Ex Vivo Continuous Glucose Monitoring With Microdialysis Technique: The Example of GlucoDay

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***Abstract*—The use of a glucose biosensor in conjunction with a microdialysis probe used to sample the interstitial fluid of the patient has been demonstrated to be extremely useful and advantageous for obtaining a continuous glucose monitoring instrument capable of detecting glycemic level in real time for a long period. The first example of this kind of instrument which was cleared for commercialization is the GlucoDay made by Menarini. The approach used by the GlucoDay presents several advantages if compared with other instruments and its features and future prospective are thoroughly discussed.**

***Index Terms*—Continuous glucose monitoring (CGM), diabetes, electrochemical sensors, microdialysis.**

I. Introduction

APPROXIMATELY 17 million people in the U.S. have diabetes, a life-long disorder that is, as yet, incurable. A number of long-term complications arise due to the damage caused by diabetes on small and large blood vessels. Blindness, kidney failure, amputation of the lower limbs, and nerve damage are only some of the major consequences of diabetes. Long periods of hyperglycaemia account for these long-term complications but also hypoglycaemia (blood glucose level below 50 mg/dl) represents a major issue in diabetes therapy since it can cause sudden coma and brain damage [1], [2]. Development of these complications can be prevented or slowed down by controlling the blood glucose level.

Recently, major efforts in diabetes diagnostic research have been dedicated to obtaining reliable analytical systems for the continuous monitoring of the glycaemic level. Due to the fact that glucose levels in blood can change rapidly (2.25 mg/dL min), the periodic finger stick tests commonly used by diabetic patients often fail to detect all hypoglycaemic and hyperglycaemic events. Automated and noninvasive blood glucose monitoring could then offer an important improvement in diabetes care management [3]–[5].

Several attempts have been made to find the most suitable means for a continuous monitoring of diabetes in order to monitor the glucose level in diabetic patients during the whole day [6]–[9] with alarms that warn the diabetic of impending high- or low-blood-sugar episodes. The potential combination of such systems with automatic insulin pumps, thus creating an implantable artificial pancreas, would make the management of diabetes both easier and more secure. Continuous glucose-monitoring (CGM) systems are intended to replace the point-in-time measurements of traditional glucose meters and strips, providing physicians with glucose-level trend information that can be particularly advantageous in the disease management [10]–[14].

If compared with conventional, intensive glucose testing performed with three to four finger pricks per day, CGM provides much more detailed information about the glucose trend throughout the day, thus helping the patients and healthcare providers to identify and prevent periods of hypo- and hyperglycemia and thus allowing them to make the best treatment decisions. For example, nocturnal hypoglycemia and postprandial hyperglycemia have been identified in many patients who utilize CGM.

Because of the importance of diabetes and of the advantages of the CGM, several publications have appeared in literature in the last 20 years. Some of these methods try to accomplish CGM utilizing a completely noninvasive technology which is mostly based on optical techniques such as near-infrared spectroscopy or light scattering [15]–[17]. On the other hand, many efforts have been made in order to obtain a reliable instrument for CGM based on the use of amperometric glucose biosensors and utilizing the so-called "minimally invasive" methods, which measure the glucose concentration in the interstitial fluid of the skin.

II. Commercial Instruments for CGM

Because of the objective difficulties related to CGM (stability of the sensor, biocompatibility, etc.), few products have actually reached the market in a successful way. Currently, there are five products cleared for the European Community (EU) or U.S. markets. Only one is based on the measurement of glucose in a noninvasive way. The **GlucoWatch G2 Biographer** device was the first system approved by the FDA for real-time readings. The instrument is worn like a watch on the wrist; the measurement is based on the use of a small current to extract glucose through the skin onto a pad containing glucose oxidase [18], [19]. The resulting peroxide is then measured coulometrically and the total peroxide related to the blood glucose concentration. The system requires a 2-h warm-up period; a single calibration is then required every 13 h at the beginning of a session. To date, data from the GlucoWatch have been suboptimal at hypoglycemic levels and other common problems are skin irritation and "skipped readings" due to perspiration [10], [11].

