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A novel amperometric biosensor based on gold nanoparticles anchored on reduced graphene oxide for sensitive detection of L-lactate tumor biomarker

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ABSTRACT

In this work, a novel amperometric biosensor based on gold nanoparticles anchored on reduced graphene oxide (RGO-AuNPs) and L-lactate dehydrogenase (LDH) was developed for the sensing of L-lactate. Firstly, the RGO-AuNPs modified screen printed electrodes were tested for NADH detection showing a wide dynamic range and a low detection limit. Next, the biosensor was constructed by incorporating both enzyme and RGO-AuNPs in a sol gel matrix derived from tetrametoxysilane and methyltrimetoxysilane. The enzyme loading, working pH, and coenzyme concentration were optimized. The biosensor linearly responded to L-lactate in the range of 10 μM–5 mM and showed a good specific sensitivity of 154 μA/mM cm^2 with a detection limit of 0.13 μM. This was accompanied by good reproducibility and operational stability. Tests on artificial serum proved that L-lactate can be determined practically without interferences from commonly interfering compounds such as urate, paracetamol and L-ascorbate. Our LDH/RGO-AuNPs/SPCE based biosensor thus performs as electrochemical device for the detection of L-lactate as a viable early cancer bio-marker.

1. Introduction

Malignant transformation of normal cells into tumor cells very frequently leads to an increased accumulation of lactate concentration in most solid tumors (Hirschhaeuser et al., 2011), as it has been reported in clinical studies on prostate and breast cancer (Lupo et al., 2010; Tessem et al., 2008). This characteristic renders L-lactate a viable early cancer bio-marker. Pathophysiologic accumulation of L-lactate has been also associated with a high risk for the formation of metastases and thus with overall low survival chances of cancer patients. Tumor metabolism by releasing a high amount of lactate to the extracellular space largely contributes to the immunologic escape. Leukocytes may be asphyxiated by the presence of lactate. The differentiation of monocytes to dendritic cells is inhibited by lactate and cytokine release from dendritic cells (Gottfried et al., 2006) and cytotoxic T cells (Fischer et al., 2007) are inactivated. Thus is becomes clear that monitoring of the L-lactate concentration is important not only for the detection of cancer in its early stage but also during the antitumoral treatment.

The development of methods for L-lactate detection has gained considerable attention in clinical diagnostics, food analysis, biotechnology and sports medicine (Palleschi et al., 1990; Sartain et al., 2006). Moreover, many methods have been reported for lactate determination, such as chromatographic and spectrometric analysis (Bariskaner et al., 2003; Wulkan et al., 2001). However, these methods are time consuming, complex to perform, expensive and require laborious sample pretreatment. Thus, there is a considerable need for the development of inexpensive, rapid and reliable methods for lactate quantification. In this sense, electrochemical sensors, specially employing sensitive enzyme-based amperometric biosensors, offer a cost-effective solution to achieve fast response and high sensitivity (Li et al., 2012).

The enzymes normally used in the development of amperometric biosensors for L-lactate detection (Ibupoto et al., 2012) are lactate oxidase (LOD) (Hirano et al., 2002; Spohn et al., 1996) and

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L-lactate dehydrogenase (LDH) (Chaubey et al., 2000; Leonida et al., 2003). The most commonly used lactate biosensor reported in the literature is based on the specific recognition of L-lactate using LDH. The LDH enzyme catalyzes the oxidation of lactate in the presence of nicotinamide adenine dinucleotide (NAD^+). to pyruvate and reduced form of nicotinamide adenine dinucleotide (NADH), which can be detected amperometrically (Pereira et al., 2007). This enzyme has some important advantages for monitoring the L-lactate in real samples. Among others, it overcomes the problem of oxygen dependency, and offers a high selectivity for L-lactate compared to LOD. Enzymatic amperometric sensors based on L-lactate dehydrogenase (LDH) are presented as an attractive approach for detection of L-lactate in biological samples (Rassaei et al., 2013) due to their simple design and good performances. However, the LDH based biosensor has some drawbacks, including the low stability of the enzyme and the electrochemical oxidation of NADH that occurs at high over potentials (Santos et al., 2002).

The main problem of the direct detection of NADH in biological samples is the interference from electroactive species such as ascorbic acid, paracetamol, uric acid, which undergo redox reactions at relatively low applied potential and thus reduce the selectivity of the biosensor. Different approaches that include the use of electron mediators (Prieto-Simón and Fàbregas, 2004), carbon nanotubes (Musameh et al., 2002; Pereira et al., 2007; Saleh et al., 2011a), conducting polymers (Chaubey et al., 2000; Rahman et al., 2009; Saleh et al., 2011b), chitosan composites (Ge et al., 2009), polyelectrolytes (Rotariu et al., 2014) were reported to improve the sensitivity and selectivity of the LDH biosensors.

Due to their large surface, excellent electrical conductivity and chemical stability, graphene based materials were lately used for many electrochemical application that includes detection of NADH (Gasnier et al., 2013; Guo et al., 2011; Kuila et al., 2011). Multiple possibilities of graphene functionalization have considerably enhanced the field of biosensing applications.

