009) were the first to develop in their work a glucose biosensor based on graphene and chitosan by fixing the mixture on a glassy carbon electrode. Their biosensor had an LOD of 20 μM and a linear range from 80 μM up to 12 mM. Alwarappan et al. (2010) used polypyrrole instead of chitosan; polypyrrole incapsulated nanolayers of graphene and GOx and was sedimented on the glassy carbon electrode. Their biosensor was capable of detecting glucose with an LOD of 3 μM and detection linear range of 3 to 40 μM. Sometimes investigators do not use pure graphene but the one modified with additional nanoparticles. In particular,

Norouzi et al. (2011) in their work used a nanographene support alloyed with zinc oxide nanoparticles immobilized in GOx. Their biosensors showed a high sensitivity to glucose with an LOD of 0.02 μM and linear range of 0.1 to 20 μM. Another biosensor based on graphene and metal nanoparticles was developed by Rafighi et al. (2016). They sedimented a thin layer of GOx on the surface of a composite from graphene, polyethyleneimine, and gold particles (GNS-PEI-AuNPs). Their biosensor was found to have a lower LOD (0.32 μM) and a broader linear range (1–100 μM) than biosensors without nanoparticle modification (Alwarappan et al. 2010). In another study, Gupta et al. (2017) used graphene quantum dots, which are zero-dimensional materials with quantum confinement and edge site effects. The developed GOx–GQD biosensor responded efficiently and linearly to the presence of glucose over concentrations ranging between 10 μM and 3 mM with an LOD of 1.35 μM.

Metal nanoparticles, as we mentioned above, are also used to improve biosensors' parameters (Nenkova et al. 2017; Lee et al. 2019). For instance, dissolved suspensions of nanoparticles are applied to detect glucose by electrochemical and optical methods (Taguchi et al. 2014).

In a study, Rassas et al. (2019) used gold nanoparticles as glucose biosensor components. A polyelectrolyte complex from chitosan–kappa-carrageenan coated with AuNP was used to immobilize GOx on the surface of a gold electrode. The obtained biosensor possessed a good reproducibility and stability with an LOD of 5 μM and a linear detection range of 10 μM to 7 mM. Silver nanoparticles were used by Hsu et al. (2011) as a matrix for immobilization of GOx. A graphite electrode coated with a complex of nanoparticles, GOx, nafion, and chitosan provided for a linear detection range of 0.5 to 6 mM. Zhang et al. (2015) reported the use of magnetic Fe_3O_4 nanoparticles for the purpose of enhanced enzyme immobilization, electrochemical activity, and additional magnetic properties. The LOD of glucose for the biosensor was 16 μM; the linear range, from 16 μM up to 26 mM.

One more form of nanoparticles used as a component of biosensors for glucose assays includes platinum nanoparticles (PtNPs). Liu et al. (2013) developed a biosensor based on PtNPs synthesized on the surface of the eggshell membrane and GOx; the biosensor was capable of detecting glucose within the linear range of 10–225 μM in blood serum samples with recoveries between 93.6 and 102.8%. Yang et al. (2017) also developed an amperometric biosensor from one-dimensional platinum nanotubes (MPtNT) for glucose assays. The LOD of the MPtNT-based biosensor is calculated to be 0.2 μM; the linear range, from 0.025 to 2.20 mM.

As can be seen from the above discussion, in recent years investigators have frequently combined various types of nanomaterials to obtain the best possible biosensor parameters. On the whole, nanotechnologies contributed to improving the sensitivity and linear ranges of detection of various glucose biosensors to sufficient levels for continuous monitoring of glucose in blood both in healthcare facilities and under conditions of home use.