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The variety of products that have been commercialized by Minimed is probably the most popular and most successful example present in the CGM market. Their products are all based on the use of a needle-shaped glucose biosensor with a glucose oxidase coating; it is inserted directly, via an insertion device, into the subcutaneous tissue. Once inserted, only the sensor remains under the skin. This method was the first one to be explored by researchers involved in the field of CGM [20]–[25]. Then, after more than 20 years of effort the continuous glucose monitoring system (CGMS) was finally released in 2001 by MiniMed (Silmar, CA, USA) [9]–[11], [26]. Following this initial success, other instruments which were based on the same principle but with new features have been commercialized.

CGMS Gold: This instrument is the development of the first CGMS [27]. It allows a 72-h implantation automatic glucose measurement every 5 min, and a two-week data-storage capability. A wire connects the sensor to the instrument and the glucose values are not shown in real time. After up to three days wearing the sensor and monitor, the patient has to return to the physician's office, where the sensor is removed and the collected data are downloaded to a computer.

CGMS Guardian: For this case, the sensor is connected to the instrument via radio frequency technology which allows to wireless communicate and send glucose values automatically to the compact monitor [28]. The monitor displays updated glucose readings every 5 min and provides alarms when levels become too high or too low. The monitor stores up to 21 days of information which can be downloaded to the computer.

MiniMed Paradigm Real-Time System: The MiniMed Paradigm Real-Time System is the first system to integrate an insulin pump with Real-Time continuous glucose monitoring (see [10] and references therein). The monitoring of glucose is performed in a way similar to that used by the Guardian system; however, in this case it is also coupled with an insulin pump. The patient can in this way, by reading the real time glucose level, take the necessary steps using the insulin pump.

The CGMS has been evaluated in an exceptional number of clinical tests which have been collected and published since its first release on the market [10]. However, despite the significant success and the good performance of these sensors for certain applications, some problems are still present. The major problem that has plagued these kinds of implantable biosensors is the gradual decrease in sensitivity and in some cases a complete loss of function within just hours of implantation. Biofouling, oxygen limitation, electrochemical interference, and GOD inactivation have been considered as explanations for this behavior. For instance, a tissue reaction to the sensor implantation may result in a limitation in the blood supply to the tissue surrounding the probe and thus in a reduced availability of glucose and oxygen [10], [11], [13].

A reliable calibration method becomes critical in the application of CGM. The use of the blood glucose value measured with conventional clinical methods or a finger-stick system must be correlated with the subcutaneous glucose concentration that is measured with these devices. All the Minimed CGM instruments currently on the market require an initial (calibration meter) finger-stick measurement and four finger-stick measurements from midnight to midnight each day. Moreover, a considerable drift of the sensor signal due to the foreign body reaction limits the number of recalibrations. For this reason, the use of the CGMS is not allowed for more than three days [29]. From several clinical studies, it has been demonstrated that the data for both the CGMS Gold and Guardian have been suboptimal at hypoglycemic levels [13].

Beyond these examples, the only other instrument cleared for sale in Europe is the GlucoDay.[1] This instrument is based on the coupling of an amperometric glucose biosensor and a microdialysis probe used to sample the interstitial fluid of the patient. This approach presents several advantages if compared with other instruments and the features and future prospective will be thoroughly discussed in this paper.

III. Microdialysis Technique As Sampling Method

The microdialysis technique arose historically in the field of neuroscience, having been introduced in 1972 by Delgado *et al.* [30] for use in brain research. A dialysis membrane usually separates two fluid compartments, where the external one usually represents the site to be sampled. The other compartment contains a liquid whose composition (ionic strength and pH, for example) tentatively matches that of the external fluid being sampled. Permeable substances present in one of these compartments, and absent in the other, will diffuse across the membrane down their concentration gradient. The availability of cylindrical membranes of very small size, the so-called hollow fibers used in artificial kidney cartridges, provided a great impulse for the development of this technique, and much of the original research work was done by Ungerstedt. He implanted "hollow fibers" into rat brains in order to mimic the function of blood vessels [31]; since then, this simple "microdialysis probe" has been markedly improved [32]–[35]. A vast number of different designs have appeared [36]–[41] resulting in probes that, because of their narrow cylindrical profile, can be handled like a needle and easily implanted into the brain and many other tissues.

