Development of a Biosensor for the Detection of Carcinoembryonic Antigen Using Faradic Impedance Spectroscopy

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A direct and simple method has been proposed to immobilize carcinoembryonic antibody (anti-CEA) on the surface of nanogold-modified glassy carbon electrode. Colloidal Au was electrodeposited onto the surface of pretreated glassy carbon electrode (GCE) as immobilization matrix to amplify response signal, then anti-CEA was absorbed on nanogold by amino group and physical absorption. The impedance change occurred on the sensor surface due to the specific immuno-interaction was utilized to determine carcinoembryonic antigen (CEA). Tests revealed significant change in the electron-transfer resistance (R_{et}) to various concentration of CEA in $Fe(CN)_6^{3-/4-}$ probe solution. The assay also demonstrates that the immunoelectrode exhibits a high level of sensitivity and satisfied response range.

Carcinoembryonic antigen (CEA) is a large cell surface glycoprotein (mol. wt 180,000) that was first described in 1965 by Gold and Freedman, an antigen expressed by gastrointestinal carcinoma.[1] CEA has been widely accepted as a useful tumor marker in the diagnosis and management today.[2–4] When CEA levels are positive (>5.0 ng/mL) in patients they could be useful prognostic indicators.[5] CEA diagnosis sometimes can be a critical issue, however more and more attention was paid to it.

Radio-immuno-assay (RIA) has been used to quantify CEA and enzyme linked immunosorbent assay (ELISA) for determines the quality. However, these traditional immuno-assay techniques are complicated multistage processes, tedious, and expensive. Thus, the development of a simple method for the determination of CEA in a real sample is needed. Therefore, our approach to these problems was to use electrochemical immunoelectrode rather than RIA, ELISA, because immunosensor techniques are sensitive, versatile, and inexpensive.[6] In this work, nanogold was used as immobilize matrix and amplified substance for the surface charge, high surface area and biocompatibility.[7] Anti-CEA was absorbed on nanogold by amino group and physical absorption.

Electrochemical impedance spectroscopy (EIS) is a rapidly developing electrochemical technique for the characterization of biomaterial-functionalized electrodes and biocatalytic transformations at electrode surfaces, and specifically for the transduction of biosensing events at electrodes or field-effect transistor devices.[8] The redox couple $Fe(CN)_6^{3-/4-}$ serves as a "probe" for the insulating properties and the density of the adsorbed layer. The formation of antigen–antibody complex will change the electrochemical impedance because the electrode is coated with a blocking layer. When antigens bind to the surface-immobilized antibodies, the access of the redox couple is hindered to a higher degree than in the absence of antigens. The many advantages of this method of immobilization included simplicity, relatively fast, inexpensive procedure and high sensitivity. For the powerful ability of EIS in interfacial properties changes upon biorecognition events, this method is promising to immobilize other antibody and detect subtle change in protein recognition.

Colloidal gold (0.24 μM) with average particle diameter of 16 nm were prepared according to literature.[9] The pretreated electrode was applied +1.5 V for 750 s in colloidal gold sols for immobilization of nanogold, then rinsed with water and pH 7.0 PBS for 20 s, respectively. Then, the electrode was immersed in the anti-CEA solution (pH = 7.0) (4 °C) for 7 h. Next the electrode was incubated in 10 mg/mL BSA for 1 h at 37 °C to block non-specific site. The finished electrode was stored at 4 °C when not in use. The schematic diagram of the biosensor and the structure of the electrode coating are shown in Figure 1.

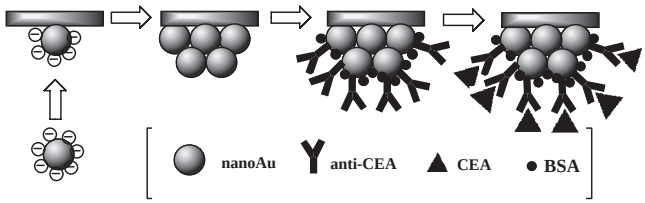

Figure 1. Schematic representation of the immobilization of anti-CEA via the activation of amine groups on the nanoAu-coated GCE.

It is well known that electrochemical impedance spectroscopy (EIS) is an effective tool for studying the interface properties of surface-modified electrodes. The typical electrochemical interface can be represented as an electrical circuit as shown in Figure 2. R_{et}, which equals the semicircle diameter at higher frequencies in the Nyquist plot of impedance spectroscopy, controls the interfacial electron-transfer rate of the redox probe between the solution and the electrode. Thus, R_{et} can be used to describe the interface properties of the electrode. Its value varies when different substances are adsorbed onto the electrode surface.

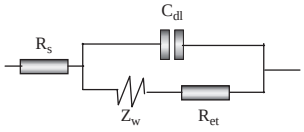

Figure 2. Equivalent electric network of the electrochemical interface. R_s, electrolyte resistance; C_{dl}, double-layer capacitance; R_{et}, electron-transfer resistance; Z_w, Warburg impedance.

Figures 3 and 4 show the dependence of impedance spectroscopy with the electro-deposition time and absorption time. Figure 3 reveals a gradual increase of covering and R_{et}. This may be due to the immobilization of nanogold onto the electrode, which forms a nanogold layer containing negative electron disperseed by $Fe(CN)_6^{4-/3-}$ probe in solution. Hence, the interfacial electron transfer is retarded, resulting in an increase

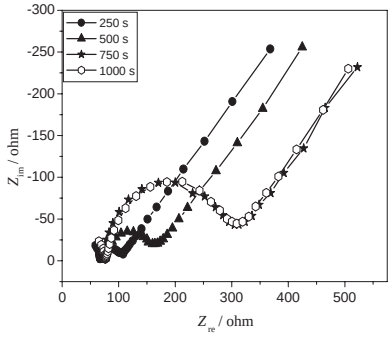

Figure 3. Optimization electrochemical deposition time of nanogold on the surface of GCE by EIS. EIS were recorded within the frequency range 50–10^6 Hz at the bias potential 0.17 V; amplitude of alternating voltage 10 mV, using 2.5 mM $Fe(CN)_6^{3-/4-}$ as the redox probe in 0.1 M phosphate buffer, pH 7.0.

