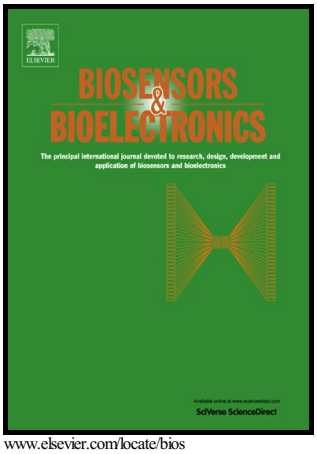


www.elsevier.com/locate/bios

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

Tingting Xu, Bo Chi, Meilin Chu, Qicheng Zhang, Shuyue Zhan, Rongjia Shi, Hong Xu, Chun Mao

PII: S0956-5663(17)30564-X
DOI: http://dx.doi.org/10.1016/j.bios.2017.08.030
Reference: BIOS9940

To appear in: *Biosensors and Bioelectronic*

Received date: 15 February 2017
Revised date: 19 July 2017
Accepted date: 12 August 2017

Cite this article as: Tingting Xu, Bo Chi, Meilin Chu, Qicheng Zhang, Shuyue Zhan, Rongjia Shi, Hong Xu and Chun Mao, Hemocompatible ε-polylysine-heparin microparticles: a platform for detecting triglycerides in whole blood, *Biosensors and Bioelectronic,* http://dx.doi.org/10.1016/j.bios.2017.08.030

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] and 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*

***** Corresponding Authors.

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

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 $\mu A \cdot mg^{-1} \cdot dL \cdot cm^{-2}$ and the

detection limit was 4.67 $mg \cdot dL^{-1}$ (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 $mg \cdot dL^{-1}$. The new constructed platform is suitable for detecting triglycerides in whole blood directly, which provides new analytical systems for clinical illness diagnosis.

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

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 [1,2].Various methods were used to detect TGs [3-7]. 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 [8,9]. 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 [10]. ε -Polylysine (ε -PL), a polypeptide which is rich of positive charges, possesses advantages of bacteriostasis, security and high solubility [11,12]. 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 [13]. 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.

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 [14,15]. Briefly, 0.5 $mg{\cdot}mL^{-1}$ 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 $mg{\cdot}mL^{-1}$, 5 $mg{\cdot}mL^{-1}$, 10 $mg{\cdot}mL^{-1}$) were added into ε -PL solution under ultrasonic condition for 5 min. Next, the mixtures were centrifuged at 15000 $r{\cdot}min^{-1}$ 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 [16]. 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 $mg \cdot mL^{-1}$ dopamine and 2 $mg \cdot mL^{-1}$ 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) [17]. 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 $mg \cdot mL^{-1}$ 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) [18-20].

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 [21]. After 1 h, the mixtures were centrifuged for 10 min at 1500 $r \cdot min^{-1}$. 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 [22]. 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 $mV \cdot s^{-1}$. 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 component was critical to the size and stability of the microparticles. In this study, TEM and DLS were used to measure the shape and size of the particles. Fig. 1A-1C showed TEM images of ε -PL-Hep microparticles, where can be observed that the microparticles were well dispersed with an average diameter of 180 nm and 200 nm while the concentrations of Hep were set at 2.5 $mg \cdot mL^{-1}$ and 5 $mg \cdot mL^{-1}$, respectively. However, the size of microparticles was out of control while the concentration of Hep increased to 10 $mg \cdot mL^{-1}$. The ζ-potentials of ε -PL-Hep microparticles were -37.3 mV, -37 mV and -26 mV, respectively, which meant that the ε -PL-Hep microparticles were moderately stable.

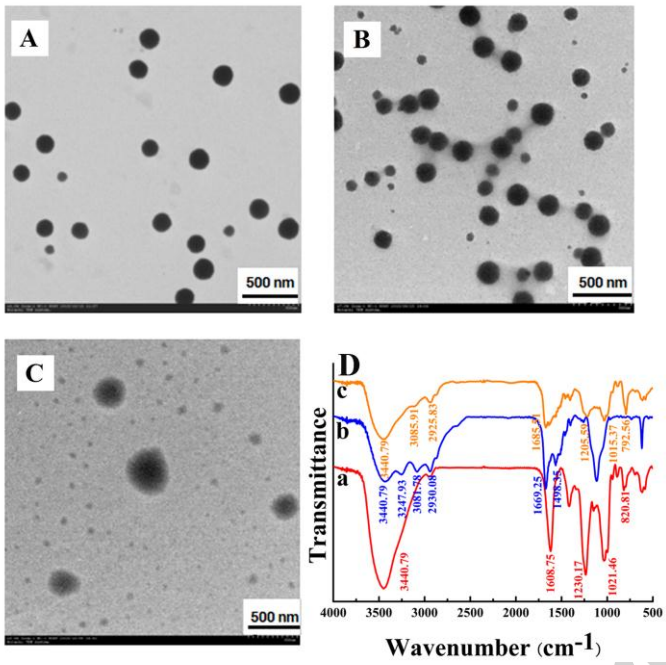


Fig. 1. TEM images of ε -PL-Hep microparticles with (A) 2.5 mg·mL^{-1} heparin, (B) 5 mg·mL^{-1} heparin, and (C) 10 mg·mL^{-1} heparin, respectively; (D) FT-IR spectra of (a) Heparin, (b) ε -PL, and (c) ε -PL-Hep microparticles.

