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A solid ionic Lactate biosensor using doped graphene-like membrane of Au-EVIMC-titania nanotubes-polyaniline

Hongying Cheng*, Cuiying Hu, Zhongling Ji, Wangcheng Ma, Hongxia Wang

School of chemical biology and materials engineering, Jiangsu Key Laboratory for Environment Functional Materials, Suzhou University of Science and Technology, Suzhou 215009, Jiangsu, PR China

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ABSTRACT

Herein, a doped graphene-like membrane was designed, which was copolymerized to be a solid ionic biosensor by using titania nanotubes (TiNTs), polyaniline (PANI), EvimCl (1-ethyl-3-vinylimidazolium chloride, EVIMC) and chloroauric acid ($HAuCl_4$). The structure of graphene-like arrangement and the copolymerization mechanism of the film were discussed in detail. Because of the high catalytic property, Lactate could be determined on the membrane catalyzed by lactate dehydrogenase (LDH) containing isocitrate dehydrogenase (NAD^+), which was immobilized onto the film by electrostatic attraction. The electrochemical response on the LDH/Au-EVIMC-TiNTs-PANI/ITO was increased by twice than the LDH/TiNTs/PANI/ITO, and exhibited two linear responses within the concentration range from 5.5×10^{-7} M to 5.55×10^{-6} M, and 5.55×10^{-6} M to 3.33×10^{-3} M, with a detection limit of 1.65×10^{-7} M ($S / N = 3$). The interference study for other common coexistence such as uric acid and hemoglobin revealed that there was no overlapping signal for the detection of Lactate on the biosensor. The developed method proved the most stability in the determination of real blood samples, and the recoveries ranged from 96.7% to 105.8% with a satisfactory result.

1. Introduction

Recently, the TiNTs with nanoscaled channel have attracted the considerable attraction, because of its high properties, including high surface area, good electronic conductivity, biocompatibility and high chemical stability (Sandoval et al., 2017; Sarswat et al., 2017; Agilan and Rajendran, 2018). Moreover, the TiNTs coupling with PANI has also been chemical oxidized offline, showing the soft skeleton, the crystalline structure (Huang et al., 2015) and the excellent electrocatalytic performance (Zhu et al., 2015). Up to now, the ionic liquids (IL) have the properties such as wide electrochemical window, good biocompatibility and high ionic conductivity. And they have been applied in modified electrodes or biosensors to bind carbon paste or carbon nanotubes (Yang et al., 2015). Apparently, the appropriate selection of IL in polymerization could also improve the rigidity and productivity of the copolymer (Wang et al., 2017) such as PANI.

The measurement of Lactate level was very important, because it could not only benefit for the differential medical diagnosis and therapeutic method, but also assure the quality in dairy products and beverages. Various methods have been reported for the determination of Lactate such as polarimetry (Feng et al., 2010), gas chromatography (GC) (Inoue et al., 2006), capillary electrophoresis (CE) (Alhusban et al., 2014), high performance liquid chromatography (HPLC) (Gómez-Mingot et al., 2012) and nuclear magnetic resonance (NMR) (Seli et al., 2008). While, most were expensive, needed complicated sample pretreatment and trained persons (Pundirn et al., 2016). At this time, biosensing methods attracted the researchers' interests, because they were simple, fast, specific and highly sensitive (Vargas et al., 2016; Naiara et al., 2016). The enzymes utilized to catalyze Lactate included lactate oxidase (LOx) (Hickey et al., 2016) and LDH (Zhang et al., 2016). Because of the diversitied forms, the LDH biosensor coupled with various supports were very widely used.

In this study, the appropriate size of TiNTs was synthesized by the hydrothermal method (Liu et al., 2014). Under the effect of polymerization initiation, the aniline, TiNTs grafted with $-NH_2$ (Wang et al., 2012), EVIMC and $HAuCl_4$ were electro copolymerized on the surface of indium tin oxide (ITO). Several morphology characterizations expressed the copolymerization mechanism, which showing a multi conjugation and a graphene-like arrangement. Under the synergistic and induction effects among the components, the reduced product of $HAuCl_4$ on the graphene-like grids showed the irregular nano scale. And the proposed biosensor presented outstanding electro conductivity, biocompatibility, the stability and the ideal reproducibility in the 400 times determination of Lactate.

* Corresponding author.
E-mail address: chenghy1015@126.com (H. Cheng).

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2. Experimental

2.1. Materials and reagents

Nano-TiO_2 powder (P-25) was purchased from Degussa Corporation (USA, 30–50 nm in diameter). EVIMC, $HAuCl_4$ and aniline were obtained from Fluka Chemical Co. (USA). (3-Aminopropyl) triethoxysilane (APTES, 99%) and 2, 2′- Azo - bisisobutyronitrile (AIBN) was obtained from Aladdin Chemical Co. Phosphate buffer solutions (PBS 0.2 mol L^{-1}) were prepared by varying the ratio of NaH_2PO_4 to Na_2HPO_4. ITO glass was purchased from Suzhou Nippon Sheet Glass Electronics Co. Ltd. (Suzhou, China). Ultra-pure water (18.2 MΩ/cm) used throughout the experiments was prepared ourselves.

2.2. Preparation of the Au-EVIMC-TiNTs-PANI/ITO

TiNTs was prepared self-synthesis by a previously reported hydrothermal method (Dai et al., 2010), and the TiNTs-NH_2 was fabricated in mixture solution at room temperature as reported (Cao et al., 2009). The detail information could be seen in the supplemental information.