The basic principles of this technique have been extensively reviewed by different authors who offered theoretical and physicochemical considerations [42]–[48].

Probe: The central component of any microdialysis system is the probe itself. An increasing number of companies supply probes ready to use and made in different shapes, dimensions, shafts materials, fibers, etc. A microdialysis probe usually consists of a small piece (2–10 mm) of a cylindrical dialysis membrane (the hollow fiber), connected to an inlet and an outlet tube of suitable dimensions. Despite the differences in design, what essentially typifies the probe is the position of the inlet and outlet tubes, and basically, two types of probes can be distinguished: in the first one, the two tubes are positioned in a serial arrangement; in the other type, the inlet and outlet tubes are concentric or parallel. Consequently, in the first type, which is normally termed a "linear probe," the hollow fiber is glued in between; in the second one, usually called a "concentric probe, " it is affixed at the tip. The linear probe is the simplest design and is the most suitable for sampling of soft tissues such as muscle [49], [50], adipose and subcutaneous tissue [51]–[53], and tumors [50], [54]–[56]. The advantages of this kind of probe are

[1]Registered trademark.

the simplicity of construction, the small dimensions and high flexibility. One disadvantage is that, when implanted *in vivo*, two holes are necessary, one for the entrance and one for the exit, thus increasing the risk of microbial attacks. The concentric probe, patented by Ungerstedt is the most used, although it is also the most difficult to construct. It can be made very thin (around 300 μm in diameter); the material of the shaft may be rigid (metal) or flexible (fused silica or plastic), and the length of the membrane at the tip can be varied from millimeters to centimeters.

Because of its small dimensions and conformation, it is easy to handle as a needle. It also presents the lowest degree of invasiveness when implanted *in vivo*, since it requires only a single insertion point. When the shaft is rigid, the probe can be easily glued to the skull of animals for intra-cerebral investigations. By contrast, the flexible conformation is the most suitable for implantation in blood vessels and in soft tissue because it permits freedom of movement without injury. This kind of probe can be assembled in larger and more robust designs that are useful for sampling from bioreactors and fermenters, where stirring may be continuous.

Membrane: The hollow fiber is the most crucial part of the microdialysis probe. It acts as a membrane, and its characteristics affect performance in the sampling step as well as the probe's suitability for the selected application. Hollow fibers are commercially available in different materials, the most common being polycarbonate (PC), regenerated cellulose (Cuprophan, CU), cellulose acetate (CA), polyacrylonitrile (PAN), polyethersulphone (PES), polysulphone (PE), and polyamide (PA). Generally, the fibers have an outer diameter between 200 and 500 μm, a wall thickness between 9 and 100 μm (some of these membranes having a supporting layer of the same material as the fiber), and a molecular weight cutoff (MWCO) ranging from 3000 to 20 000 Da, although larger cutoff limits are available. The MWCO characterizes the dialysis performance by controlling the size of the molecules that will be retained by the membrane. This parameter is related to the size of the pores in the membrane and to the pore distribution. However, other characteristics of a molecule such as its shape, charge, degree of hydration, the nature of the solvent, pH and ionic strength, in addition to the molecular weight, are important parameters to be considered. For *in vivo* implantation and for bioprocess application, both sterilizability and biocompatibility are required, although the latter is a vague concept for which a variety of definitions exist.

Sampling Considerations: As mentioned before, microdialysis is a sampling technique controlled by diffusion. Because a fluid continuously perfuses the probe, equilibrium conditions will not be reached, and only a fraction of the actual concentration of the analytes present in the medium surrounding the probe will be collected. The amount of analyte collected by the fiber is normally defined as "recovery." The "relative recovery" is the ratio between the concentration in the dialysate (C_{in}) and the concentration outside the probe (C_{out}) and is the value obtained if volume concentrations are used, while the absolute amount of mass removed from the medium of interest per time interval is defined as "absolute recovery."