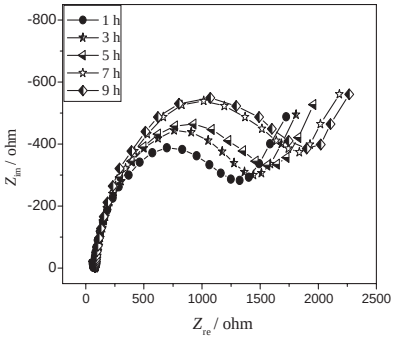

Figure 4. Optimization anti-CEA absorption time on the surface of nanogold modified GCE by EIS. The impedance spectra were recorded as described in the caption of Figure 3.

in the electron-transfer resistance. After 750 s, the value of R_{et} is no obvious change with increasing the time, indicating no increase of amount of nanogold upon GCE surface. Figure 4 shows the optimized condition of anti-CEA absorption time, the value of R_{et} increase with the anti-CEA absorbed time. After 7 h, no obvious increase of R_{et} was observed again. So 7 h was chosen as optimization absorption time.

When the antigen bound the antibody immobilized on the electrode, there would be an additional layer that increased the impedance response. Figure 5 shows the calibration curve of the immunoelectrode obtained using different concentration CEA standard samples. A good linear relationship was observed between the change of R_{et} and the CEA samples concentration, and the linear range was from 2 to 80 ng/mL with a detection of 0.6 ng/mL (estimated to be three times the standard deviation of zero-dose response), the senstivity of the present sensor is sufficient for the practical uses.

Some antigen serum samples (AFP, α-fetoprotein; IgG, human immunoglobulin G; C_3, complement III; HBsAg, hepatitis B surface antigen; PSA, prostate special antigen) were used to evaluate the selectivity of the electrode. The results were listed in Table 1. The R_{et} shift were calculated from the R_{et} shift of the electrode in assay solution containing 10 ng/mL CEA and different concentrations of interfering antigens compared with the impedance reading of the electrode in the same assay solution containing only 10 ng/mL CEA. The interferential degree of other antigen can be judged from the R_{et} shift caused by the

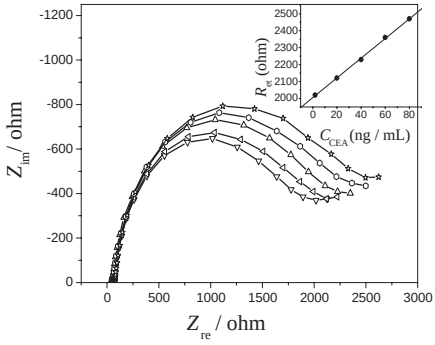

Figure 5. Nyquist plots of electrodes for variable antigen concentrations. Inset: relationship between antigen concentration and the value of R_{et}. The impedance spectra were recorded as described in the caption of Figure 3.

Table 1. The interference of CEA immunosensor by other antigens in human serum

Interferential antigens	AFP	IgG	HBsAg	C_3	PSA
Upper limit concentration[a] (ng/mL)	200	1600	1000	1100	80
Shift in R_{et}[b] (ohm)	30	50	15	46	32

[a]The upper limit concentration of interferential antigens means more than 20 times normal concentration in healthy human serum. [b]The shift value caused by the interferential antigens.

upper limit concentration of interferential antigens. In our experiments, the six kinds of substance tested did not cause interference with the electrode nearly.

Finally, a series of experiments with the addition of known concentration of CEA into four tested human serum samples was conducted. The recovery was in the range 97.2–102.3%, which demonstrate the proposed method satisfied the requirement for the test of human serum.

In this paper, we have described a new immunoelectrode for determination of CEA. This electrode is easy to prepare, has high selectivity and sensitivity. The electrode could be applied to determination of content of CEA in human serum. The method can also be used to immobilize some positively charged biomolecules to develop biosensors and bioreactors.

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References

1 P. Gold and S. O. Freedman, *J. Exp. Med.*, **121**, 439 (1965).
2 J. P. Kohler, D. Simonowitz, and D. Paloyan, *Am. Surg.*, **46**, 449 (1980).
3 H. J. Staab, F. A. Anderer, and T. Brümendorf, *Br. J. Cancer*, **44**, 652 (1981).
4 H. J. Wanebo, B. Rao, and C. M. Pinsky, *N. Engl. J. Med.*, **299**, 448 (1978).
5 H. J. Staab, F. A. Anderer, and T. Brümendorf, *Br. J. Cancer*, **45**, 718 (1982).
6 L. G. Andrey, A. Plamen, W. Michael, and W. Ebtisam, *Biosens. Bioelectron.*, **13**, 113 (1998).
7 C. R. Martin and D. T. Mitchell, *Anal. Chem.*, **70**, 322A (1998).
8 L. Alfonta, I. Willner, D. J. Throckmorton, and A. K. Singh, *Anal. Chem.*, **73**, 5287 (2001).
9 G. Frens, *Nat. Phys. Sci.*, **241**, 20 (1973).

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