FT-IR spectra of Hep, ε -PL and ε -PL-Hep microparticles were exhibited in Fig. 1D. A broad stretching band appeared around 3500 cm^{-1} due to the hydroxyl (–OH) groups (curve a). A sharp peak was observed at 1608.75 cm^{-1} due to the carboxylic groups of Hep. In addition, the appearance of the peaks at 1230.17 cm^{-1}, 1021.46 cm^{-1} and 820 cm^{-1} may be attributed to the characteristic adsorption of C–O–C and S=O groups respectively [23]. In curve b, broad peak in the range from 3600 to 3000 cm^{-1} was attributed to the overlap peaks of amine (–NH_2, –NH–) and hydroxyl (–OH) groups stretching vibrations. It could also be found that two shoulders near 1600 cm^{-1} corresponded to the strong Amide I (~1669 cm^{-1}) and Amide II (~1498 cm^{-1}) absorbance bands of ε -PL [24,25]. After the occurrence of electrostatic self-assembly between Hep and ε -PL, all the characteristic peaks of the two substances were retained, this showed that ε -PL-Hep microparticles were successfully formed.

3.2. Blood compatibility analysis

3.2.1. Characterization of in vitro anticoagulation properties of ε -PL-Hep

microparticles.

Blood plasma APTT, PT and TT were widely used to detect the abnormality of blood plasma [26,27]. The data were shown in table 1. It could be easily found that either the Hep or ε -PL-Hep microparticles kept excellent blood compatibility. Both APTT and TT of Hep were exceeded the measurement limit at 50 μg·mL^{-1}. However, ε -PL-Hep microparticles possessed relatively mild anticoagulation as the blood plasma samples of APTT, PT and TT were 28.5, 12.7 and 25.2 s after injection of 50 μg·mL^{-1} ε -PL-Hep microparticles, respectively. Considering of the shortcomings of excess Hep use [28-30], this development will bring a new conception to anticoagulant therapy.

Table 1 APTT/PT/TT of test samples

Sample	APTT(s)	PT(s)	TT(s)
Control	26.2±0.2	12.6±0.1	14.6±0.6
1 mg/mL Heparin	/	51.5±0.3	/
500 μg/mL Heparin	/	46.1±0.6	/
50 μg/mL Heparin	/	14.4±0.2	/
1 mg/mL ε -PL-Hep microparticles	/	13.1±0.8	/
500 μg/mL ε -PL-Hep microparticles	37.4±1.5	12.8±0.3	38.1±0.9
50 μg/mL ε -PL-Hep microparticles	28.5±0.8	12.7±0.7	25.2±1.2

/ means exceeding the measurement limit of the clot-detection instrument.

3.2.2. In vitro Hep release studies from ε -PL-Hep microparticles

The release studies *in vitro* were performed in PBS (Fig. 2A). During the first 36 h, Hep was released relative quickly, while the curve became flat during the following hours. After 96 h, the quantity of Hep released from the microparticles was close to 100%. The long-term sustained Hep release provided possibilities to the moderate

long-term anticoagulation capability of the microparticles.

3.2.3. Hemolysis assay

The hemolysis ratio is another important data reflects the bio-compatibility of materials. It increases with the breakage of the red blood cells' membrane and the release of the hemoglobin and other internal components into the surroundings. In our research, the hemolysis ratios of ε -PL-Hep microparticles were all less than 5% at different concentrations, which indicated that ε -PL-Hep microparticles could be regarded as blood compatible materials (Fig. 2B) [31,32].

3.2.4. Anti-biofouling property of the modified GCE surface

Anti-biofouling property of ε -PL-Hep microparticles was evaluated by whole blood adhesion tests. The SEM results indicated that a lot of blood cells and platelets were adhered on the blank electrode substrate while the surface of electrode substrate modified with Lipase-ε -PL-Hep microparticles was comparatively clean (Fig. 2C, 2D). According to this phenomenon, it was believed that few thrombus could form onto the surface of ε -PL-Hep microparticles, which proved that the anticoagulation of ε -PL-Hep microparticles can suppress blood-cell adhesion efficiently [33].

