Biosensors and Bioelectronics 99 (2018) 571–577

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Hemocompatible ε-polylysine-heparin microparticles: A platform for detecting triglycerides in whole blood

Tingting Xu[a,b], Bo Chi[a], Meilin Chu[b], Qicheng Zhang[b], Shuyue Zhan[b], Rongjia Shi[b], Hong Xu[a,*], Chun Mao[b,*]

[a] State Key Laboratory of Materials-Oriented Chemical Engineering, College of Food Science and Light Industry, Jiangsu National Synergetic Innovation Center for Advanced Materials, Nanjing Tech University, Nanjing 211816, China
[b] National and Local Joint Engineering Research Center of Biomedical Functional Materials, Jiangsu Key Laboratory of Biofunctional Materials, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210023, China

ARTICLE INFO

Keywords:
Hemocompatible ε-polylysine-heparin microparticles
Electrostatic self-assembly
Biosensor
Lipase
Triglycerides

ABSTRACT

Triglycerides are clinically important marker for atherosclerosis, heart disease and hypertension. Here, a platform for detecting triglycerides in whole blood directly was developed based on hemocompatible ε-polylysine-heparin microparticles. The obtained products of ε-polylysine-heparin microparticles were characterized by fourier transform infrared (FT-IR) spectra, transmission electron microscopy (TEM) and ζ-potential. Moreover, the blood compatibility of ε-polylysine-heparin microparticles was characterized by in vitro coagulation tests, hemolysis assay and whole blood adhesion tests. Considering of uniform particle size, good dispersibility and moderate long-term anticoagulation capability of the microparticles, a Lipase-(ε-polylysine-heparin)-glassy carbon electrode (GCE) was constructed to detect triglycerides. The proposed biosensor had good electrocatalytic activity towards triglycerides, in which case the sensitivity was 0.40 μA mg^{-1} dL cm^{-2} and the detection limit was 4.67 mg dL^{-1} (S/N = 3). Meanwhile, the Lipase-(ε-polylysine-heparin)-GCE electrode had strong anti-interference ability as well as a long shelf-life. Moreover, for the detection of triglycerides in whole blood directly, the detection limit was as low as 5.18 mg dL^{-1}. The new constructed platform is suitable for detecting triglycerides in whole blood directly, which provides new analytical systems for clinical illness diagnosis.

1. Introduction

It is of great importance to analyze total triglycerides (TGs) level in the human body, which is a clinically important marker for atherosclerosis, heart disease and hypertension (Vijayalakshmi et al., 2008; Zhang et al., 2015). Various methods were used to detect TGs (Dhand et al., 2009; Solanke et al., 2009; Wu et al., 2014; Narang and Pundir, 2011; Minakshi and Pundir, 2008; Moldoveanu and Chang, 2011). Among them, electrochemical biosensors are rather available for analysis because of their simpleness, sensitivity, and specificity.

At present, many researches about the detection of blood components are usually conducted in serum separated by centrifuging. However, this method always needs complex centrifugal equipments and consumes more time to prepare samples, which may cause pollution. What's more, the detection results in serum cannot express the real situation in whole blood (Chen et al., 2013; Rivera et al., 2012). Therefore, it is of great significance to detect the blood analyte directly in the whole blood. However, when the traditional TGs biosensor is directly employed in the whole blood, the formed biofouling on the electrode surface will adversely impact the electron transfer from the enzyme to electrode redox center. Hence, it is vital to form an anti-biofouling surface of electrode, which can be obtained by using anti-biofouling biomaterials with micro/nanosize.

