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Liquid crystal biosensor for detecting ischemia modified albumin

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Abstract Ischemia modified albumin detection has great significance in early diagnosis and treatment of myocardial ischemia. In this study, anti-IMA was immobilized on the surface of a glass slide using self-assembled DMOAP and PEI, the binding of antibody and antigen lead to topology-geomorphologic changes on the slide surface, which induced 5CB molecules orientation transformation and modulated light transmittance. Results showed that we can detect concentrations as low as 50 μg/mL IMA. Moreover, this method can be realized with label-free, easy operation without dependence on equipment, and can be observed by the naked eye.

Introduction

Acute coronary syndrome (ACS) is a common disease in the cardiovascular system with extremely high mortality and morbidity, so early diagnosis of ACS is important to improve patients' prognosis and quality of life [1]. Currently, the laboratory examination, ECG, and clinical manifestations are the popular methods in ACS early diagnosis, and are still unsatisfactory [2]. Although necrotic biochemical markers, such as creatine kinase isoenzyme MB (CK-MB), myoglobin (Myo), and cardiac troponin (cTn), are regarded as a useful tool for clinical treatment [3], these makers are only released from myocardial cells after irreversible lesions. In addition, cTn can be detectable in patients about 6 h after heart disease occurs. When they have ischemia symptoms, the level of cTn is under clinical decision limits such that patients have to

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stay in hospital for repeated tests [4]. The incidence of cardiovascular events may be further reduced if we can diagnosis myocardial ischemia and plan therapeutic interventions earlier [5]. Therefore, seeking for a reliable biochemical marker of myocardial ischemia appears particularly important before cTn is generated.

At present, researchers have aroused interest for a new myocardial ischemia protein, ischemia modified albumin (IMA), which increases rapidly in 5–10 min after myocardial ischemia happens, and increases continuously during ischemia [6]. IMA provided an effective basis for detection of the early myocardial ischemia, and FDA approved IMA as a new clinical diagnosis of myocardial ischemia in 2003 [7]. The albumin cobalt binding test (ACB test) is one of the classical techniques in the IMA assay, and Bar-Or firstly established the manual spectrophotometric method [8]. However, there are still shortcomings in this method. For instance, IMA cannot be detected directly, as it binds with the cobalt ion through measuring the capacity of blood HSA. More false-positive results by the ACB method can cause interference with the test; there was no way to detect IMA when the blood albumin concentration was lower than 34 g/L. DTT, the staining agent of ACB, was not stable with a low melting point and easily volatilized in the air, which threatened human health [9]. Another classical method, ELISA, also had disadvantages, for example, the testing time to determine IMA was longer than 1 h, and the operation process and procedure is complex in general [10].

A liquid crystal (LC) biosensor, which is also called an electronic eye, has been regarded as a novel sensor technique [11]. LC molecules demonstrated the orientation order on special sensitive membrane surfaces, when specific biological molecules were bonded on membranes; it disturbed the orderly orientation of LC molecules, and caused changes in image color and brightness of the sensor as seen through a polarized microscopic view. In 1998, Abbott began to apply LC molecules as a sensing element for biochemical substance analysis and initially developed the field of the LC biosensor [12]. After further research, he revealed the self-assembly behavior of phosphor lipid molecules between the liquid phase and water phase, inducing the liquid crystal molecules in a particular orientation. A principle based on characteristics of the binding reaction between biological molecules that affected LC alignment for changing the polarization image was proposed. Such liquid crystal biosensors can be used for detecting cells, proteins, polypeptides, IgGs, and organic phosphorus [13–15]. Moreover, the LC sensor has a wide range of advantages such as simple operation, rapid response, high sensitivity, and low cost, which makes it stand out among other sensors. For these reasons, LC applications have ranged from the initial detection of organic gases and small molecules to biological macromolecules [16, 17]. Ho and Chen [16] introduced a LC-based biosensor for human serum albumin detection. IMA was a similar protein, therefore, we came up with the idea. Moreover, studies on myocardial ischemia with LC biosensors are not available so far.

In this study, a simple IMA determinant method based on the LC biosensor has been reported. The binding between IMA and anti-IMA changed topological topography on the surface induced LC from an initial vertical homogeneous alignment orientation into disorder, and modulated light transmittance. Compared with other methods, the LC biosensor can be in the ascendancy, because of its simple operation, excellent selectivity, free labeling, and low cost.