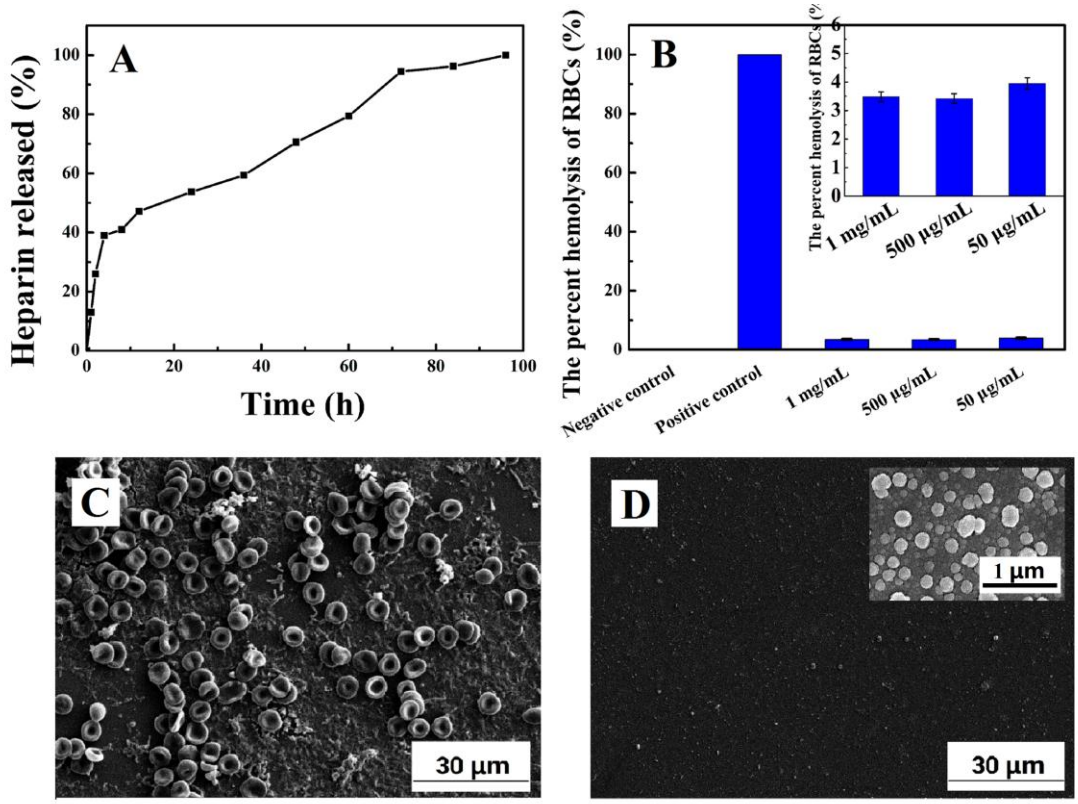
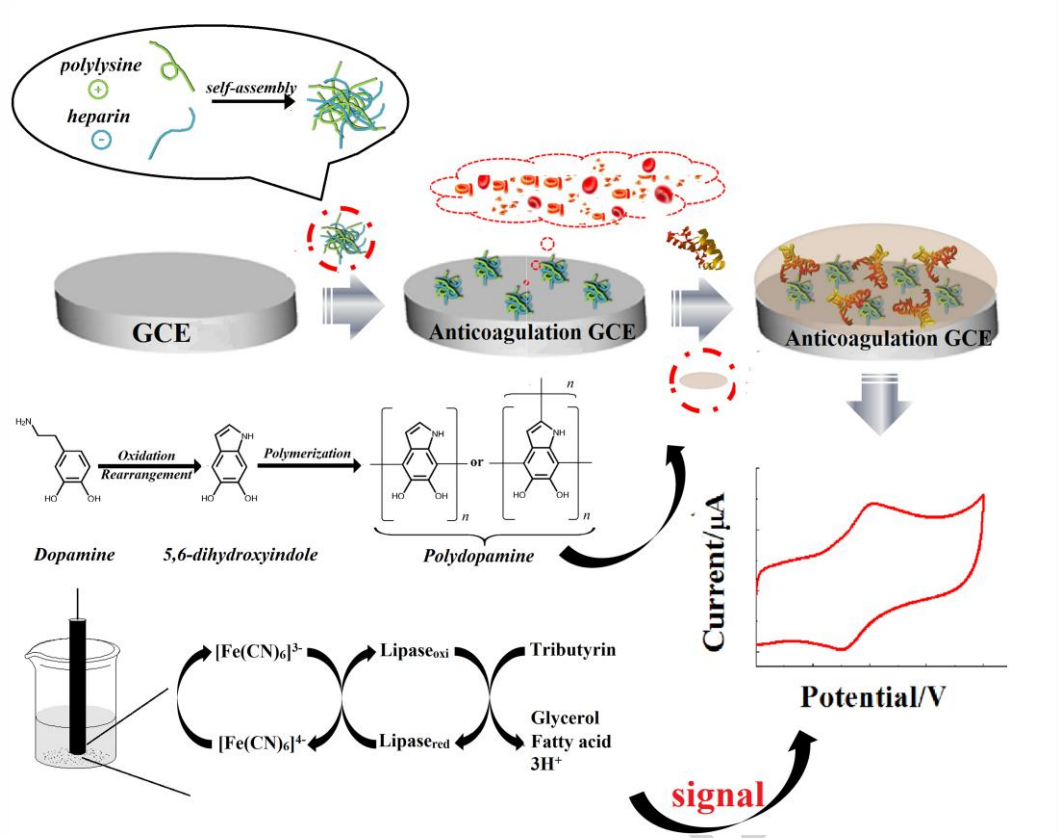