Under a given set of experimental conditions, most of these parameters remain constant and a calibration can be performed. Often, after sampling, a successful separation of the analyte of interest from a complex matrix is the first goal of an analysis. One of the key points of microdialysis lies precisely in the fact that these two steps, the sampling and the separation, at least from macromolecules, are obtained at the same time.

IV. CGM Using Amperometric Biosensors Coupled With Microdialysis Probe

The possibility of monitoring changes in subcutaneous glucose concentration by the microdialysis method was first demonstrated by Lonnroth [57]. Other reports followed [58]–[63], but in all of these, measurements were made in a discontinuous way using traditional techniques. The microdialysis technique was subsequently adopted by the same groups (i.e., Pfeiffer and Shichiri) which had initially developed the *in vivo* needle sensors, now employing these same sensors in a flow system connected to a microdialysis probe for sampling [64]–[71].

Schoonen and Schmidt have combined the microdialysis method with a miniaturized Clark-type oxygen electrode [72]. In this system, the enzymes GOD and catalase were contained in solution in a reservoir and continuously perfused at a fixed rate into the microdialysis probe and then to the sensor cell. All the glucose diffusing into the probe was catalytically converted and the difference between the basal level of oxygen and the value after its depletion by the enzymatic reaction was correlated with the glucose concentration. In subsequent studies, a successful implant functioning up to nine days was reported [73], but a significant day-by-day variation of *in vivo* calibration factors was also noted. Moreover, the possibility of enzyme leakage toxic to humans could not be excluded.

On the basis of the results from this group, the Roche Diagnostic Company has recently produced a wearable apparatus that has been tested in 23 diabetic patients for up to 72 h. The extra-corporeal unit displays a glucose value every minute. The signal is corrected for the time needed for fluid transport from the microdialysis probe to the sensor (31 min). The one-point calibration mode was used after an initial equilibration time of about 4.7 h. Continuous monitoring was feasible for at least three days with no time-dependent decline in sensitivity to glucose being noted [74].

In the early 1990s, our group also developed a system for continuous glucose monitoring, coupling microdialysis with a flow cell containing an electrochemical glucose biosensor [75], [76]. The initial experiments were carried out with bench-sized instrumentation and involved studies both *in vitro* and *in vivo* on animals and humans. In these studies, a number of hospitalized patients with different pathologies (but all subjected to an oral glucose load) were monitored. The one-point *in vivo* calibration method was adopted, assuming that the constant value of current observed before the glucose load was proportional to the concentration measured in the blood. The subcutaneous glucose values were compared with blood samples that were taken every 30 min during 3 h of monitoring and analyzed for glucose by standard laboratory procedures. The subcutaneous values measured with the sensor correlated well with the blood glucose levels. In 15 experiments, there were only three cases in which a delay was observed between changes in blood glucose

