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Comparative Performance of Different Nanostructured Electrochemical Sensors on Insulin Detection

Fabrizio Mastrantonio · Francesco Valgimigli · Lucia Grassi · Paolo Cappa · Giovanni De Micheli · Sandro Carrara

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Abstract New advancements in the management of type I diabetic patients (T1DM) call for a new generation of monitoring systems based upon an array of metabolic biosensors including sensors for insulin detection. Blood insulin values are always less than 270 pM; therefore, specifically designed insulin sensors have to provide a limit of detection in the range of few pico-molar units. At present, electrochemical sensors are one of the best options to provide low-cost technologies for diabetes mellitus monitoring and treatment. Moreover, modern nanotechnology permits to improve further their performances by providing new materials, structured at the nanoscale (i.e., carbon nanotubes), which alter the original sensor properties when used for both in vivo and in vitro biosensing applications. A recent article in literature has proposed a solution based on electrochemical sensors that promise performances in line with above-mentioned insulin detection requirements. However, data presented in this paper show that such a promise is still overestimated, and an electrochemical sensor capable of insulin detection within the physiological range is still beyond the present state of the art even when adopting nanostructured working electrodes.

F. Mastrantonio (✉) · F. Valgimigli
A.Menarini Diagnostics, Florence, Italy
e-mail: fmastrantonio@menarini.it

L. Grassi · P. Cappa
Department of Mechanical and Aerospace Engineering, Faculty of Civil and Industrial Engineering, Sapienza University of Rome, Rome, Italy

P. Cappa
e-mail: paolo.cappa@uniroma1.it

G. De Micheli · S. Carrara
Laboratory of Integrated Systems, EPFL—École Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

S. Carrara
e-mail: sandro.carrara@epfl.ch

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1 Introduction

The control and personalized therapy of patients with metabolic disorders, including diabetes mellitus (DM), represents one of the major targets of the current research in biomedical engineering [1]. In order to achieve this goal, it is important to detect simultaneously different endogenous and exogenous metabolites (1) to evaluate the patient health conditions, (2) to further individualize the established therapy, and (3) to verify the response to the administered treatment. In particular, the above-mentioned approach to long-term DM management requires a multiple-analyte sensing strategy that includes parameters such as glucose, lactate, and insulin [2]. Continuous glucose monitoring is key to verifying the achievement of the established glycemic goals (preprandial and bedtime/overnight), to determine the patient's glycemic variability and to reduce the risk of hypoglycemic events at night. On the other hand, lactate is an important substrate to monitor the metabolic stress in patients with several pathological conditions, and a large body of clinical evidences shows a tight association between hyperlactatemia and mortality in many diagnostic groups. Finally, insulin sensing is of great importance for clinical diagnostics because it serves as predictor of diabetes, insulinoma, and trauma. It is well known that biological actions of insulin are essential for regulation and maintenance of glucose homeostasis. Insulin resistance (typically defined as decreased sensitivity or responsiveness to the metabolic actions of insulin) plays an important role in the pathophysiology of diabetes. Insulin resistance is also associated with obesity as well as hypertension, coronary artery disease, and dyslipidemias which are all risk factors of the

metabolic syndrome, a cluster of metabolic disturbances triggered by overweight and physical inactivity. Therefore, the monitoring of insulin secretion from pancreatic β cells to observe the temporal patterns of secretion, as well as the real-time measurements of the insulin/glucose ratio, would be of paramount importance to quantify insulin sensitivity and resistance in humans in order to investigate the pathophysiology and epidemiology of major public health problems and to follow the clinical course of the patients on various therapeutic regimens.

As far as glucose sensing technology is concerned, current technology can offer good results in terms of sensitivity, linear dynamic range, and detection limits due to the large amount of research and industry investments in the area [3]. Whilst, for other metabolites, technology still needs to be improved in order to guarantee the expected analytical performances of dedicated biosensors. Besides other analytes, for insulin detection too, the ideal solution should be based on electrochemical sensors because of their portability, low-cost readers and test strips, and for being the present technology of choice for glucose monitoring.