Fig. 2. (A) Heparin release from ε -PL-Hep microparticles; (B) Hemolysis test results of ε -PL-Hep microparticles; SEM images of (C) blank substrate, (D) electrode substrate modified with Lipase/ε -PL-Hep microparticles exposed to human whole blood for 30 min, respectively. Inset was the partial enlarged SEM images of the electrode substrate modified with Lipase/ε -PL-Hep microparticles.

3.3. Electrochemical characterization of the biosensors

3.3.1. Mechanism of TGs sensing



Scheme 1 Schematic illustration of construction of the tributyrin biosensor.

Scheme 1 showed proposed biochemical reaction at the lipase/ε -PL-Hep/GCE during tributyrin detection wherein lipase hydrolyses tributyrin resulting in the production of glycerol, fatty acid and protons. The pH value of the buffer medium decreased due to the generated protons, and the electrochemical signal of the biosensor changed. With the increase of the concentration of tributyrin, the response current of the biosensor increased, which could be attributed to pH sensitive behavior of the lipase/ε -PL-Hep/GCE[34]. The present employed biosensor for detection of TGs was fabricated with dopamine fixing ε -PL-Hep microparticles and lipase on the GCE. With the adhesion of dopamine, the ε -PL-Hep microparticles and lipase could be tightly stick to the surface of the GCE and were also regarded as a versatile platform for later detection. Because the catechol/quinone groups of polydopamine could react with the thiol groups or the terminal amino of lipase and ε -PL-Hep microparticles [35], polydopamine-assisted grafting of ε -PL-Hep microparticles and lipase was obtained by dropping of the mixture of lipase and dopamine onto the

surface of (ε -PL-Hep)-GCE.

3.3.2. Optimization of the TGs biosensor

The performance of the TGs biosensor could be affected by the following experimental parameters such as scan rate, dopamine, lipase and ε -PL-Hep microparticles concentrations. The fabricated biosensor had various response currents to different parameters. Therefore, a scan rate of 100 $mV \cdot s^{-1}$, a concentration dopamine of 1 $mg \cdot mL^{-1}$, a concentration lipase of 2 $mg \cdot mL^{-1}$, and a concentration ε -PL-Hep microparticles of 30 $mg \cdot mL^{-1}$ were thought of as the optimal conditions (Fig. S1).

3.3.3. Direct electrochemistry of Lipase–(ε -PL-Hep)–GCE

The CVs curve is one of the most effective tools to characterize the assembly procedure of biosensors [36]. Fig. 3A described the CVs of (ε -PL-Hep)-GCE, Lipase-Hep-GCE and Lipase-(ε -PL-Hep)-GCE measured in PBS. (ε -PL-Hep)-GCE (curve a) showed no redox peak, indicating that the ε -PL-Hep microparticles were not reacted in the range of potential. Meanwhile, a weak voltammetric signal of the Lipase-Hep-GCE (curve b) was exhibited in the potential ranging from -0.2 to 0.6 V. Nevertheless, due to the direct electron transfer of Lipase, a pair of approximately symmetrical redox peaks of Lipase-(ε -PL-Hep)-GCE (curve c) was present at the potentials of 51 and 262 mV, suggesting that the bioactivity of lipase was retained with the presence of ε -PL-Hep microparticles.

Since EIS can also give the information about the stepwise assembly of the biosensors, the results of modified biosensors were also monitored [37]. Different modification stages were shown in Fig. 3B via EIS. The impedance spectrum of the bare MGCE was demonstrated in curve a. Compared to the bare GCE (curve a), the R_{et} of the (ε -PL-Hep)-GCE was increased (curve b). Due to the presence of the insulating lipase, the R_{et} of the Lipase-(ε -PL-Hep)-GCE (curve c) was increased obviously. That means the successful fabrication of Lipase-(ε -PL-Hep) modified biosensors. Furthermore, the inset of Fig. 3B displayed the equivalent circuit of the

electrochemical system which involved charge-transfer resistance (Rct), Warburg impedance (Zw), solution resistance (Rs) and double layer capacitance (Cd). The charge-transfer resistances (Rct) of (a) bare GCE, (b) (ε -PL-Hep)-GCE and (c) Lipase-(ε -PL-Hep)-GCE were calculated to be 324.3 Ω, 452.6 Ω and 603.4 Ω, respectively, which was consistent with the result of the Nyquist diagrams.