Heparin (Hep) is most well known for its anticoagulant activity, so it has been widely exploited in the clinic to prevent thromboembolic disease (Lu et al., 2013). ε-Polylysine (ε-PL), a polypeptide which is rich of positive charges, possesses advantages of bacteriostasis, security and high solubility (Zhang et al., 2013; Orive et al., 2006). Combining the advantages of both, new ε-PL-Hep microparticles were prepared via electrostatic self-assembly approach in this study. They were used to decorate the electrode based on both advantages of sustained Hep release and larger exposed surface areas (Li et al., 2009). Finally, a Lipase-(ε-PL-Hep)-GCE was constructed to detect the TGs in whole blood directly. The proposed biosensor had good electrocatalytic activity towards TGs.

* Corresponding authors.
E-mail addresses: xuh@njtech.edu.cn (H. Xu), maochun@njnu.edu.cn (C. Mao).

http://dx.doi.org/10.1016/j.bios.2017.08.030
Received 15 February 2017; Received in revised form 19 July 2017; Accepted 12 August 2017
Available online 15 August 2017
0956-5663/ © 2017 Elsevier B.V. All rights reserved.

2. Materials and methods

2.1. Materials

Lipase was purchased from Solarbio Co., Ltd. (China). Triglycerides (TGs) (98.5%), porcine heparin (Hep) (Mw 8000–12000), ascorbic acid (AA), cysteine (Cys), uric acid (UA), and β-D-(+)-Glucose (Glu) were obtained from Sigma-Aldrich Co. Ltd. (USA). ε-Polylysine (ε-PL) (Mw 3500–5000) was purchased from GL Biochem Ltd. (China). All solutions were prepared by twice-distilled water. All other chemicals (AR grade) were used as received.

2.2. Synthesis of ε-PL-Hep microparticles

The ε-PL-Hep microparticles were prepared via electrostatic self-assembly approach (Liu et al., 2015, 2014a). Briefly, 0.5 mg mL^{-1} ε-PL solution was prepared by 0.01 M phosphate buffered saline (PBS, pH 7.4). Then, a same volume of different concentrations of Hep solution (2.5 mg mL^{-1}, 5 mg mL^{-1}, 10 mg mL^{-1}) were added into ε-PL solution under ultrasonic condition for 5 min. Next, the mixtures were centrifuged at 15,000 r min^{-1} for 15 min. The precipitation was washed twice with PBS.

2.3. Characterization of ε-PL-Hep microparticles

Transmission electron microscopy (TEM) images of ε-PL-Hep microparticles were obtained to study the morphologies of the microparticles with an H-7650 interface high-resolution transmission electron microscope (HITACHI, Japan). Size and ζ-potential of ε-PL-Hep microparticles were measured by dynamic light scattering (DLS). The structures of ε-PL-Hep microparticles were determined by Fourier transform infrared (FT-IR) spectra using a Cary 5000 Fourier transform infrared spectropho-tometer (VARIAN, USA).

2.4. Fabrication of Lipase-(ε-PL-Hep) modified biosensors and TGs measurements

Prior to the modification, the glassy carbon electrode (GCE) was polished with 0.3 and 0.05 μm aluminum slurries and then rinsed thoroughly with double-distilled water (Zhou et al., 2015). The GCE was successively sonicated in ethanol and double-ionized water, respectively, and then dried at room temperature. Next, 8 μL of the prepared ε-PL-Hep microparticles solution was dropped onto the pretreated GCE surface and dried in air. To immobilize lipase, 8 μL of the mixture of 1 mg mL^{-1} dopamine and 2 mg mL^{-1} lipase was dropped onto the surface of the (ε-PL-Hep)-GCE and kept overnight. Due to the self-polymerization of dopamine, the ε-PL-Hep microparticles and the lipase could be fixed on the GCE tightly. After that, the GCE modified by Lipase-(ε-PL-Hep) was prepared. The prepared electrodes were stored in a refrigerator at 4 ℃ before use. In the presence of lipase, the TGs were hydrolyzed and transformed into glycerol and butyric acid which affected the solution pH value. The value was represented in proportion to the amount of TGs.