Moreover, the nanostructuration of the electrochemical sensor working electrodes provides excellent performance improvements by using carbon nanotubes [4], metallic [5] and semiconducting [6] quantum dots, metallic bismuth [7], and other nano-materials.

The insulin value in blood usually varies from 30 to 55 pM as basal values and rises up to not more than 269 pM as maximum value reached during the oral glucose tolerance tests after 30 min in healthy people [8]. To target these values, literature has recently proposed new advancements in electrochemical sensors based on nanostructured electrodes. In particular, the use of silicon carbide nanoparticles on glassy carbon electrodes has been suggested as an effective solution for the electrochemical detection of insulin with sensors that could reach a detection limit of 3.3 pM and a linear range of detection up to 600 pM [9].

The aim of this work is to investigate the electrochemical behavior of silicon carbide nanoparticles on screen-printed carbon past electrodes on the electrochemical detection of insulin in order to assess the feasibility of developing such a sensor. Our findings and the related comparison with other nano-materials employed in the electrochemical sensing of insulin demonstrated that the electrochemical detection of insulin is still beyond the state of the art of the electrochemical nano-sensors.

2 Materials and Methods

Three different biosensors were developed by using commercially available carbon paste screen-printed electrodes (SPEs) (DropSense, Spain). SPE modifications were carried out by using multiwalled carbon nanotubes (MWCNT) (DropSense, Spain) available as powder, or silicon carbide nanoparticles (SiC NP) (NanoAmor, Texas), also available as powder. Nanostructuring was achieved by drop casting of 30 μl of MWCNT-chloroform 1 mg/ml solution (2 μl each time) or, in the other case, 30 μl of SiC-chloroform 1 mg/ml solution (2 μl each time) onto the working electrode, and subsequent drying of the mixture. The solutions were created by dispersion of the two powders in chloroform and sonication (2 h) in order to break macroaggregates. Then, the electrodes were rinsed out with Milli-Q, and they were conditioned for five voltammetry cycles (low potential=−0.1; high potential=1; scan rate, 100 mV/s) before the first use. All the functionalized electrodes were stored at +4 °C when not used.

The electrochemical response of electrodes was investigated by cyclic voltammetry under aerobic conditions. Electrochemical measurements were performed by using Versastat 3 potentiostat (Princeton Applied Technologies). For calibration and investigation of the detection limit, the signal was acquired by using cyclic voltammetry (from −0.1 to 1 V; scan rate, 100 mV/s) first with PBS solution (100 μl) and then with solutions at different concentrations under quiescent conditions. A different electrode was used for each measurement due to the impossibility of implementing any electrode cleaning procedures

Scanning electron micrographs were also acquired with the SUPRATM 40 (Zeiss) instrument to gather FE-SEM images with a nominal resolution of 1.5 nm at 10 kV in order to verify the quality of the obtained sensing surfaces as well as to compare the different obtained electrodes.

3 Results and Discussion

The SEM images were used to investigate how the nanomaterials change the geometrical surface of the screen-printed electrodes based upon carbon past. Figure 1 shows one of the working electrodes structured by using the silicon SiC NP, while Fig. 2 shows how the electrode is modified by