3.3.4. Response behavior of the TGs biosensor

The relationship between amperometric response and concentration of the TGs was studied by DPV technique in 0.1 M PBS with various concentrations of TGs under the optimized conditions. The calibration curve which response currents were proportional to various TGs concentrations was illustrated in Fig. 3C, indicating that a linear relationship was obtained (the concentration ranging from 50 $mg \cdot dL^{-1}$ to 300 $mg \cdot dL^{-1}$).The linear regression equation was I (μA) = 0.0295 c ($mg \cdot dL^{-1}$) + 1.1607 (R^2 = 0.9952), in which I was the value of current and c was the concentration of TGs. It was estimated that the electrode effective area was approximately 0.073 cm^2 (Figure S2), in which case the sensitivity was 0.40 $\mu A \cdot mg^{-1} \cdot dL \cdot cm^{-2}$. The detection limit was 4.67 $mg \cdot dL^{-1}$(S/N = 3).

The analytical performance between some reported TGs biosensors and the fresh prepared biosensor made by ourselves were compared with each other. As was shown in Table S1, the proposed biosensor of which the sensitivity was higher and the detection limit was lower exhibited more excellent advantages compared to the previous reported models. Based on the good performance, the ε -PL-Hep microparticles could maintain enzymatic activity due to the provided good biocompatible microenvironment.

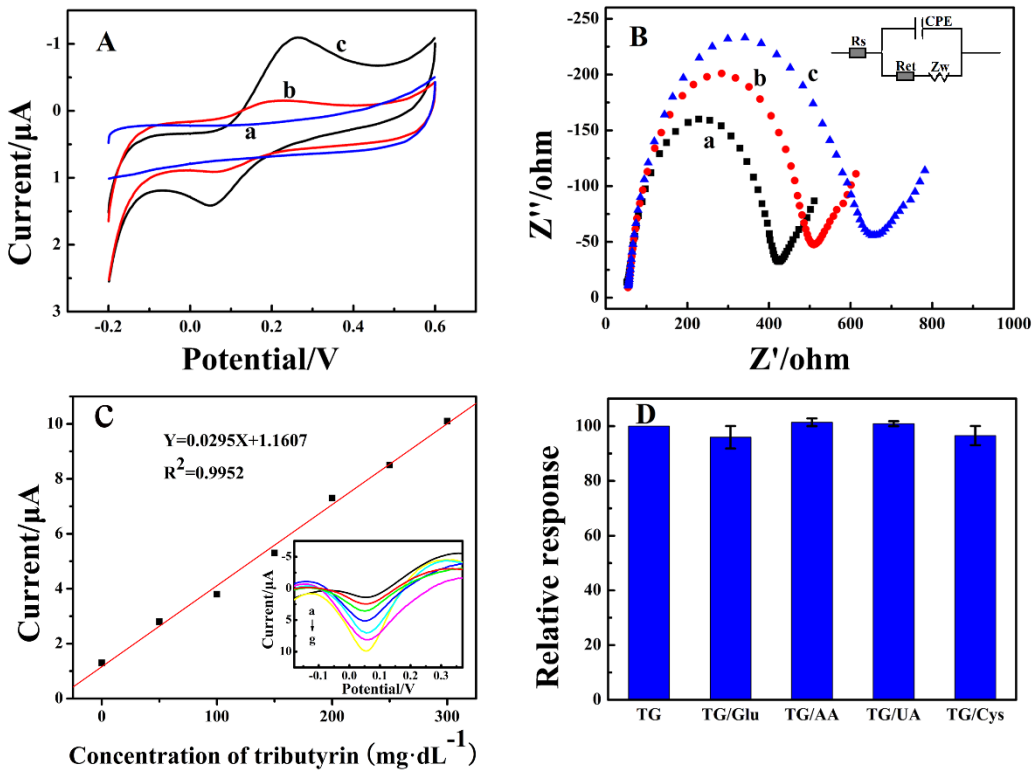


Fig. 3. (A) CV of (a) (ε -PL-Hep)-GCE, (b) Lipase-Hep-GCE, and (c) Lipase-(ε -PL-Hep)-GCE in PBS; (B) Electrochemical impedance characterization: (a) bare GCE, (b) (ε -PL-Hep)-GCE, (c) Lipase-(ε -PL-Hep)-GCE; inset was the equivalent circuit; (C) calibration curve for tributyrin obtained at the Lipase-(ε -PL-Hep)-GCE biosensor; and the inset: DPVs obtained at Lipase-(ε -PL-Hep)-GCE in PBS with various concentration of tributyrin (from a to g) 0, 50, 100, 150, 200, 250, 300 mg·dL^{-1}; (D) The specificity of ε -PL-Hep microparticles modified biosensor toward TGs, TGs+Glu, TGs+AA, TGs+UA, and TGs+Cys.

