BLOOD INSULIN LEVEL BIOSENSOR FOR ATHLETES

BIOSSENSOR PARA NÍVEL DE INSULINA SANGUÍNEO EM ATLETAS

BIOSENSOR PARA EL NIVEL DE INSULINA EN SANGRE DE DEPORTISTAS

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ABSTRACT

Introduction: The pancreas releases insulin to assist the human body in utilizing blood glucose. It regulates metabolism by promoting the absorption of glucose into the blood. Objective: This work aimed to create an electrochemical biosensor based on magnetic graphene nanomaterial to measure insulin levels in athletes' blood. Method: A magnetic graphene nanocomposite created by graphene oxide (GO) and Fe-Ni bimetallic oxides on a glassy carbon electrode was synthesized using the electrochemical deposition method (GCE). Results: The immediate electrical deposition of Fe-Ni bimetallic oxide nanoparticles with the spherical shape on the GO nanosheet without aggregations was validated by structural characterizations of Fe-Ni/GO/GCE using XRD and SEM. The electrochemical results for insulin determination showed good sensitivity and anti-interference capability. The applicability and accuracy of the proposed electrochemical sensor to detect insulin were explored by blood serum samples from sportsmen. Conclusion: The results assigned acceptable RSD values (3.31% to 4.30%) and confirmed the feasibility of the proposed sensor for detecting athletes' blood insulin. ***Level of evidence II; Therapeutic studies - investigation of treatment outcomes.***

Keywords: Biosensing Techniques; Nanoparticles; Insulin; Doping in Sports.

RESUMO

Introdução: A insulina é liberada pelo pâncreas para auxiliar o corpo humano na utilização da glicose sanguínea. Ela regula o metabolismo, promovendo a absorção da glicose pelo sangue. Objetivo: O objetivo deste trabalho foi criar um biossensor eletroquímico baseado em nanomaterial de grafeno magnético para medir os níveis de insulina no sangue dos atletas. Método: Em um eletrodo de carbono vítreo, um nanocomposto magnético de grafeno criado por óxido de grafeno (GO) e óxidos bimetálicos de Fe-Ni foi sintetizado usando o método de deposição eletroquímica (GCE). Resultados: A deposição elétrica imediata de nanopartículas de óxido bimetal Fe-Ni com a forma esférica na nano folha GO sem agregações foi validada por caracterizações estruturais de Fe-Ni/GO/GCE utilizando XRD e SEM. Os resultados eletroquímicos para determinação da insulina demonstraram boa sensibilidade e capacidade anti-interferência. A aplicabilidade e precisão do sensor eletroquímico proposto para detectar insulina foram exploradas por amostras de soro sanguíneo dos esportistas. Conclusão: Os resultados designados para os valores aceitáveis de RSD (3,31% a 4,30%) confirmaram a viabilidade do sensor proposto para detecção de insulina sanguínea de atletas. ***Nível de evidência II; Estudos terapêuticos - investigação dos resultados do tratamento.***

Descritores: *Técnicas Biossensoriais; Nanopartículas; Insulina; Doping nos Esportes.*

RESUMEN

Introducción: El páncreas libera insulina para ayudar al cuerpo humano a utilizar la glucosa en sangre. Regula el metabolismo, favoreciendo la absorción de la glucosa por la sangre. Objetivo: El objetivo de este trabajo fue crear un biosensor electroquímico basado en un nanomaterial de grafeno magnético para medir los niveles de insulina en la sangre de los deportistas. Método: Sobre un electrodo de carbono vítreo, se sintetizó un nanocompuesto de grafeno magnético creado por óxido de grafeno (GO) y óxidos bimetálicos de Fe-Ni mediante el método de deposición electroquímica (GCE). Resultados: La deposición eléctrica inmediata de las nanopartículas de óxido bimetálico Fe-Ni con forma esférica sobre la nano plancha de GO sin agregaciones fue validada por las caracterizaciones estructurales de Fe-Ni/GO/GCE mediante XRD y SEM. Los resultados electroquímicos para la determinación de la insulina mostraron una buena sensibilidad y capacidad anti-interferencia. La aplicabilidad y la precisión del sensor electroquímico propuesto para detectar la insulina se exploraron con muestras de suero sanguíneo de deportistas. Conclusión: Los resultados asignados a valores aceptables de RSD (3,31% a 4,30%) confirmaron la viabilidad del sensor propuesto para detectar la insulina en sangre de los atletas. ***Nivel de evidencia II; Estudios terapéuticos - investigación de los resultados del tratamiento.***