2.5. Blood compatibility analysis

2.5.1. In vitro coagulation tests

Whole blood was obtained from a healthy rabbit. Different concentrations of ε-PL-Hep microparticles were incubated in 1.5 mL platelet-poor plasma at 37 ℃ for 1 h , followed by investigating the coagulation times including activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) with a Rayto-2204C Semi automated coagulometer (USA) (Mao et al., 2009). All the tests were performed in triplicate.

2.5.2. In vitro Hep release studies of ε-PL-Hep microparticles

The release of Hep was performed by dialysis 10 mL of 0.1 mg mL^{-1} mixtures (Spectra/Por CE, MWCO=14000) in 30 mL PBS at 37 ℃. 10 mL dialysate was moved out at appropriate time intervals (1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 36 h, 48 h, 72 h and 96 h). The concentrations of Hep were detected by toluidine blue O (TBO) (Foo et al., 2007; Liu et al., 2014b, 2013).

2.5.3. Hemolysis assay

Healthy rabbit blood was collected in tubes and washed three times with 10 mL PBS. After 2% red blood cells suspension being prepared, different concentrations of ε-PL-Hep microparticles were incubated with it at 37 ℃. The red blood cells were also incubated with PBS and twice-distilled water as negative control and positive control, respectively (Liu et al., 2007). After 1 h, the mixtures were centrifuged for 10 min at 1500 r min^{-1}. The values at 545 nm were chosen to express the results. The formula was as follow.

$$\text{Percent hemolysis}(\%) = \left(\frac{\text{sample absorbance} - \text{negative control absorbance}}{\text{positive control absorbance} - \text{negtive control absorbance}}\right) \times 100$$

2.5.4. Whole blood adhesion tests

The surface sections of blank GCE and Lipase-ε-PL-Hep microparticles modified substrate were placed into the 24-well microplates and 1 mL of PBS was added into each well for 24 h equilibration. Next, 1 mL whole blood was added into each well. The samples were taken out after being incubated at 37 ℃ for 30 min. After that, they were rinsed three times with PBS, followed by being fixed with 2.5% glutaraldehyde of PBS for 30 min. Then samples were rinsed three times with PBS and dehydrated with ethanol/water solutions (50%, 60%, 70%, 80%, 90%, 95% and 100% of ethanol) for 30 min each and air dried (Hou et al., 2010). At last, the samples were observed under scanning electron microscope (SEM, JEOL JSM-6300, Japan).

2.6. Electrochemical characterization of the biosensors

Electrochemical workstation (Shanghai Chenhua Co. Ltd., China) was used to characterize the electrochemical performances of Lipase-(ε-PL-Hep)-GCE. All electrochemical experiments were carried out in a three-electrode cell. Cyclic voltammograms (CVs) were conducted by a three-electrode cell with the modified GCE as a working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as a reference electrode in 0.1 M PBS purged with N_2 bubbling for 5 min before electrochemical measurements at a scan rate of 100 mV s^{-1}. The electrochemical impedance spectroscopy (EIS) measurements were conducted in the frequency ranging from 1 Hz to 100 kHz with 5 mV AC amplitude. Differential pulse voltammetry (DPV) tests were conducted with pulse amplitude of 0.05 V and pulse width of 0.2 s. Various concentrations of TGs solution were added with intense stirring, then CVs was conducted until the currents kept steady, and DPV was instantly performed. So the relationship between response currents and various concentrations of TGs solution was obtained.

In order to evaluate the feasibility of the proposed biosensor for clinical application, the electrochemical performance of the novel electrode we proposed for detection of TGs in the whole blood was also investigated.

3. Results and discussion

3.1. Characterization of ε-PL-Hep microparticles

The formation of ε-PL-Hep microparticles was mediated via electrostatic self-assembly, and the concentration ratio of each compo-

Download English Version:

https://daneshyari.com/en/article/5031372

Download Persian Version:

https://daneshyari.com/article/5031372

Daneshyari.com