3.3.5. Anti-interference and long shelf-life of TGs biosensor

The anti-interference for TGs biosensor was verified by glucose (Glu), ascorbic acid (AA), uric acid (UA), and cysteine (Cys) for the sake of precisely detecting the TGs in human blood. 5 mM Glu, 0.2 mM AA, 0.2 mM UA, and 1 mM Cys were respectively mixed with TGs (200 mg·dL^{-1}), which were on the basis of normal concentration levels of these substances in blood. Meanwhile, the changes of detection current were 4.1%, 1.4%, 0.9% and 3.5%, respectively (Fig. 3D), suggesting

that the interferences merely have ignorable influences with regard to this method of detecting TGs [25].

The shelf life of the proposed biosensor has been studied for several weeks by measuring electrochemical current response at a regular interval of 1 week. The electrode retained 89% of its initial activity even after a month when stored in refrigerated conditions (4 ℃)，which proved that the enzyme electrode we fabricated was stable enough.

3.3.6. Amperometric determination in whole blood

In order to evaluate its applicability, the biosensor was used for determinating the concentrations of TGs in whole blood. DPV curves of the Lipase-(ε -PL-Hep)-GCE with the successive addition of TGs in whole blood were illustrated in Fig. S3. For the sake of fully mixing the added TGs, appropriate stirring was used for more than 5 min. With the continuous addition of TGs, the peak current of the DPV curves gradually increased. Meanwhile, an anodic peak was measured at 0.051 V. At the same time, the inset in Fig. S3 showed the calibration curve, illustrating the correlation between current response and TGs concentration, and a regression equation was I (μA) = 0.0331c (mg·dL^{-1}) + 0.2230 (R^2=0.9998). A detection limit of 5.18 mg·dL^{-1} (S/N = 3) was observed. The anticoagulant surface provided by ε -PL-Hep microparticles resulted in nice electrical conductivity in whole blood. What's more, different concentrations of TGs were added to the diluted blood and analyzed by our biosensors according to this calibration curve. Compared with the spiked samples, the values of TGs got by our biosensor were reliable. The relative errors were all lower than 6%, which indicated that the developed biosensor could be potentially used for the detections of clinical blood samples (Table 2).

Table 2 Results of the tributyrin assay and the recovery test for blood samples

No.	Tributyrin added (mg • dL^{-1})	Tributyrin found (mg • dL^{-1})	Relative errors (%)

0	0	84.5	\
1	5	89.8	6
2	25	110.7	4.8
3	50	131.8	5.4

4. Conclusion

In this case, uniform ε -PL-Hep microparticles were synthesized, which exhibited moderate long-term anticoagulation capability. Moreover, a Lipase-(ε -PL-Hep)-GCE electrode was constructed to detect the TGs which could be used in whole blood directly. The proposed biosensor had good electrocatalytic activity towards TGs, meanwhile, the detection limit was as low as 5.18 $mg \cdot dL^{-1}$ in whole blood. The data obtained from the biosensor showed good agreement with those from a biochemical analyzer in hospital. Based on this method, a new platform has been constructed for novel biosensors directly used in the whole blood, which provides new analytical systems for clinical illness diagnosis.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (No. 31401588, 51641104 and 51403103), Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, Huaian Key Research and Development Program (HAG201621), Jiangsu Key Technology RD Program (BE2016010), the Priority Academic Program Development of Jiangsu Higher Education Institution, and Major projects of Natural Sciences of University in Jiangsu Province of China (14KJA150006).

References

[1] A. Vijayalakshmi, Y. Tarunashree, B. Baruwati, S.V. Manorama, B.L. Narayana, R.E.C. Johnson, N.M. Rao, Enzyme field effect transistor (ENFET) for estimation of triglycerides using magnetic nanoparticles, Biosens. Bioelectron. 23 (2008)

1708-1714.

[2] Q.M. Zhang, D. Berg, S.M. Mugo, M.J. Serpe, Lipase-modified pH-responsive microgel-based optical device for triglyceride sensing, Chem. Commun. 51 (2015) 9726-1928.

[3] C. Dhand, P.R. Solanki, K.N. Sood, M. Datta, B.D. Malhotra, Polyaniline nanotubes for impedimetric triglyceride detection, Electrochem. Commu. 11 (2009) 1482–1486.

[4] C. Wu, X.Y. Liu, Y.F. Li, X.Y. Du, X. Wang, P. Xu, Lipase-nanoporous gold biocomposite modified electrode for reliable detection of triglycerides, Biosens. Bioelectron. 53 (2014) 26-30.

[5] J. Narang, C.S. Pundir, Construction of a triglyceride amperometric biosensor based on chitosan–ZnO nanocomposite film, Int. J. Biol. Macromol. 49 (2011) 707-715.