15.5 Continuous Monitoring of Glucose

Continuous monitoring of glucose is based on the measurement of glucose concentration not less than each 5 min for a prolonged period of time (more than 24 h). Continuous monitoring provides a more detailed information on the character and tendencies of glucose-level change and makes it possible to identify periods of nocturnal hypoglycemias and postprandial hyperglycemias, to adjust sugar-reducing therapy, and to introduce changes to the diet and physical-activity schedule. Continuous monitoring of the glucose level in blood ex vivo was proposed by Albisser et al. in 1974, and in 1982, a system for in vivo monitoring was developed (Shichiri et al. 1982). At present, two types of continuous glucose monitoring systems are used: continuous subcutaneous glucose monitoring and continuous monitoring of glucose in blood. Still, due to a possible contamination of the electrode with proteins, a risk of thromboembolism, and coagulation factors, most of these systems do not measure blood glucose directly. For this reason, subcutaneously implantable needle-type electrodes were developed to measure the concentration of glucose in extracellular fluid (Yoo and Lee 2010). Extracellular fluid contains glucose at a concentration similar to that in blood (McGarraugh 2009). Sensors for glucose concentration measurements in extracellular fluid use either subcutaneous microdialysis probes or microneedles (Lucarelli et al. 2012; Corrie et al. 2015); microneedles are more popular and are used on the market more often. These devices continuously monitor glucose levels, and using the trackable glucose profile, automatically inject insulin at hyperglycemic peaks and glucagon at hypoglycemic troughs. A low concentration of O_2 (required for operation of GOx) in extracellular fluid causes problems with long-time stability of the biosensor. The problems were overcome by using a special membrane for modulating the inflow of glucose and uptake of O_2. Based on this technology, a device named GlucoWatch was developed (Cygnus, Inc., USA). Its operation, however, was fraught with some problems: application of an electric field caused skin irritations, and additional calibrations were necessary using blood tests with fingerprick, and the measurement accuracy was disturbed by patient sweating (Vashist 2012). On top of that, GlucoWatch could not monitor glucose in short periods of time, because glucose was extracted and measured at different times. For this reason, the device was discontinued soon after its launch in 2002, and in 3 years after that the company closed down.

The first needle-type enzyme electrode for subcutaneous implantation was described by Shichiri et al. (1982). The first commercial needle-type glucose biosensor began to be sold by Minimed (Sylmar, CA, USA). Nevertheless, it did not provide real-time data, and the results of every 72-h monitoring should be obtained via a technician (Gross et al. 2000). Later, its analogues appeared: SEVEN and G4 Platinum of Dexcom (San-Diego, CA, USA), iPro2 of Medtronic MiniMed (Dublin, Ireland), Freestyle Navigator and Libre of Abbott (Abbott Peak, Illinois, USA). These devices display updated concentrations of glucose in real-time mode

approximately each 3–5 min. The sensor is disposable and can be used from 3 to 14 days (Boscari et al. 2018; Dungan and Verma 2018).

Thus, the continuous glucose monitoring systems are convenient for outpatient, inpatient, and home use. At present, investigators test novel types of continuous glucose monitoring systems and try to overcome the shortcomings of the existing devices (Sola-Gazagnes et al. 2019). This technology is a step forward on the way to creating implantable devices for automated human health monitoring, for example, artificial pancreas, which can simulate the natural insulin control process in the organism.

15.6 Non-invasive Methods of Glucose Analysis

As we mentioned above, invasive methods of research are inconvenient for the end user; for this reason, many investigators have focused on the development of non-invasive glucose monitoring sensors. The first developed non-invasive sensors were based on optical methods of analysis (Oliver et al. 2009) and measured the change of the physical properties of light in extracellular fluid or in the ocular anterior chamber. Nevertheless, to date those developments have not found broad commercial application.

Many investigations focus on the search for a relation between glucose levels in blood and in other biological fluids that require no invasive sampling, such as tears, saliva, and sweat (Corrie et al. 2015). Glucose sensors in the form of contact lenses were developed for monitoring glucose levels in lacrimal fluid (Chu et al. 2011; Yao et al. 2011). The essence of the method is that an electrochemical sensor with immobilized GOx is screen-printed on the plastic surface of the lens. The sensors demonstrated a high signal speed, high sensitivity to glucose, and a good reproducibility within the range of low concentrations of glucose. However, it is necessary still to overcome such problems as a more efficient suppression of interference, total biocompatibility for wearable contact lenses, and integration of sensors with readout and communication chains.