Before modifying, pieces of ITO glass (1 cm × 5 cm) were cleaned and dried. Then 5uL 0.05 M AIBN was dropped onto the surface and dried in the atmosphere. About 0.0075 g EVIMC (0.01 M) and 10 mg TiNTs-NH_2 were added into 5 mL 0.01 M HCl. After being ultra-sonicated for 30 min, 50uL aniline was added and stirred for 30 min again. Successively, after adding 50uL $HAuCl_4$ in the above mixture in ice bath, the pretreated ITO was subjected to electrochemical polymerization by repeatedly cycling from 0.0 V to + 1.2 V for 15 cycles.

2.3. Fabrication and electrochemical characterization

The enzyme of LDH containing NAD^+ was dropped onto the film of Au-EVIMC-TiNTs-PANI and then was air dried and stored in vacuum at 4 °C designated as LDH/Au-EVIMC-TiNTs-PANI/ITO. For a comparative purpose, other modified electrodes were fabricated as LDH/TiNTs/PANI/ITO, LDH/TiNTs/ITO, and LDH/PANI/ITO.

The Cyclic voltammograms (CVs) and Electrochemical Impedance Spectroscopy (EIS) of the modified electrodes were recorded in 25 mL 0.1 M PBS(pH = 7) containing 5 mM $Fe(CN)_6^{3-/4-}$ and 0.1 M KCl. And the amperometric detection was conducted containing various concentrations of Lactate.

2.4. Pretreatment of blood samples and detection of Lactate

After collection of volunteers', the blood samples were pretreated by heparin to separate the plasma in the cell to avoid the interference from glycolysis. Before detection, 0.5 mL of pretreated plasma was diluted in 5.0 mL of PBS solution. Then the traditional colourimetric method (λ = 550 nm) and this proposed method were used to detect the Lactate to evaluate the practicability of the biosensor.

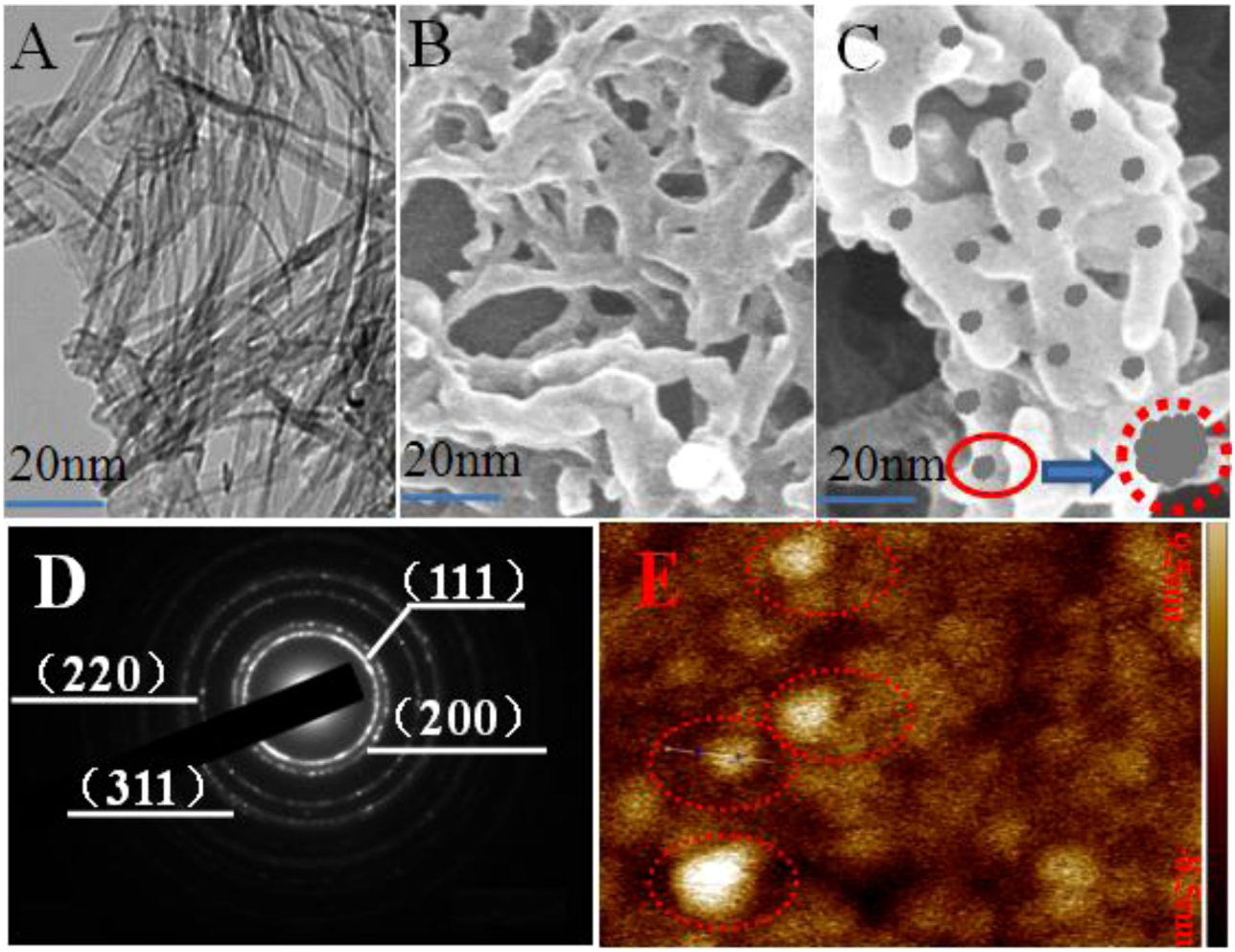

Fig. 1. (A-C) The TEM image of TiNTs, SEM of TiNTS-PANI and Au-EVIMC-TiNTS-PANI, respectively. (D-E) The corresponding SEAD and AFM pattern, respectively. Inset in (C) was the nano-Au SEM shape.