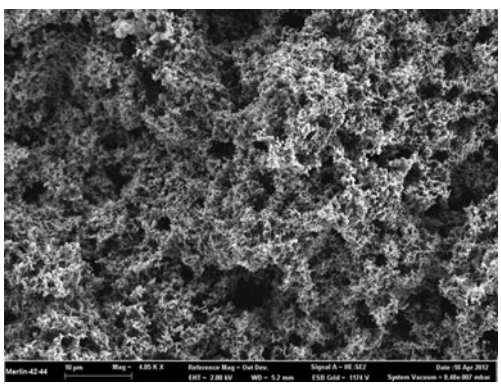

Fig. 1 SEM image of silicon carbide nanoparticles onto screen-printed electrodes

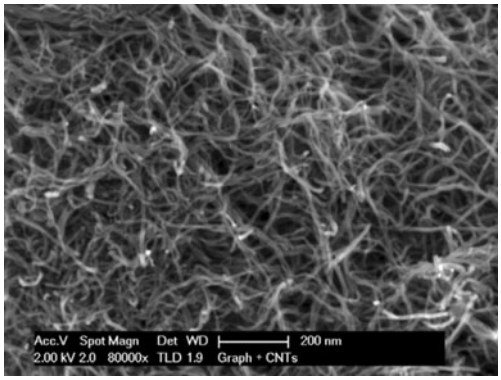

Fig. 2 SEM image of multiwalled carbon nanotubes onto a screen-printed electrode

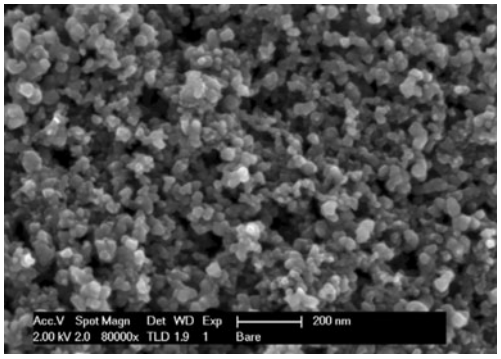

Fig. 3 SEM image of a bare screen-printed electrode

using the MWCNT. In both cases, the electrode geometrical surfaces are massively modified by the presence of the nano-materials compared to the original bare surface, as shown in Fig. 3. The modified electrodes (Figs. 1 and 2) clearly show a more structured surface that may offer a larger electrochemical active area for insulin electrochemical sensing. In fact, the electrochemical characterization of the obtained surfaces points this out in the case of MWCNT.

During the experiment, several measurement series were gathered for three types of electrodes:

- Bare graphite electrode (Fig. 3)
- Graphite electrode modified with multiwalled carbon nanotubes (Fig. 2)
- Graphite electrode modified with nanoparticles of silicon carbide (Fig. 1)

Figure 4 shows typical voltammograms gathered on the bare electrode. The figure clearly shows the expected Faradaic current located near +600 mV and related to the insulin direct detection. Similarly, cyclic voltammograms for different concentrations of insulin were obtained on the electrode modified either with multiwalled carbon nanotubes or with nanoparticles of silicon carbide.

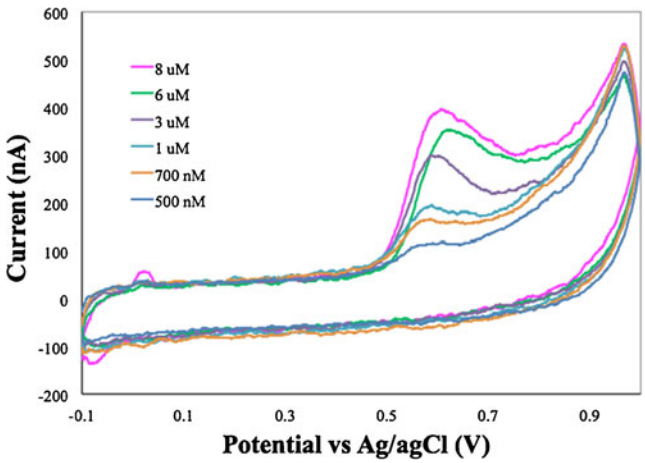

Fig. 4 Cyclic voltammograms on insulin at different concentrations as gathered on a bare screen-printed electrode