Descriptores: *Técnicas Biosensibles; Nanopartículas; Insulina; Doping en los Deportes.*

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INTRODUCTION

Insulin is released by the pancreas that aids the human body inside the utilization of glucose in the blood. Insulin has a role in preventing blood sugar levels from rising too excessive.[1,2] Human insulin is made up of two protein molecules called A-chain and B-chain, which are joined together via two disulfide links and contain 21 and 30 amino acid residues, respectively[3]. Furthermore, diabetes is a chronic illness with life-threatening consequences that arises when the pancreatic does not make enough insulin or if the body's insulin is not used correctly, leading in elevated blood glucose levels. Uncontrolled diabetes causes hyperglycemia, or high blood sugar, which causes long-term damage to several of the body functions, including the neurons and blood vessels.[4,5]

Maintaining basal insulin sets during activity and athletics has also been a significant difficulty.[6] Insulin-independent glucose absorption by muscles remained high for two hours after exercise. Insulin sensitivity improves after exercise as well. Although steroids spawn muscle mass, insulin stimulates glycogen production, insulin suppresses catabolism both muscle and liver via increasing glycogen and protein synthesis and stimulating the entrance of glycogen and organic acids in muscle cells before such an event, resulting in improved stamina.[7] The ability of an athlete to store glycogen determines his or her stamina. Insulin is also widely utilized by bodybuilders as a performance enhancer due to its supposed anabolic qualities, such as inspiration of glycogen synthesis, which is critical for muscle repair after exercise.[8] As a result, the Olympic Committee prohibited insulin in 1998. Athletes with diabetes who require insulin can use it if they have a medical exemption.

As a result, not only is the amount of insulin in the human body crucial, but so are the amount and timing of insulin delivery. Spectrophotometric assesses,[9,10] mass spectrometry analyses,[11] and electrochemical methods have all been used in investigations to determine the insulin level in clinical samples.[12-15] Electrochemical biosensors, for example, are devices that can convert electrochemical and biochemical data, such as analyte, in analytical signals. The benefits of these sensors include high repeatability, a wide linear range, high sensitivity, and limit of detection. Furthermore, research has demonstrated that altering electrode surface may improve the surface-to-volume ratios and thus the efficacy of electrochemical biosensor. As a result, the focus of this research was on developing an electrochemical sensor application of magnetic graphene composites for detecting insulin in athlete human plasma.

METHOD

Experimental details

The magnetic GO nanocomposite was added to the GCE using the electrochemical deposition process.[16,17] 50 mg of GO nanosheets were distributed in 50 ml of 0.1M phosphate buffer solutions (PBS) to make the electrochemical electrolyte. The PBS is made from an equal mixture of Na_2HPO_4 and NaH_2PO_4. During the 40 min sonication phase, the scattered GO was exfoliated. To disperse suspension GO, 5 mM $FeCl_3$ and 2 mM $NiCl_2.6H_2O$ were added. The GCE was continuously polished in Al_2O_3 before being sonicated in a mix of distilled water and ethanol for 15 min before being modified. The electrochemical synthesis and analyses were carried out in three-electrode electrochemical cell with Ag/AgCl/3M KCl reference-electrode, platinum mesh as an auxiliary--electrode, and cleaned GCE as working-electrode. During the electro deposition, the prepared electrolyte was gently agitated.

Blood serum specimens from athletes were used as genuine samples to test the precision and obtained from the proposed biosensors for insulin measurement. Five participants, aged 20 to 24, submitted real human serum 60 min after glucose delivery. The blood samples were centrifuged at 2000 rpm for 20 minutes before being measured, and supernatants were mixed to 0.1M PBS in an equal volume ratio. After that, under ideal conditions, the developed sensor was used to determine the levels of insulin into the produced real samples. The insulin concentration in prepared actual samples was determined by amperometric analysis employing Fe–Ni@GO/GCE at 0.1V. Amperometry assays were made out in 0.1M PBS using an Autolab potentiostat/galvanostat. X-ray diffraction (XRD) and scanning electron microscopy (SEM) were used to examine the morphological and structural features of electrodeposited nanomaterials.

This work was conducted based on the Declaration of Helsinki principle. The participants signed the Free and Informed Consent Form (EHIC).