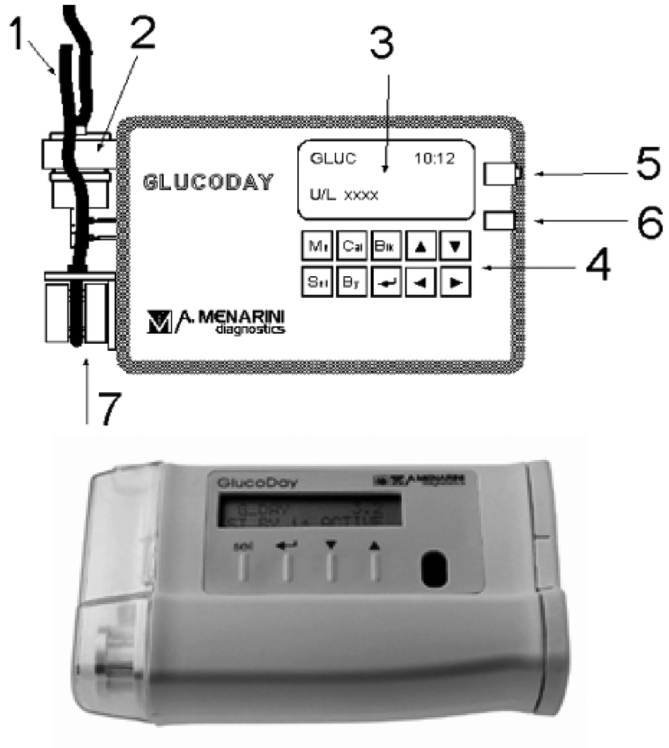

Fig. 1. (1) Fluidic line; (2) wall jet flow cell; (3) display; (4) keyboard; (5) RS232 interface to PC; (6) 12 V connector; (7) microperistaltic pump; (8) battery compartment (top). Picture of the instrument (bottom). With permission from Poscia *et al.* [78].

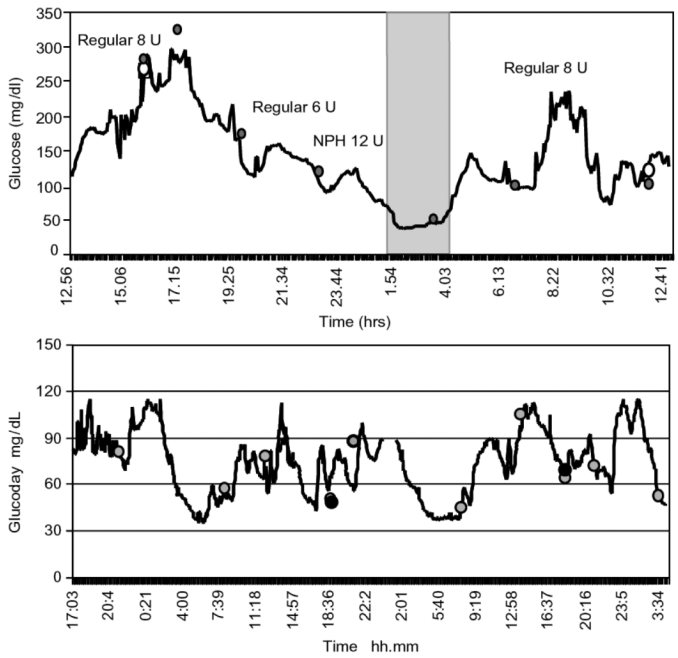

Fig. 2. Monitoring of 24 h (top) and 58 h (bottom) *in vivo* with GlucoDay on diabetic patient. When indicated, patient assumed some amounts of insulin, both in regular and fast (NPH-neutral protamine Hagedorn insulin-) formulation. Gray box highlights nocturnal episode of hypoglycaemia. Gray dots represent finger-stick controls, while black dots correspond to blood measurements (with permission of A. Menarini Diagnostic, Florence, Italy).

and related sensor measurements. The delay was calculated to be about 30 min [77].

The miniaturization of the whole instrumentation was achieved and finally several prototypes of the portable device described have been produced, including the instrument, GlucoDay.

V. GlucoDay

GlucoDay instrument, now commercialized by A. Menarini Diagnostics (Florence, Italy) is composed of a linear subcutaneous probe that is connected to a wearable unit about the the size of a walkman. The latter is comprised of a programmable micropump providing flow rates from 10 to 100 μl/min; a wall-jet cell with a glucose biosensor; a disposable fluid line which includes a pressure sensor; a microcontroller for pump speed programming, signal acquisition, and data storage; an LC display which shows glucose values every second; a keyboard; and an RS232 interface and 9-V battery.

Data can also be visualized continuously on a computer through an infrared communicating port. The system takes a glucose value every second and stores an averaged value every 3 min, for a total of 480 measurements per day. Two plastic bags, one for the buffer reservoir and one for the waste, complete the apparatus for a total weight of about 250 g. Alarms are also present to warn of hypo- or hyperglycaemic events. A picture of the instrument is shown in Fig. 1.