Materials and methods

Reagents and materials

IMA, N,N-dimethyl-N-octadecyl (3-aminopropyl) trimethoxysilyl chloride (DMOAP), and polyetherimide (PEI) were purchased from Sigma-Aldrich company (St. Louis, USA). LC 4-cyano-4-pentylbiphenyl (5CB) was obtained from Shijiazhuang Huarui Technology Co. Ltd (Hebei, China). Anti-IMA was provided by Shanghai Gaochuang Chemical Technology Co., Ltd (Shanghai, China). Glass slides were purchased from the Fan Pai company (Haimen, China). Copper grids (200 mesh, 20 μm thick) were obtained from Beijing tech Mirror Company Limited (Beijing, China). Other reagents were of analytical grade. Anti-IMA was dissolved in KOAc buffer (10 mmol/L, PH = 5.6), and IMA in 1 × PBS buffer (containing 0.01 % Tween-20). All the solutions were treated with ultrasonic degassing before proceeding any further.

Substrate preparation

Glass slides were cut into 2 cm × 2 cm size, then cleaned with piranha solution (containing 70 % H_2SO_4 and 30 % H_2O_2, v%) at 80 °C for 1 h. After that, the slides were rinsed with copious amount of deionized water and ethanol, and dried with nitrogen. The treated glass slides were then immersed in aqueous solution containing 1 % DMOAP, left to stand for 30 min under room temperature, cleaned with water, and dried with nitrogen, with oven drying at 110 °C for 1 h. Sequentially, the slides were exposed to an ultraviolet (UV) light for 2 min and air-dried to reserve. The DMOAP-treated assembly glass slides were immersed in 0.005 % PEI solution for 1 h, followed by large amounts of deionized water and dried under nitrogen gas. The slides were then kept in a dry and ventilated place before use.

Immobilization of anti-IMA

Firstly, anti-IMA 1 mg/ml was dissolved in 1 × PBS buffer (pH = 7.4) and prepared in different concentrations, then dipped in a small amount of anti-IMA on to glass slides, reacting at room temperature for 1 h. Then, the slides were washed with 1 × PBS buffer solution (pH = 7.4, containing 0.01 % Tween-20) and rinsed with deionized water, and finally dried with nitrogen.

Detection of IMA

Different concentrations of IMA were dissolved in 1 × PBS buffer solution with 0.01 % Tween-20 (pH = 7.4) and dipped on the middle of glass slides, then rinsed with deionized water, and dried with nitrogen. The LC cell was made up of two glass slides, a piece of 20 μm thick copper grid was placed in the center of the surface, and 5CB molecules were added on the grid with a pipette. The upper slide only assembled with DMOAP was covered on the other slides, with four clips that

fixed four sides. The LC cell was heated in constant thermostats (38 °C about 5 min till the LC was finally filled in its entire cavity and showed isotropic arrangement (40 °C). The LC cell slowly cooled down to room temperature (28 °C), then LC molecules went from the isotropic state into the nematic state, and induced LC vertically on the self-assembly film.

Results and discussion

Detection principle

The design diagram is provided in Fig. 1. A liquid crystal cell was made up of two glass slides. DMOAP was fixed on the upper slide, and mixed self-assembled DMOAP/PEI was immobilized on the bottom slide, which was prepared for binding anti-IMA. When a LC cell was inserted with 5CB molecules and placed in a polarized microscope, LC molecules were uniformly placed with a vertical orientation that light could not filter through the cell, and the optical signal displayed as a uniform black image. After combining with IMA, the immobilized molecules were enlarged, and this resulted in undulating topography on the surface. Since the orientation of the LC was sensitive to surface topography, which originated from characteristics of birefringence, the LC molecules would be effectively inducing LC molecules from a uniform vertical position to a horizontal or inclined orientation. The incident light transmitted through the change of orientation area, resulting in several bright spots appearing on the black background.