RESULTS AND DISCUSSION

The morphological properties of GO and Fe–Ni@GO/GCE are shown in Figure 1. Figure 1a depicts a highly rippled, wrinkled, and crumpled layer of ultra-thin GO nanostructure on the GCE surface with a porous structure. Figure 1b displays that spherical nanoparticles of Fe-Ni bimetal oxides deposited homogeneously on GO nanosheets without aggregations, signifying a great porosity and wide electroactive surfaces for analyte ions diffusion, which can speed up the redox process.[18]

Figure 2 shows XRD pattern of surface powder of produced GO and Fe–Ni@GO on GCE surfaces. In crystalline structure of GO, there is indeed a prominent peak at 10.68° that is connected to the (001) planes of GO, as shown in Figure 2.[19] The XRD pattern of Fe_2O_3@GO nanostructures show the (001) planes of GO and key diffraction patterns at 24.08°,33.22° ,35.73°,40.97°,49.63°,54.02°, and 62.51°, respectively, that are attributed to rhombohedral of Ferrite through planes of (012),(104),(110),(113),(024). Diffraction peak of Ferrite, NiO, and GO are seen in the XRD analysis of Fe–Ni@GO nanocomposite films in Figure 2, confirming the simultaneously electrochemical processes of GO nanostructures, NiO, and Ferrite nanoparticle on GCE surfaces.

The amperometric analysis and calibration curve of Fe–Ni@GO/GCE to serial additions of 100μM insulin in 0.1M PBS at 0.1V are depicted in Figure 3. It is demonstrated by the proposed electrode's extremely quick reactions to successive insulin administrations. Furthermore, linear range

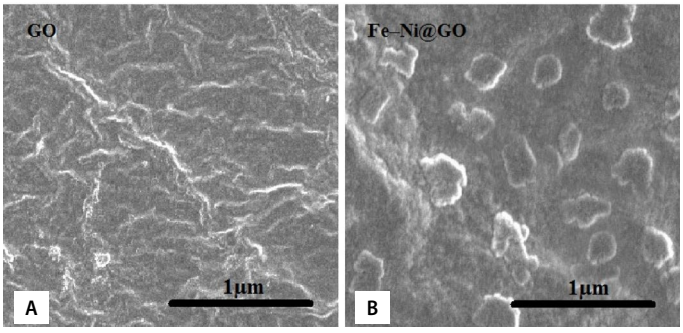

Figure 1. SEM images of GOand Fe–Ni@GO/GCE.

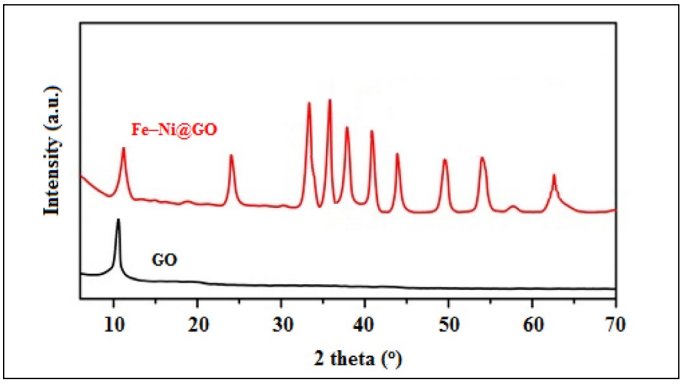

Figure 2. XRD patterns of prepared GO and Fe–Ni@GO.

obtained at 10-1500 µM. When compared to the other published insulin biosensor in Table 1, the acquired sensitivity is 0.3225µA/µM by a limit of detection of 0.005µM. As shown that Fe–Ni@GO/GCE has comparable limit of detection values and a wider linear range for determining insulin, which is linked to the high catalytic capability of nanomaterials and magnetic nanoparticles because of a synergistic effect among both Fe–Ni and GO bimetal oxides through magnetic nanocomposite.[20]

Fe–Ni@GO/practical GCE's potential for determining insulin in human serum samples is examined. The blood serum of 5 people between the ages of 20 and 24 who had an intropin injection. The amperometric analysis and achieved calibration curve of Fe–Ni@GO/GCE to serial additions of 5g/ml insulin solution are shown in Figure 4. Figure 4 and Table 1 show that the insulin level in the produced sample of the first Participant is 0.791ng/ml, which is similar to the insulin ELISA kit's results. These experiments were repeated on the remaining 5 athletes, with the average of 5 times insulin readings for each sample reported in Table 1. As can be seen, there was good agreement among amperometry and