The actual glucose sensor (a platinum electrode covered by three membranes: cellulose acetate, nylon net with covalently linked GOD, and a polycarbonate protective membrane) is located in a miniaturized wall-jet cell. The sensor exhibits excellent analytical performances, with a linear range extending up to 27 mM, thanks to the microdialysis dilution effect which was estimated to be 1:10 for the probe length used and for the flow rate set by the instrumentation. Long-term stability tests revealed that the biosensor still maintains its initial activity after incubations of 4 weeks at 45 °C, 11 weeks at 37 °C, and 32 weeks at room temperature. From these results, a shelf life of more than two years at 2 °C–8 °C can be extrapolated [78], [79]. The system was first tested on rabbits, then on human volunteers, and in Fig. 2 profiles of subcutaneous glucose monitoring up to 12 and 58 h are shown.

In these trials, a one-point *in vivo* calibration of the instrument was performed, testing the blood and/or capillary glucose levels and correlating this value with the current output obtained by the instrument. Studies undertaken to optimize the calibration procedures showed that the best accuracy was obtained performing the calibration at 60 min, or better yet 120 min, after the insertion of the fiber. This is the time necessary for the microdialysis probe to reach equilibrium under the skin and for the tissue to recover from the insult of fiber insertion. In Fig. 2 (above), 24-h run is displayed. During the first 24 h, the glucose was monitored by the GlucoDay and verified by seven finger-stick measurements. Moreover, two venous blood checks were also performed, at the beginning and at the end of the experiment. The patient was able to proceed with his normal life during the trials.

It is interesting to note how the nocturnal hypoglycaemia seen on the first day, as evidenced by the gray box in the figures, was completely remedied the following night by the adjustment of the dosage of a quick formulation of insulin.

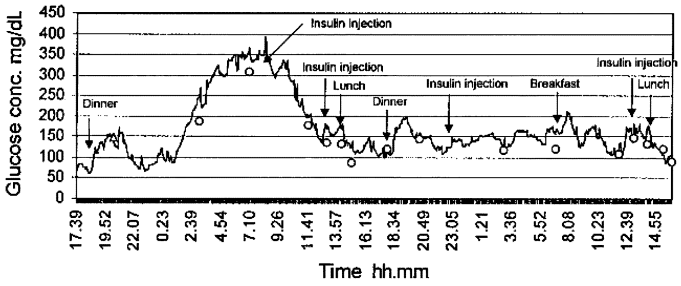

Fig. 3. A 48-h continuous monitoring in diabetic patient (type 1). Line: GlucoDay venous blood sample.

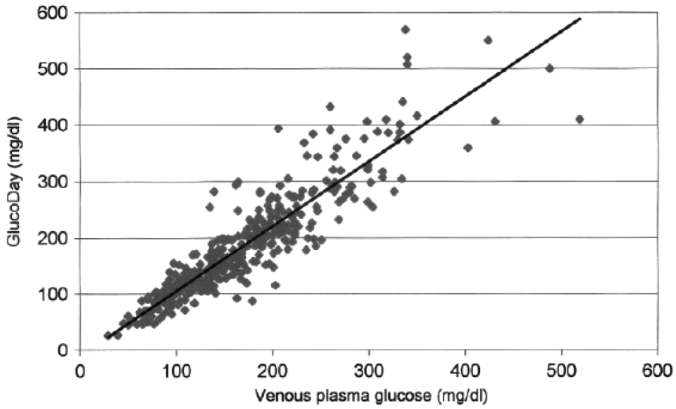

Fig. 4. Correlation between the GlucoDay and venous glucose in the whole population tested during the multicenter test performed with Glucoday ($\mathrm{n} = 60, \mathrm{r} = 0.9, \mathrm{r}^2 = 0.817$). With permission from Maran *et al.*, 2002 [82].

Fig. 3 shows another 48-h continuous monitoring of a diabetic patient (type 1) performed with one venous blood calibration (after 2 h) and with 17 different glucose measurements in venous plasma. Again, a very good correlation with venous plasma samples, with an overall bias percentage within 15%, was observed. A similar correlation was observed with venous blood samples (less than 15%) demonstrating no differences in correlation between blood and plasma levels. The data obtained during these experiments always showed a good correlation with capillary samples. Moreover, it can be highlighted that this good correlation is obtained by using only one calibration point performed 2 h after the microfiber insertion.

