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A novel piezoelectric immunosensor for detection of carcinoembryonic antigen

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Abstract

A simple, rapid, and highly sensitive immunosensor for the direct determination of carcinoembryonic antigen (CEA) in human serum using a piezoelectric crystal has been developed and optimized. In order to improve sensitivity of the immunosensor, a protein A-based orientation-controlled immobilization method for antibodies was adopted together with an immunoreactive accelerant of polyethyleneglycol (PEG) used to amplify the signal response of frequency. Human normal serum was utilized as a reference background. The linear range for CEA concentration obtained by the end-point method was 66.7–466.7 ng/mL. Clinical samples from cancer patients were analyzed by the proposed piezoelectric immunoassay, and the analytical results were reasonably comparable with those obtained by the chemiluminescence immunoassay (CLIA). The proposed immunosensor provides a new promising method for the highly sensitive immunoassay of CEA in clinical laboratory.

Keywords: Carcinoembryonic antigen; Piezoelectric immunoassay; Protein A; PEG

1. Introduction

Carcinoembryonic antigen (CEA), a kind of glycoprotein found in colorectal carcinomas, is an important tumor marker responsible for clinical diagnosis of colorectal, pancreatic, gastric, and cervical carcinomas [1]. Various immunoassays, such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and chemiluminescence immunoassay (CLIA) were developed for CEA, which commonly has a very low serum level. Yet these methods suffer from disadvantages such as using complex time-consuming procedures and/or requiring potentially dangerous or expensive materials. As the detection of serum CEA level is of great importance for clinical diagnosis of colorectal, pancreatic, gastric, and cervical carcinomas, it is of considerable interest to research further for new sensitive and accurate means for CEA assay.

A piezoelectric device is portable, simple, cost effective, and suitable for real-time monitoring of bio-specific interaction such as antigen–antibody with high sensitivity and specificity. It may also eliminate some expensive and time-consuming purification procedures in many cases. Moreover, the advance in biosensor technology, specifically in immunosensor systems, suggests that new means for the detection of CEA could be developed. Actually, the piezoelectric crystal immunosensors were widely used to the quantification of bio-molecular interaction [2–7]. Recently, increasing interest has been focused on the studies of tumor markers. A few kinds of tumor markers such as carbohydrate antigen-125 [8], carbohydrate antigen-19-9 [9], and CEA [10] have been investigated with electrochemical immunosensor. Up to now, use of the piezoelectric immunosensor for the detection of CEA has not been reported. In this study, a piezoelectric immunosensing method for determination of CEA in human serum using piezoelectric immunosensor has been initially provided.

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It is well known that the immobilization of antibodies on the surfaces of base transducers is a key procedure in the construction of the immunosensors. In general, protein immobilization on the biosensor surface can be performed by building an inert hydrophobic base layer to cover the surface prior to coupling the antibody using a cross-linking agent. The base layer can be formed by various materials such as 3-(aminopropyl)triethoxysilane (3-APTES), polyethylenimine (PEI), protein A, etc. 3-APTES, PEI, and polymeric layers, however, need to be pre-activated by glutaraldehyde and other cross-linkers. Moreover, cross-linking produces a three-dimensional multilayer matrix that creates diffusion barriers and transport limitations, resulting in long immunoreaction time, poor repeatability, and low sensitivity [7]. Furthermore, antibodies bound with a cross-linker, i.e., glutaraldehyde, may lack desirable orientations, which may presumably decrease the antigen-binding ability of antibodies. It is well recognized that the immobilization of antibodies using protein A can provide ideal immunoreaction properties. On the one hand, protein A can specifically bind the F_C region of antibody molecules with their F_{ab} region maximally exposed towards the antigen solution. In other words, the monolayer formed by protein A is orientation-controlled [11] and it allows analyte to bind the active sites of protein A easily. Accordingly, an improved detection sensitivity and reproducibility of sensor can be expected. On the other hand, the formation of protein A monolayer is relatively easy and less dependence on special technique and equipment. For these reasons, protein A-based orientation-controlled immobilization was utilized to bind CEA antibodies.

Polyethyleneglycol (PEG), a water-soluble polymer with the ability of dehydration, was also used as the immunoreaction enhancer to increase immunosensing response signal in the present work. In addition, main experimental conditions were optimized. The analytical characteristics of the created immunosensor were investigated in detail. Subsequently, the proposed immunosensing system was used to evaluate several clinical serum samples obtained from cancer patients with results compared with those given by chemiluminescence immunoassay used in current clinical laboratory.