The average values obtained for linear dynamic range, sensitivity, and detection limit are reported in Table 1 where the errors are expressed as standard deviations. The results show a good detection limit and sensitivity for the graphite sensor at concentrations lower than 8 μM. On the other hand, the same electrode does not show a linear response ($R^2 \approx 0.90$). Moreover, in the presence of silicon carbide nanoparticles, a $R^2 = 0.96$ (average) is obtained, but the sensor has lower sensitivity (the worst of those calculated) and the highest detection limit, indicating that this kind of nanostructuring has no major advantages. Finally, the third sensor, nanostructured with MWCNTs, has a wider linear dynamic range than the other sensor types and the best sensitivity response with a detection limit slightly higher than that obtained with the bare electrode. However, this is not due to a limited sensitivity of the sensor itself, but rather to an increase in the measurement error caused by the large capacitive current usually recorded on MWCNTs. The best analytical performances were achieved in the presence of MWCNTs. Linear dynamic range is, in fact, 0–25 μM with a sensitivity of (457.62±128.24) μA/(cm^2·mM) and a limit of detection of (1.29±0.18) μM. On the other hand, the SiC NP does not show the expected electrochemical improvements in the considered range, from 0.7 to 8 μM; the sensitivity was registered as limited to 133.74±14.60 μA/(cm^2·mM) with a detection limit of only 1.97±0.20. The latter results were worse than those obtained on the bare screen-printed surfaces, which

Table 1 Comparison of sensitivities and limit of detection in the case of the tested nano-materials

	Graphite	Graphite+ SiC NP	Graphite+ MWCNT
Measured range (μM)	0.5–8	0.7–8	6–25
Sensibility (μA/(cm^2·mM))	306±16	134±15	458±128
Limit of detection (μM)	0.94±0.04	1.97±0.20	1.29±0.18

showed a sensitivity of 305.66±15.57 μA/(cm^2·mM) and a limit of detection of 0.94±0.04 μM.

These results are in line with those obtained in the detection of other electrochemical active species where carbon nanotubes were found to be the best nano-material to enhance the sensor properties, as well as in the case of hydrogen peroxide [10] or in the etoposide electrochemical detection [11]. This research has shown that none of the nano-materials tested in this study could improve the performance of an electrochemical sensor enough to detect the physiological concentration of plasmatic insulin (maximum, 269 pM [8]) that usually occurs in healthy subjects.

4 Conclusions

This experimental research has been carried out to assess whether different nano-materials proposed in literature should be considered for the electrochemical detection of plasmatic insulin. The latter represents a crucial parameter in the monitoring of patients with metabolic disorders, such as diabetes mellitus or insulinoma, and it is of paramount importance to devise the proper pharmacological plan and ensure a completely personalized therapy (i.e., intensive insulin infusion administered to diabetic patients in critically ill conditions). Two types of nanostructures were considered: silicon carbide nanoparticles and multiwalled carbon nanotubes. In both cases, the performances measured on the obtained nano-based sensors were not enhanced enough to enable insulin electrochemical detection in the physiological range. Our findings demonstrate that, to the best of our knowledge, the detection of insulin in the physiological range is still beyond the capability of electrochemical sensors at the state-of-the-art technology, even considering the improvements provided by nano-materials that have succeeded in other clinical and medical diagnostic applications such as drug detection in human plasma [12]. The effect of other electroactive species existing in blood or other multicomponent human liquids that may generate interference on the detection of insulin (e.g., uric acid, glucose, lactates, L-cysteine, and cholesterol) is usually mitigated or eliminated by covering the surface of the sensing electrodes with polymer films (e.g., Nafion) [9]. The structuring of carbon nanotubes also fosters *per sé* the selective determination of electroactive species [13]. However, the aim of this paper is to demonstrate that the state-of-the-art electrochemical sensors for the detection of insulin in human samples are not yet sensitive enough in the concentration range they must target. Therefore, the problem of specificity will be addressed when an appropriate electrochemical sensor will be available for the accurate detection of insulin.

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