These preliminary *in vivo* experiments showed a satisfactory system performance as well as demonstrating other positive features: stability of the sensitivity, reproducibility, one point calibration, small and light walkman-like instrument (245 g), and an ease of use. Moreover, the microfiber insertion was well tolerated by the human volunteer and no local infection was reported after its removal. These features allowed the GlucoDay to enter into a multicenter European clinical trial to evaluate the accuracy in monitoring glucose levels in type 1 and type 2 diabetic patients. A total of 70 diabetic patients (43 type 1 and 27 type 2 diabetic patients, 38 women and 32 men) from seven different centers participated in this study [80].

Nine venous blood samples were collected during the 24-h period as follows: 1 h after the insertion, usually performed in the morning; before lunch; 1, 2, and 3 h after lunch; before dinner; 2 h after dinner; and at 3:00 A.M. and at 7:00 A.M. the next morning. The lag time between subcutaneous glucose values and venous plasma glucose concentration has been estimated *in vivo* to be ca. 3 min.

Among 70 diabetic patients, 60 successfully completed the 24-h monitoring, and their data were analyzed by an independent statistician. No complications at the site of implantation were observed. Both the fiber insertion and the wearing of the device were well tolerated by all patients. As shown in Fig. 4, subcutaneous glucose concentrations in this group of patients were well correlated with plasma levels. Error grid analysis of findings in 60 patients showed that 97% ($n = 381$) of the values were in zones A and B, and only 3% ($n = 9$) were in zone C, with a single value in zone D. No values in zone E were detected. Bias percentage relative to references showed a difference of -2% in the hypoglycemic range ($>$68 mg/dl), 6.9% in the euglycemic range ($>$68–180 mg/dl), and 11.2% for the hyperglycemic range ($>$180 mg/dl).

The multicenter study demonstrated that the GlucoDay system was accurate compared with a conventional glucose determination and was associated with little or no discomfort for the patient thus confirming that GlucoDay was suitable for continuous monitoring of subcutaneous glucose levels and clearly demonstrates a good performance of the system. However, in the whole experiment, the device was only used for 24 h, though the GlucoDay is approved for a maximal monitoring time of 48 h.

To address the question if the device also shows good performance over a 48-h monitoring span, a new clinical study was recently performed. A sample of 28 patients with diabetes was studied. GlucoDay glucose values were compared with laboratory standard blood glucose measurements. In this study a mean monitoring time of 45.7 h with a total of 484 paired readings was achieved, thus being a suitable study for an estimation of the real performances of GlucoDay during a two-day continuous monitoring [81].

Also, in this study, very good results were obtained with GlucoDay instrument; of the 463 paired readings, 95.5% were in the clinically acceptable A and B zones of the EGA. No differences with regard to the participants' sex with regard to accuracy were observed. The system's performance during the first 24 h was equivalent to that achieved during the second day of monitoring: there was no systematic deterioration of sensor performance from the initial one-point calibration.

To summarize, a similar performance of 48-h monitoring with the GlucoDay device has been observed as previously reported in the multicenter test with a monitoring time of 24 h, even if accuracy was slightly lower. No differences, however, were observed in the performance during the first day of monitoring versus the second [81].

After the EC approval following the multicenter test, the GlucoDay was tested to establish its accuracy compared with a standard reference method of glucose measurement during insulin-induced hypoglycemia, subsequent recovery, and the post-hypoglycemic hyperglycemic phase [82].

Normal subjects without diabetes were studied in this paper with the infusion of insulin and glucose to mimic hypoglycemic and hyperglycemic periods. The model of "slow fall" hypoglycemia, with a rate of fall of approximately 0.65 mg/dL/min,

has been adopted to mimic the clinical condition of hypoglycemia induced by hyperinsulinemia in the postprandial situation. Data obtained from the eight subjects showed that 94% of the GlucoDay measurements were in the acceptable zones of the grid (85% in zone A, 9% in zone B, and 6% in zone D), thus indicating that GlucoDay monitors changes in plasma glucose during hypoglycemia with a degree of accuracy similar to that shown by other CGM devices. Plasma glucose fall was correctly detected by the GlucoDay by the correlation between the Beckman analyzer and GlucoDay rates of fall. Of note, during the fall towards the hypoglycemic plateau, the GlucoDay never gave erroneous or contradictory information (i.e., plasma glucose is rising). Moreover, the glycemic nadir, time spent in hypoglycemia, and rate of fall and rate of rise of plasma glucose were accurately represented by the GlucoDay.