In the present work, a novel amperometric biosensor based on gold nanoparticles anchored on reduced graphene oxide (RGO-AuNPs) and L-lactate dehydrogenase (LDH) was developed for the sensing of L-lactate. In the first step, the RGO-AuNPs modified screen printed electrodes were tested for NADH detection showing a large linear range and a low limit of detection. Then, the biosensor was developed by incorporating both enzyme and RGO-AuNPs in a sol gel matrix derived from tetrametoxysilane and methyltrimetoxysilane. A scheme describing the working principle of the LDH/RGO-AuNPs/SPCE biosensor can be found in Fig. 1. The common interfering electroactive compounds such as ascorbic acid, uric acid and paracetamol have been studied.

2. Materials and methods

2.1. Materials

Graphite powder, sulfuric acid (H_2SO_4), sodium nitride ($NaNO_3$), potassium permanganate ($KMnO_4$), sodium citrate, tetrachloroauric (III) acid ($HAuCl_4$), sodium borohydride ($NaBH_4$), nicotinamide adenine dinucleotide reduced disodium salt (NADH) and NAD+, potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6]$) were obtained from Sigma-Aldrich and used without further purification. Tetramethoxysilane (TMOS), methyltrimethoxysilane (MTMOS), polyethylene glycol (PEG600) were purchased from Fluka. All other chemicals used were of analytical grade. Phosphate buffer solution (PBS, 0.1 M, containing 0.1 M KCl, pH=7.5) was prepared by mixing solution of Na_2HPO_4, NaH_2PO_4 and KCl. All solutions were prepared in ultrapure water (Millipore, 18 MΩ cm).

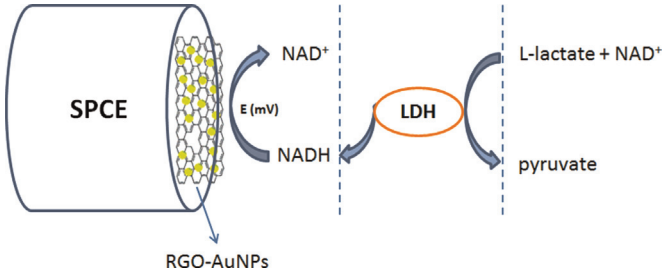

Fig. 1. Schematic representation of working principle of the LDH/RGO-AuNPs/SPCE biosensor.

2.2. Instrumentation

The metal loading in the graphene was determined by inductive Coupled Plasma Spectroscopy (ICPS), using a Jobin-Yvon 2000 Ultrace Analyzer. Size and distribution of Au nanoparticles were studied by transmission electron microscopy (TEM), using a JEOL-2000 FXII equipment working at 200 kV. X-ray photoelectron spectroscopy (XPS) was carried out on an ESCAPlus Omicron spectrometer using a monochromatized Mg X-ray source (1253.6 eV). Data were analyzed using Casa XPS software package.

Cyclic voltammetry (CV) and amperometric measurements were performed using a computer-controlled μ-Autolab type III and electrochemical impedance spectroscopy (EIS) experiments with an Autolab PGSTAT 101. Nova 1.10 software was used to gather data. The screen-printed carbon electrodes (SPCE, Dropsens DRP-110) consisted of a three-electrode system having a carbon working electrode (4 mm diameter), an Ag pseudo-reference electrode and a carbon counter electrode. All potentials are reported vs. Ag pseudo-reference electrode and all experiments were carried out at room temperature (22 °C), using a 5 mL cell.

2.3. Thermal exfoliation/reduction of graphite oxide

Graphite oxide was first prepared using a modified Hummer's method from graphite powder by oxidation with $NaNO_3$, H_2SO_4 and $KMnO_4$ in an ice bath as reported elsewhere (Hummers and Offeman, 1958; Vallés et al., 2012). In brief, 170 mL of concentrated H_2SO_4 was added to a mixture of graphite flakes (5.0 g) and $NaNO_3$ (3.75 g), and the mixture was cooled in an ice bath, and stirred for thirty minutes. $KMnO_4$ (25 g) was slowly added and stirred for another thirty minutes. The reaction was then warmed to 35 °C, and stirred for two more hours. Water (250 mL) was slowly added, and then 30% H_2O_2 (20 mL). The mixture was stirred for an hour, filtered, and the obtained powder was repeatedly washed with 400 mL of HCl:H_2O (1:10), and dried. Next, 300 mg of the synthesized graphite oxide was heated at 700 °C for 15 min under Ar atmosphere affording the graphene-like material (RGO).

2.4. Synthesis of hybrid material RGO-AuNPs

In a typical experiment, 5.8 μmol of sodium citrate were added to 20 mL $HAuCl_4$ aqueous solution (5.2×10^{-5} M). The mixture was stirred at room temperature until clear solution is observed. Then, 70 μL of freshly prepared cold (0 °C) $NaBH_4$ in water (0.1 M) were added dropwise. Next, 10 mg of thermally exfoliated graphene (RGO) were added, and the mixture stirred at room temperature until a complete loss of color was observed (60 min). Finally, the dispersion was vacuum filtered, washed with distilled water (300 mL), and dried overnight at 80 °C.

2.5. Modification of the screen-printed electrodes

We have dispersed 0.5 mg of RGO-AuNPs in 1 mL water to form 0.5 mg/mL RGO-AuNPs suspension under stirring and sonication