2. Experimental

2.1. Materials

CEA antibody, protein A, and human normal serum album were purchased from Zhongshan Biotechnology Company (Beijing, China). Purified CEA antigen of human serum and serum of cancer patients were provided by Hunan Provincial Tumor Hospital. PEG (molecular weight 6 kDa) was bought from Changsha Chemical Reagents (Hunan, China). Phosphate buffered saline (PBS) was prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, 0.24 g of KH_2PO_4, and 1.5 g of PEG in 800 mL of distilled water, and finally adjusting pH to 7.2 with HCl, but PBS (pH 7.2) used for diluting antibody was made merely by Na_2HPO_4 and KH_2PO_4. All other reagents and solvents were of analytical reagent grade and double-distilled water was used throughout.

2.2. Apparatus

The piezoelectric crystals used in this study were gold deposited AT-cut with 9 MHz basic resonance (Model JA5B, purchased from Cheng XI Radio Equipments). The quartz crystal is connected into a homemade transistor logic circuit (TTL). The frequency shift was monitored by a frequency counter (Model SS7201, Shi Jia Zhuang Wireless Factory). The antibody-modified piezoelectric crystal, one side of which was sealed with an O-ring silicone rubber covered by a plastic plate, was mounted on the detection cell in which an optimized buffer solution was gently agitated by a magnetic stirrer (Model JB-2, Shanghai Analytical Instruments, Shanghai, China).

2.3. Preparation of immunosensing surfaces

The antibody-modified piezoelectric crystal was prepared as follows. The crystal was soaked in 1.2 M NaOH solution for 10 min, rinsed by distilled water, then soaked in 1.2 M HCl solution for 10 min, rinsed with distilled water again, and finally, dried in air. Protein A (0.2 g/L) solution were dropped onto the gold electrode. Then, the electrode was incubated for 40 min at 37 °C, washed by PBS and distilled water, dried in air in succession. CEA antibody solution (1:2) was spread on the central surface of the crystal, incubated 40 min at 37 °C, washed with PBS and distilled water, the sensor coated by a CEA antibody film on the surface of the crystal was ready for use.

2.4. Measurement procedure

One complete measuring cycle includes following steps. First, the resulting crystals were mounted in a piezoelectric crystal cell filled with 3 mL of the PBS. The frequency responses decrease slowly until equilibrium (frequency shift <2 Hz) was reached (it took about 10 min). The stable frequencies of the crystal immobilized with antibody were measured. Second, purified samples of CEA serum of variable concentrations were injected into the cell with the stable crystal frequencies were recorded, respectively. Third, the human normal serum was used as a reference and measured in the same way.

3. Result and discussion

3.1. Characterisation of sensor performance

In order to provide an immunosensor with a good performance, the solution ratio of CEA to PBS, the incubation time and the incubation temperature for the antibody immo-

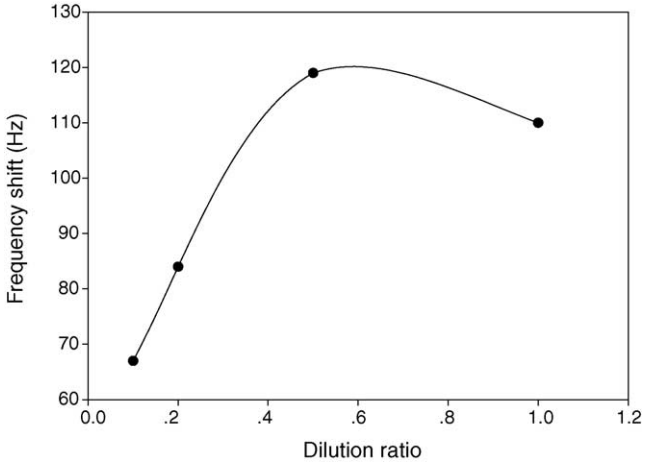

Fig. 1. Relationship between antibody dilution ratio and frequency shift of CEA serum sample at concentration 200.0 ng/mL. The background value has been subtracted.

bilization were investigated. Similarly, the influence of the immunoreaction time was also studied.

The amount of antibody immobilized on the crystal largely influences on the sensitivity of the immunosensor. In this study, it was controlled by dilution ratio of antibody solution in the immobilization process. The dilution factors (1:1, 1:2, 1:5, and 1:10) of antibody were examined and the results obtained are shown in Fig. 1. The dilution ratio of 1:2 produced a maximum signal (119 Hz) and was adopted for further work.