This important study, even if not very extensive as to number of patients, has proven the accuracy of the GlucoDay glucose sensor in an experimental model of hypoglycemia mimicking the postprandial condition.

Another interesting study, aimed to comparing the reliability of GlucoDay and of the CGMSgold (needle-type sensor) made by MiniMed, recently appeared in literature [83]. The study, even if with a limited number of patients, is in our opinion important to assess the peculiar characteristics of the two most used approaches applied for the CGM.

The study was particularly focused on the hypothesized nocturnal hypoglycemic drift of the needle-type sensor and the delay during rapid glucose changes of the microdialysis sensor. The sensors perform similarly during the day and night, without significant differences in accuracy. The hypothesized delay for the microdialysis system has been quantified (7 min) by the horizontal shift. This delay can be corrected for retrospectively but has implications for prospective use of microdialysis systems as an alarm for hypo- or hyperglycemia.

In this study, the microdialysis-based sensor (GlucoDay) has demonstrated to be more accurate than the needle-type sensor (CGMS) especially during the hypoglycemic period, thus demonstrating it to be a valuable adjunctive tool for glucose trend analysis.

Recently, our group has reported preliminary results obtained with the use of novel planar glucose biosensors as electrochemical probes to be coupled with a microdialysis fiber for continuous glucose monitoring in order to develop an improved GlucoDay instrument [84], [85]. The sensors were produced using the "screen printing" technique and showed a high degree of reproducibility together with a low cost and the possibility of mass production. Prior to enzyme immobilization the electrodes were chemically modified with ferric hexacyanoferrate (Prussian Blue). This modification allows the detection of the hydrogen peroxide produced by the enzymatic reaction catalysed by GOD at low applied potential (ca. 0.0 V versus Ag/AgCl), thus limiting electrochemical interferences. The layer of Prussian Blue (PB) showed high stability under the working conditions (pH 7.4). Also, after 1 year of storage dry at RT no loss of activity was observed demonstrating a high stability of the PB layer in storage conditions. The assembled glucose biosensors showed high sensitivity towards glucose together with a long-term operational and storage stability. On the basis of these characteristics, it was then suggested that such biosensors could be used in conjunction with a microdialysis probe for a continuous monitoring of glucose for clinical purposes and for this reason the sensor was further evaluated in terms of its eventual clinical application. Problems related to temperature and oxygen dependence were investigated and their effect on the linear range of the sensor was studied. The sensors were also tested in conjunction with a microdialysis probe, with serum samples, and in animal testing, and positive and encouraging results were observed. *In vivo* experiments with dogs for ca. 20 h have demonstrated the feasibility of the system proposed suggesting the usefulness of successive clinical studies on diabetic patients. Presently, the new system, based on the use of a screen printed electrode as sensor surface, will be tested in clinical trials, to confirm the possibility of its use in conjunction with a microdialysis probe.

VI. Conclusion

CGM instruments are seen as the future of the diabetes management because of their advantages over intermittent glucose monitoring. These include the possibility of fully understand the glycemic trend of a diabetic patient and thus avoid the undetected hypo- or hyper-glycemic period [10], [11], [13]. Also, CGM seems to be particularly advantageous to adjust therapy or document the physiological state of a patient.

During the last few years, several devices have been proposed for continuous subcutaneous glucose analysis *in vivo*. Among the few examples commercially available for CGM, the use of a glucose biosensor in conjunction with a microdialysis probe seems to be extremely useful and advantageous. The first example of this kind of instrument which was cleared for commercialization is the GlucoDay. This review has highlighted the basic principles, the performances, and the clinical studies which have demonstrated the feasibility of this instrument for this kind of application.

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