[6] Minakshi, C.S. Pundir, Construction of an amperometric enzymic sensor for triglyceride determination, Sens. Actuat. B 133 (2008) 251–255.

[7] S.C. Moldoveanu, Y.P. Chang, Dual analysis of triglycerides from certain common lipids and seed extracts, J. Agric. Food Chem. 59 (2011) 2137-2147.

[8] Y. Chen, Y. Yang, Y.F. Tu, An electrochemical impedimetric immunosensor for ultrasensitive determination of ketamine hydrochloride, Sens. Actuat. B 183 (2013) 150-156.

[9] L.C.R. Rivera, J.L.A. Sánchez, P.L. Sánchez, I. Katakis, C.K. O'Sullivan, Amperometric immunosensor for the determination of IgA deficiency in human serum samples, Biosens. Bioelectron. 33 (2012) 134-138.

[10] G. Lu, F.R. DeGuzman, S.J. Hollenbach, M.J. Karbarz, K. Abe, G. Lee, P. Luan, A. Hutchaleelaha, M. Inagaki, P.B. Conley, D.R. Phillips, U. Sinha. A specific antidote for reversal of anticoagulation by direct and indirect inhibitors of coagulation factor Xa, Nat. Med. 19 (2013) 446-451.

[11] D.D. Zhang, Y.M. Zhang, L. Zheng, Y.Z. Zhan, L.C. He, Graphene oxide/poly-L-lysine assembled layer for adhesion and electrochemical impedance detection of leukemia K562 cancer cells, Biosens. Bioelectron. 42 (2013) 112-118.

[12] G. Orive, S.K. Tam, J.L. Pedraz, J.P. Halle, Biocompatibility of alginate-poly-L-lysine microcapsules for cell therapy, Biomaterials 27 (2006) 3691-3700.

[13] Y. Li, L. Tang, J. Li, Preparation and electrochemical performance for methanol oxidation of pt/graphene nanocomposites, Electrochem. Commun. 11 (2009) 846-849.

[14] Y. Liu, J. Zhang, J. Wang, Y. Wang, Z. Zeng, T. Liu, J.Y. Chen, N. Huang, Tailoring of the dopamine coated surface with VEGF loaded heparin/poly-L-lysine particles for anticoagulation and accelerate in situ endothelialization, J. Biomed. Mater. Res. A 103 (2015) 2024-2034.

[15] T. Liu, Y. Liu, Y. Chen, S. Liu, M.F. Maitz, X. Wang, K. Zhang, J. Wang, Y. Wang, J.Y. Chen, N. Huang, Immobilization of heparin/poly-(L)-lysine nanoparticles on dopamine-coated surface to create a heparin density gradient for selective direction of platelet and vascular cells behavior, Acta. Biomater. 10 (2014) 1940-1954.

[16] C.Y. Zhou, D.L. Liu, L. Xu, Q.L. Li, J. Song, S. Xu, R.Q. Xing, H.W. Song, A sensitive label–free amperometric immunosensor for alpha-fetoprotein based on gold nanorods with different aspect ratio, Sci. Rep. 5 (2015) 9939-9946.

[17] C. Mao, L.C. Jiang, W.P. Luo, H.K. Liu, J.C. Bao, X.H. Huang, J. Shen, Novel blood-compatible polyurethane ionomer nanoparticles, Macromolecules 42 (2009) 9366-9368.

[18] H.L. Foo, A. Taniguchi, H. Yu, T. Okano, S.H. Teoh, Catalytic surface modification of roll-milled poly(ε-caprolactone) biaxially stretched to ultra-thin dimension, Mater. Sci. Eng. C 27 (2007) 299-303.

[19] T. Liu, Z. Zeng, Y. Liu, J. Wang, M.F. Maitz, Y. Wang, S.H. Liu, J.Y. Chen, N. Huang, Surface modification with dopamine and heparin/poly-L-lysine nanoparticles provides a favorable release behavior for the healing of vascular stent lesions, ACS Appl. Mater. Inter. 6 (2014) 8729-8743.

[20] X. Liu, Z. Yue, T. Romeo, J. Weber, T. Scheuermann, S. Moulton, G. Wallace, Biofunctionalized anti-corrosive silane coatings for magnesium alloys, Acta. Biomater. 9 (2013) 8671-8677.

[21] M. Liu, X.L. Yue, Z.F. Dai, L. Xing, F. Ma, N.Q. Ren, Stabilized

hemocompatible coating of nitinol devices based on photo-cross-linked alginate/heparin multilayer, Langmuir 23 (2007) 9379-9385.

[22] X.M. Hou, X.B. Wang, Q.S. Zhu, J.C. Bao, C. Mao, L.C. Jiang, J. Shen, Preparation of polypropylene superhydrophobic surface and its blood compatibility, Colloids Surf. B: Biointerf. 80 (2010) 247-250.