The effect of incubation time is shown in Fig. 2. With increasing incubation time, the frequency response increased and then reached an approximately constant value when the incubation time was longer than 40 min.

The influence of incubation temperature was studied in the temperature range from 25 to 45 °C. From the results shown in Fig. 3, the maximum response occurred at an incubation temperature of 37 °C.

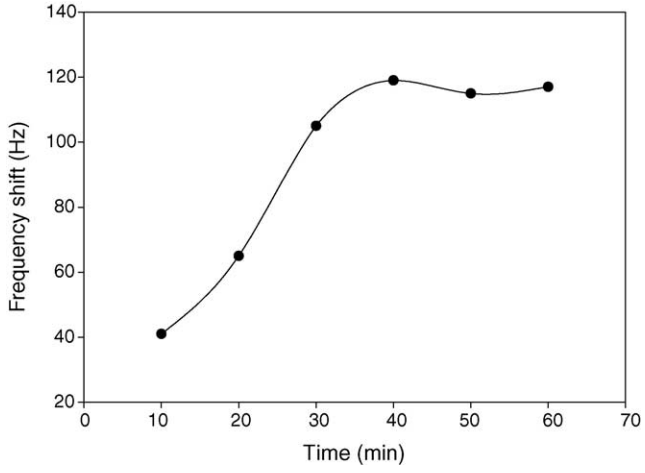

Fig. 2. Relationship between incubation time for immobilizing antibody and frequency shift of CEA serum sample at concentration 200.0 ng/mL. The background value has been subtracted.

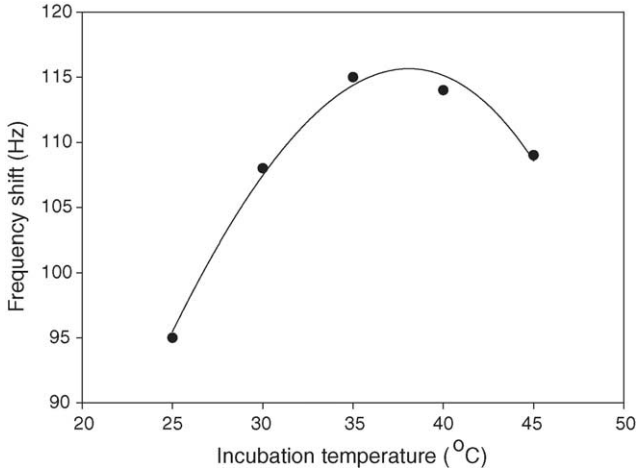

Fig. 3. Relationship between incubation temperature and frequency shift of CEA serum sample at concentration 200.0 ng/mL. The background value has been subtracted.

For determining the desirable time for immunoreaction, the immunosensor was soaked in CEA serum samples. Fig. 4 shows that frequency change of a crystal increased with the immunoreaction time between antibody and antigen and reached a plateau at reaction time of 20 min.

3.2. Amplification effect of PEG

In our experiment, an amount of 1.5 g/L of PEG in assay buffer medium was adopted to improve the immunoagglutination. The immunoreaction performance amplified by PEG and the one not amplified by PEG are shown in Fig. 5. Obviously, PEG results in an enhanced response of frequency shift and shorter time needed for approaching equilibrium. The amplification mechanism has been studied in detail in literature [12,13]: the presence of PEG would decrease the

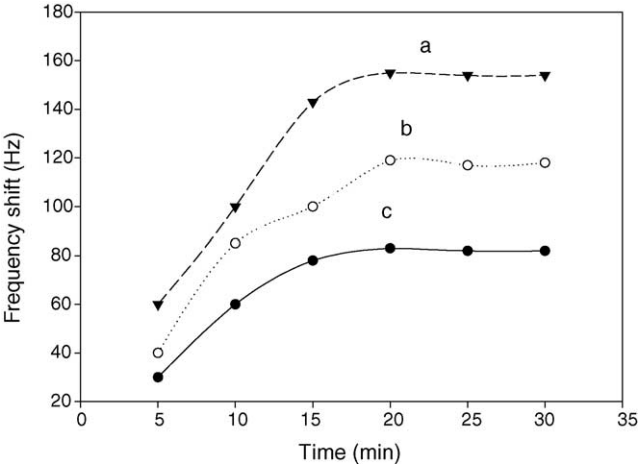

Fig. 4. Relationship between immunoreaction time and frequency shift of CEA in serum samples at different concentrations: (a) 266.7 ng/mL, (b) 200.0 ng/mL, and (c) 133.3 ng/mL. The background value has been subtracted.