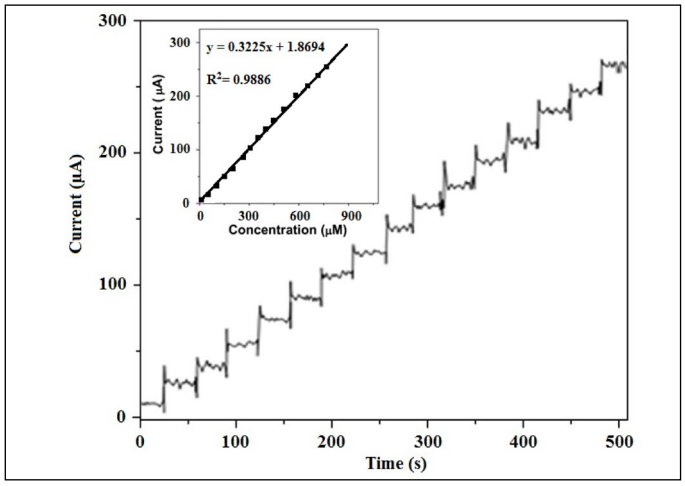

Figure 3. Amperometric analysis and calibration curve of Fe–Ni@GO/GCE to consecutive adding 100µM insulin into 0.1M PBS at 0.1V.

Table 1. Results of insulin content measurements by amperometry and ELISA methods in prepared plasma blood serum from 5 participants aged 20 to 24 years who received an intropin injection.

Participant No.	Insulin level in prepared blood samples(ng/ml)			
	Amperometry analysis		ELISA analysis	
	Fe-Ni@GO/GCE	RSD (%)	ELISA kit	RSD (%)
1	0.791	±3.31	0.788	±3.41
2	0.822	±4.30	0.826	±4.38
3	0.796	±4.22	0.812	±4.02
4	0.823	±4.19	0.811	±3.93
5	0.831	±3.33	0.822	±3.81

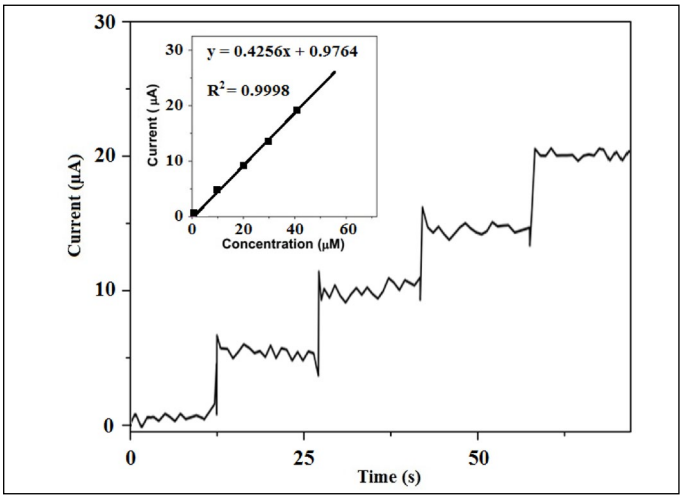

Figure 4. Amperometric analysis and calibration curve of Fe–Ni@GO/GCE to consecutive adding 10µg/ml insulin into prepared blood samples at 0.1V.

ELISA measurement systems, and the attained RSD ranges from 3.31% to 4.30%, indicating that both techniques, particularly Fe–Ni@GO/GCE as an accurate and reliable electrochemical insulin biosensor in medical and pharmaceutical analyses, have acceptable accuracy.

CONCLUSIONS

The construction of an electrochemical biosensor on Fe–Ni@GO composite as a magnetic graphene nanomaterial for the monitoring of insulin levels in athletes' blood samples was presented in this paper. On GCE, Fe-Ni@GO was prepared using the electro deposition method. The simultaneously electrodeposited Fe-Ni bimetal oxide particles in spherical--shaped onto 2D wrinkled stacks of ultra-thin GO nanostructure without even any aggregation was validated by morphological and structural characteristics of Fe–Ni@GO/GCE. Electrochemical analyses revealed good constancy and selectivity of Fe–Ni@GO/GCE to oxidation insulin with a linear ranges between 10-1500µM, detection limit of 0.005µM, and sensitivity of 0.3225µA/µM when compared to other observed insulin electrochemical sensors, with similar detection limit value and a wider linear range of Fe-Ni@GO/GCE to detection insulin. The study's findings on the functional capability of Fe-Ni@GO/GCE for determining insulin in able to prepare real samples of athletes' blood serum of 5 people aged 20 to 24 years who received insulin injection revealed that there was good accordance between ELISA and amperometry measurement systems, and the achieved acceptable accuracy of the both methods, particularly Fe-Ni@GO/GCE, indicating that Fe-Ni@GO/GCE as electrochemical insulin sensor is accurate and reliable in pharmaceutical analyses.

The author declare no potential conflict of interest related to this article

AUTHORS' CONTRIBUTIONS: The work is conceived and executed by Xu Deng. The author is fully responsible for execution and writing of this manuscript.

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