[23] C. Chauvierre, M.C. Marden, C. Vauthier, D. Labarre, P. Couvreur, L. Leclerc, Heparin coated poly(alkylcyanoacrylate) nanoparticles coupled to hemoglobin: a new oxygen carrier, Biomaterials 25 (2004) 3081-3086.

[24] G. Yang, B.L. Zhang, J. Wang, S.B. Xie, X. Li, Preparation of polylysine-modified superparamagnetic iron oxide nanoparticles, J. Magn. Magn. Mater. 374 (2015) 205-208.

[25] S.K. Tam, J. Dusseault, S. Polizu, M. Menard, J.P. Halle, L. Yahia, Physicochemical model of alginate-poly-L-lysine microcapsules defined at the micrometric/nanometric scale using ATR-FTIR, XPS, and ToF-SIMS, Biomaterials 26 (2005) 6950-6961.

[26] A. Tripodi, V. Chantarangkul, P.M. Mannucci, Acquired coagulation disorders: revisited using global coagulation/anticoagulation testing, Brit. J. Haematol. 147 (2009) 77-82.

[27] M. Levi, C.H. Toh, J. Thachil, H.G. Watson, Guidelines for the diagnosis and management of disseminated intravascular coagulation, Brit. J. Haematol. 145 (2009) 24-33.

[28] R. Karinen, E.L. Øiestad, W. Andresen, G. Wethe, A. Smith-Kielland, A. Christophersen, Comparison of ethanol and other drugs of abuse concentrations in whole blood stored in venoject® glass and plastic and venosafe™ plastic evacuated tubes, J. Anal. Toxicol. 34 (2010) 420-428.

[29] R. Lever, W.T. Lo, M. Faraidoun, V. Amin, R.A. Brown, J. Gallagher, C. Page, Size-fractionated heparins have differential effects on human neutrophil function *in vitro*, Br. J. Pharmacol. 151 (2007) 837-843.

[30] T. Arndt, Carbohydrate-deficient transferrin as a marker of chronic alcohol abuse: a critical review of preanalysis, analysis, and interpretation, Clin. Chem. 47 (2001)

13-27.

[31] D. Fischera, Y.X. Li, B. Ahlemeyerc, J. Krieglsteinc, T Kissel. *In vitro* cytotoxicity testing of polycations: influence of polymer structure on cell viability andhemolysis, Biomaterials 24 (2003) 1121-1131.

[32] H.T. Chen, M.F. Neerman, A.R. Parrish, E.E. Simanek, Cytotoxicity, hemolysis, and acute *in vivo* toxicity of dendrimers based on melamine, candidate vehicles for drug delivery, J. Am. Chem. Soc. 126 (2004) 10044-10048.

[33] B.S. Smith, S. Yoriya, L. Grissom, C.A. Grimes, K.C. Popat, Hemocompatibility of titania nanotube arrays, J. Biomed. Mater. A 2 (2010) 350-360.

[34] W. Zhang, Y. Tang, J. Liu, Y. Ma, L. Jiang, W. Huang, et al. An electrochemical sensor for detecting triglyceride based on biomimetic polydopamine and gold nanocomposite, J. Mater. Chem. B 2 (2014) 8490-8495.

[35] H. Lee, S.M. Dellatore, W.M. Miller, P.B. Messersmith, Mussel-inspired surface chemistry for multifunctional coatings, Science 318 (2007) 426-430.

[36] Y. Wang, Y.Y. Shao, D.W. Matson, J.H. Li, Y.H. Lin, Nitrogen-doped graphene and its application in electrochemical biosensing, ACS Nano 4 (2010) 1790-1798.

[37] C. Sun, X.H. Chen, Q.R. Han, M. Zhou, C. Mao, Q.S. Zhu, J. Shen, Fabrication of glucose biosensor for whole blood based on Au/hyperbranched polyester nanoparticles multilayers by antibiofouling and self-assembly technique, Anal. Chim. Acta 776 (2013) 17-23.

[38] P.R. Solanke, C. Dhand, A. kaushik, A.A. Ansari, K.N. Sood, B.D. Malhotra, Nanostructured cerium oxide film for triglyceride sensor, Sens. Actuat. B 141 (2009) 551-556.

Highlights

- By a simple electrostatic self-assembly method, ε-polylysine-heparin microparticles which possess uniform particle size, good dispersibility and moderate long-term anticoagulation capability have been compounded.
- The ε-polylysine-heparin microparticles were used to fabricate a Lipase-(ε-polylysine-heparin)-GCE electrode and proposed

Lipase-(ε-polylysine-heparin)-GCE electrode had strong anti-interference ability as well as a long shelf-life.

- Combining the moderate long-term anticoagulation capability and the microsized effect, the proposed biosensor had good antithrombosis ability and could be directly used in whole blood, which provides new analytical systems for clinical illness diagnosis.