Influence of DMOAP/PEI

The aim of self-assembled DMOAP/PEI was to induce LC molecules perpendicular to uniform orientation on the treated glass slides and to provide a sufficient number of amino for anti-IMA settling. So it was well worth noting the effects of the DMOAP/PEI mixture ratio on the LC orientation. As shown in Fig. 2, when the PEI
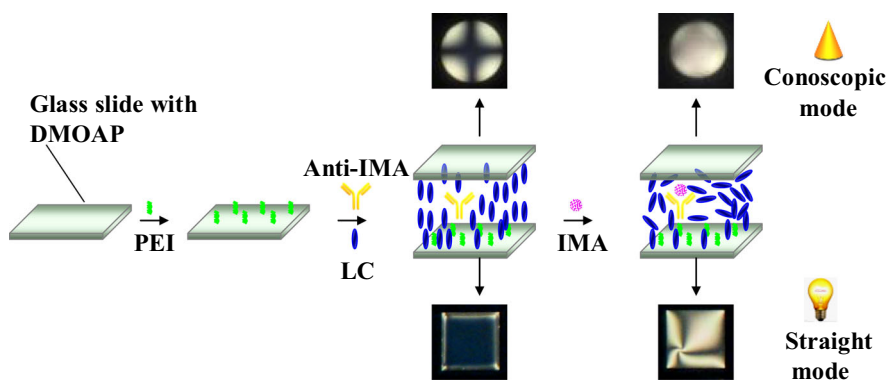
Fig. 1 The principle of LC-based biosensor for IMA detection
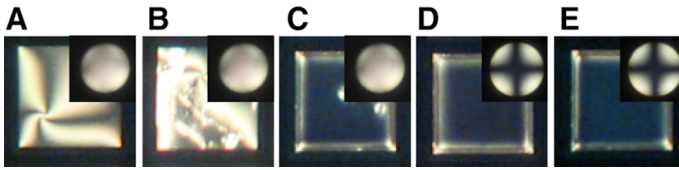
Fig. 2 Optical appearance under a cross polarizer of LC cells with 5CB in different volume ratios of DMOAP/PEI volume ratio **a** 1:2, **b** 1:1, **c** 2:1, **d** 3:1, **e** 5:1

proportion was higher, the number of DMOAP was limited so that it could not effectively induce LC molecules into vertical orientation, and an obvious brief regent texture was seen as well. As the DMOAP volume ratio increased, most of the LC molecules had a vertical orientation, and bright spots gradually became smaller, while the majority of the image area was black. When the volume ratio further increased to 3:1, the bright spots completely disappeared and the response signal showed a uniform dark background. Meanwhile, a cross diffraction could be observed under conoscopic mode, indicating that LC molecules were in homogeneous vertical orientation. The higher proportion of DMOAP/PEI (>3:1), and the less amount of PEI fixed on the slides was unfavourable for the activation of aminos. Therefore, the volume ratio of DMOAP/PEI was optimally selected to be 3:1.

Immobilization of anti-IMA

As the orientation of LC molecules was sensitive to the surface topographic change and anti-IMA was a probe to bind specifically with IMA that directly disturbed the ordered arrangement of 5CB, so immobilization of antibody was an important issue in the immunoassay for IMA. We dipped different concentrations of anti-IMA on DMOAP-coated glass slides and fabricated them into LC cells. Results are demonstrated in Fig. 3. When the anti-IMA concentration was 100 μg/mL, a large amount of protein adsorbed on glass slides to disrupt LCs transitions and a bright spot appeared. This phenomenon indicated that a higher concentration may help to control the LC order. However, some antibodies attached to the surface because of physical adsorption; the following washed surfactant solution could be easily removed. Such a condition will directly affect the binding of antibody and antigen. Therefore, we washed the glass slides, and the concentrations decreased. The bright area gradually dimmed down and finally turned into a uniform dark ground. We obtained the critical concentration with 10 μg/mL.

LOD of IMA

Solutions with different concentrations of IMA were firstly dipped on to the glass slides fixed with anti-IMA, and then made into LC cells with DMOAP-modified glass slides, and, lastly, the assembled cells were observed under a polarized light microscope. Results are shown in Fig. 4. When the IMA concentration increased,
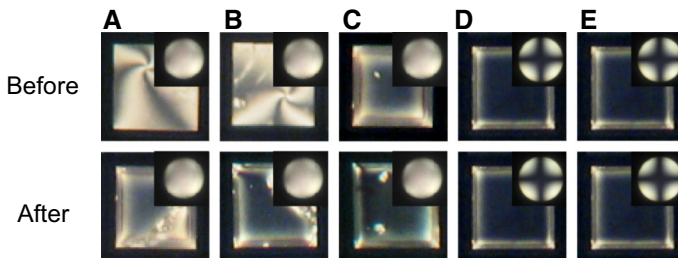
Fig. 3 Polarized images of LC cells fabricated with different concentrations of anti-IMA (μg/mL) **a** 100, **b** 50, **c** 15, **d** 10, **e** 0, before and after washing the glass slides
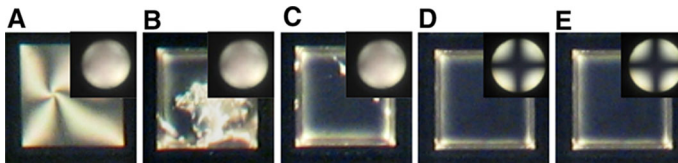
Fig. 4 Optical appearances under cross polarizer of LC cells with 5CB at different concentrations of IMA (μg/mL) **a** 500, **b** 100, **c** 50, **d** 10, **e** 0