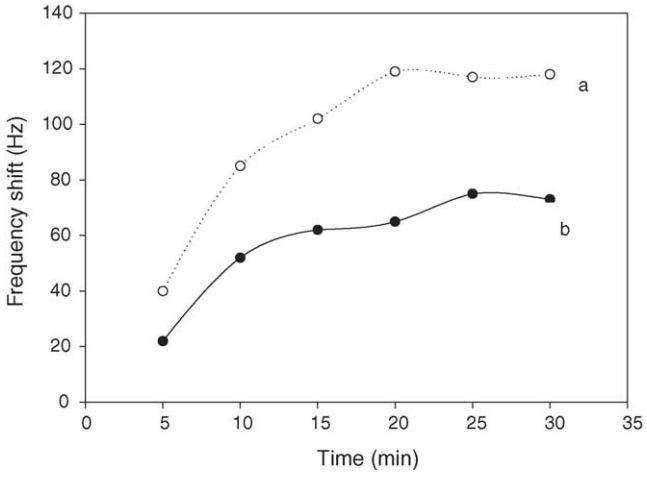

Fig. 5. Comparison of the frequency shift of system between with PEG (a) and without PEG (b). The concentration of CEA is 200.0 ng/mL. The background value has been subtracted.

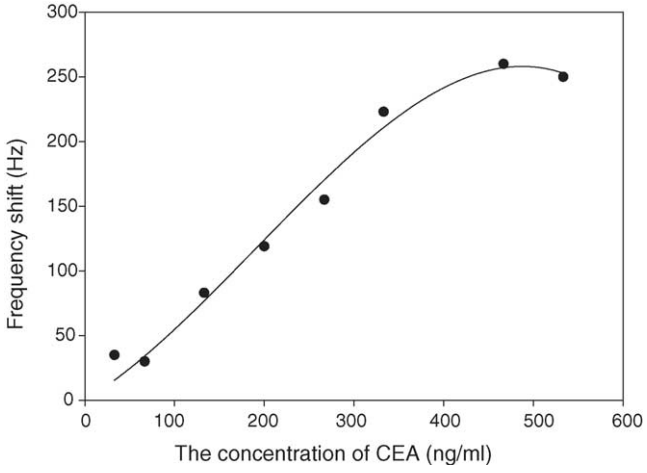

Fig. 6. Correlation between CEA concentration and frequency shift with CEA serum. The background has been subtracted.

hydration, strengthen the electrostatic attraction, and increase the entropy by randomization of formerly orientated water of hydration expelled into the bulk phase.

3.3. Detection of CEA levels and clinical application

Under optimized immunoassay conditions, the calibration graph for CEA determinations was as shown in Fig. 6. The background value has been subtracted from frequency responses. The calibration curve of relationship between the frequency shift and CEA concentration possesses a linear relationship in the range of 66.7–466.7 ng/mL. The detection limit was 66.7 ng/mL. The developed immunosensor was finally evaluated on real serum samples obtained from tumor patients. The CEA levels of five patient samples were detected by the proposed piezoelectric immunosensor and also analyzed using CLIA, which is a conventional method for serum analysis in this county, then detected, respectively, by piezoelectric immunosensor proposed in this paper. The results and the relative deviations between the two methods are shown in Table 1. The results of both methods were in reasonable agreement. It seems feasible to use the method to analyze the CEA concentration in human serum in clinical diagnosis.

Table 1
Comparison of serum CEA levels determined using two methods

Serum samples	1	2	3	4	5
Immunosensor (ng/mL)	93.5	158.2	211.3	372.7	417.8
CLIA (ng/mL)	77.2	142.3	231.5	337.6	445.9
Relative deviation (%)	7.9	11.3	−8.7	10.3	−6.3

4. Conclusion

In this paper, a simple, rapid and highly sensitive piezoelectric immunoassay has been initially proposed and applied to directly detect CEA in human serum. Use of polyethyleneglycol demonstrates an increased sensitivity and a lowered detection limit. The non-specific interference was eliminated by subtracting background value. Particularly, the immobilization of antibody on the surface of crystal by protein A is simple and successful and therefore the new assay can determine CEA in human serum without multiple washing and separation steps. Accordingly, such a promising technique can be applied in clinical diagnosis.

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