Saliva is yet another attractive object of research, because its samples are easy to obtain. Besides, simultaneously with glucose level measurements, one can have information on other significant compounds, such as lactate or cholesterol. Still, accurate measurement of glucose concentration can be prevented by the occurrence of various postprandial impurities in saliva. As a rule, the assessment of glucose in saliva requires it to be filtered to discard large biomolecules. Such sensors can be incorporated into, for example, mouthguards (Kim et al. 2014). In Arakawa et al. (2016), a system of a platinum electrode coated with a GOx membrane and an Ag/AgCl electrode was incorporated into plastic mouthguards to measure the level of glucose in artificial saliva for 5 h. However, contact lenses and mouthguards are inconvenient for long-term use due to their unfavorable impact on the eyes and mouth cavity. Besides, the volume of these biological fluids is limited, which does

not make it possible to use them for continuous monitoring of glucose levels in the organism.

The use of sweat is promising in this respect. Sweat glands are distributed all over the body, and the level of glucose in sweat changes rapidly enough to reflect the physiological conditions in the main part of the body (Sakaguchi et al. 2013). Various sorts of sensors have been developed for convenient glucose-level monitoring in sweat during training. Wearable wristbands (Gao et al. 2016) and disposable sensors (Lee et al. 2017) can continuously monitor glucose level changes in sweat, remaining attached to the skin for long periods of time. Such devices offer a simple monitoring of the level of glucose in sweat, because the fluid's capillary channel in the sensing strip efficiently adsorbs sweat. Devices in the form of plasters have also been developed (Cho et al. 2017); they include not only a biosensor but also an enzyme fuel cell to feed the entire device at the expense of the endogenous substrates of the organism. The fully integrated and self-powered smartwatch for continuous sweat glucose monitoring is an even more complex device (Zhao et al. 2019). It includes a GOx-based biosensor, a signal processor, and a display to output information about the level of glucose.

Depending on the sweat generation conditions, its parameters, such as temperature and acidity, may vary, which has a negative effect on measurement accuracy. For instance, during physical exercises, the pH of sweat drops down from 6.3 to 4–5 depending on the amount of lactic acid released, and the temperature of sweat directly depends on ambient temperature (Jadoon et al. 2015). To eliminate distortions, temperature and pH sensors are integrated with glucose sensors, which increases the accuracy of glucose monitoring by providing corrections based on precalibrated data. A temperature increase leads to enhance bioreceptor's enzymatic activity and to overrate the readings of the device. For a more accurate assessment of glucose in blood via sweat, it is necessary to introduce individual correlation coefficients for glucose concentrations for each person under different measurement conditions (Lee et al. 2017). Besides, under ordinary conditions sweat is not released by itself in amounts required for measurements. Its release should be stimulated by either physical exercises or by additional chemical agents, which can be inconvenient or unrealizable for many potential users. Also, the section of skin in the vicinity of the biosensor requires to be cleaned periodically, because residual glucose and exogenous contaminants may negatively affect the measurement accuracy.

15.7 Conclusion

Over the almost 60 recent years since the development of the first glucose-assay biosensor, significant changes have occurred both in the requirements to these devices and the technology of their production. The newest achievements in the fields of medicine, biotechnology, chemistry, physics, and cybernetics are used to create modern glucose monitoring devices. Various sorts of nanomaterials and nanoparticles that enable increasing the sensors' sensitivity and enhancing their

long-term stability are combined in the receptor element of the biosensor. Biosensor supports from flexible polymer materials enable continuous monitoring of glucose by means of wearable devices with wireless data transfer. Non-invasive methods are potentially more in demand but are still less accurate as compared with direct blood glucose monitoring methods and often require individual adjustments for each patient. As the number of diabetics constantly increases, and there is no efficient treatment yet, the demand for glucose monitoring systems shall remain invariably high. The most important tasks for investigators in the near future are to increase the biosensor's lifetime and to overcome the physical/physiological factors that affect the accurate registration of glucose concentration in the human organism.

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