antigen–antibody interactions were enhanced, which disordered the vertical orientation of LC molecules and increased birefringence behavior. The bright region became more obvious in the final image. On the contrary, as the concentrations reduced, the optical image gradually tended to be black, and there were only several little bright spots left when the concentration reached 50 μg/mL, indicating that the concentration of IMA was determined to be as low as 50 μg/mL.

We compared the proposed LC sensor with other present methods used in cardiac diseases. As demonstrated in Table 1, ACB was an indirect assay with blood albumin levels no less than 34 μg/mL; a direct method such as ELISA with binding partners showed excellent LOD with 28.6 μg/mL, and the SPR sensor with AuNPs could detect 100 ng/L. Until now there has been no other LC biosensor reported IMA assay, and so we made a new attempt in this field. Meanwhile, with the variations of light intensity in optical images, the signals can be observed by the naked eye. The LC sensor has realized on-site and rapid detection, which provides a simple strategy for the detection of IMA.

Specificity

In order to verify the selectivity of this method, we compared optical signal responses of HSA and human IgG, which have protein structures similar to the IMA. The three proteins were made into a LC cell as mentioned; we detected concentrations of 500, 100, 50, and 10 μg/mL, and we used average grey values to assess the LC response via optical images. Results are displayed in Fig. 5. In 10 μg/ mL, these three proteins were 16 as a grey value, which showed the dark ground

Table 1 The comparison of the IMA LC biosensor with other methods

Method	Detection mode	Detection limit (μg/mL)	References
ACB	Indirect assay	>34 (blood albumin)	[18]
ELISA	Direct assay	28.6	[10]
SPR	Direct assay	100	[19]
LC biosensor	Direct assay	50	This work
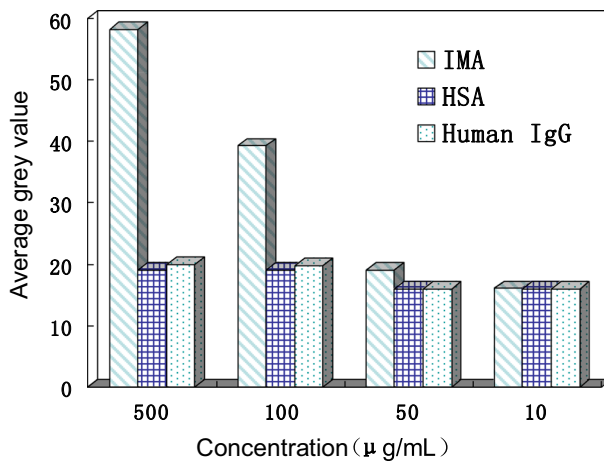
Fig. 5 Selectivities of LC biosensor to IMA, HSA and human IgG

value. With higher concentration, the average grey value from HSA and human IgG modified LC cells displayed almost identically at about 20, indicating that most of the areas were black except for some weak spots. This phenomenon may be caused by small amounts of HSA and human IgG, which was still not fully estimated. In contrast, the IMA-treated cell was quite different with obvious color texture (high grey value), which meant that IMA reacted on the surface causing an optical response. To sum up, the specificity of antigen antibody binding revealed that the LC biosensor reached high selectivity for IMA detection.

Conclusions

A new LC biosensor was studied on the basis of IMA antigen–antibody binding, which induced a LC molecular orientation transformation and resulted in optical signal changes on a glass surface. The morphology was observed by use of a polarizing microscope. The proposed sensor combined high specificity of antibody and sensitive optical signal amplification of LC molecules, presented low LOD,

high specificity, simple operation, and low cost characteristics, and more importantly, such a method would be helpful to ACS sufferers.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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