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Comparison of surface plasmon resonance and capacitive immunosensors for cancer antigen 125 detection in human serum samples

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ABSTRACT

This paper presents a comparison between surface plasmon resonance (SPR) and capacitive immunosensors for a flow injection label-free detection of cancer antigen 125 (CA 125) in human serum. Anti-CA 125 was immobilized on gold surface through a self-assembled monolayer. Parameters affecting the responses of each system were optimized. Under optimal conditions, SPR provided a detection limit of 0.1 U ml^{-1} while 0.05 U ml^{-1} was obtained for the capacitive system. Linearity for SPR was between 0.1 and 40 U ml^{-1} and 0.05–40 U ml^{-1} for capacitive system. These immunosensors were applied to analyze CA 125 concentrations in human serum samples and compared with conventional enzyme linked fluorescent assay (ELFA). Both systems showed good agreement with ELFA ($P < 0.05$). Moreover, these immunosensors were very stable and provided good reproducible responses after regeneration, up to 32 times for SPR and 48 times for capacitive system with relative standard deviation lower than 4%. The SPR immunosensor provided advantages in term of fast response and real-time monitoring while capacitive immunosensor offered a sensitive and cost-effective method for CA 125 detection.

1. Introduction

Cancer antigen 125 (CA 125) is a tumor marker for ovarian cancer. It is present in 80% of nonmucinous for ovarian cancer and circulates in the serum of patients (Endo et al., 1988). For healthy human, the concentration levels of CA 125 are lower than 35 U ml^{-1} (Wilder et al., 2003). The determination of CA 125 level in human serum is very useful to clinical diagnoses since it can give information of the disease stage, monitoring the progress of ovarian cancer patients following cytoreductive surgery and chemotherapy.

For clinical laboratory, CA 125 levels are often measured by radiometric (Marguerite et al., 1987; Mcquarrie et al., 1997) and enzyme immunoassay (Dai et al., 2003; Wu et al., 2007; Yan et al., 1999; Biomerieux® sa, 2004). These conventional immunoassays are time-consuming, required several separation steps and special equipped laboratories and/or skilful personnel (He et al., 2003; Lin and Ju, 2005). In view of these developments of an alternative specific method for the determination of CA 125 which is relatively easy to operate is interesting. One approach is immunosensor.

Immunosensors combine the natural specificity of antibody and antigen reaction with high sensitivity of various physical transducers. Most immunosensors for CA 125 are labeled with enzyme (Dai et al., 2003; Fu et al., 2008; Wu et al., 2007) and the analysis procedure requires several steps (Bange et al., 2005). Label-free immunosensor is more attractive since it directly detects changes in physical properties owing to the antibody–antigen binding on the transducer surface. For label-free, there was only one report on the analysis of CA 125 by using differential pulse voltammetry, provided a detection limit of 1.8 U ml^{-1} and a linear range of 10–30 U ml^{-1} (Tang et al., 2006).

Several transducers have been developed for label-free immunosensors. Capacitive and surface plasmon resonance transducers have recently attracted a lot of interest especially for direct detection of biomolecular interactions (Berggren and Johansson, 1997; Dudak and Boyaci, 2007; Limbut et al., 2006b; Loyprasert et al., 2008; Mazumdar et al., 2008; Teramura and Iwata, 2007; Yin et al., 2006). SPR biosensor has become particularly powerful because of its high surface sensitivity, real-time monitoring, and

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kinetic analysis (Campagnolo et al., 2004; Toyama et al., 1998) while the main advantages of capacitive biosensor are its high sensitivity, simple to operate, and relatively inexpensive.

In the present work, we employed both surface plasmon resonance and capacitive transducers for the direct detection of CA 125 using anti-CA 125 immobilized on a gold surface by self-assembled monolayer. Parameters affecting the response of both transducers were optimized then their performances were compared. To show real application, human serum samples were tested by both systems under optimum conditions. The results were then compared to enzyme linked fluorescent assay (ELFA). To the best of our knowledge this is the first time SPR and capacitive systems are investigated for direct detection of CA 125. This is also the first time that the performances of these two systems were compared under optimum conditions and applied to detect analyte concentration in real samples.

2. Experimental

2.1. Materials

Monoclonal anti-cancer antigen 125 (anti-CA 125) and cancer antigen (CA 125) from human were obtained from US biological (USA), anti-alpha-fetoprotein antibody (anti-AFP) and alpha-fetoprotein (AFP) were obtained from Dako (Denmark) and anti-human carcinoembryonic antigen (anti-CEA), carcinoembryonic antigen (CEA) obtained from Sigma (St. Louis, USA), 11-mercaptoundecanoic acid from Aldrich (MO, USA), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) from Aldrich (Steinheim, Germany), 1-dodeccanethiol ethanolic acid from Aldrich (Milwaukee, USA), ethanolamine from Merck (Darmstadt, Germany), bovine serum albumin (BSA) from Fluka (Steinheim, Germany). All other chemicals used were of analytical grade. All buffers were prepared with distilled water treated with a reverse osmosis-deionized system. Before used, the buffers were filtered through an Albet® nylon membrane filter (Albet, Spain), pore size 0.20 μm, with subsequent degassing.

2.2. SPR immunosensor

2.2.1. Anti-CA 125 immobilization

A gold disk (ϕ = 2.5 cm; 50 nm thick gold-coated BK-7 glass plate, Eco Chemie B.V., Netherlands) was cleaned with piranha solution (3:1 mixture of H_2SO_4 and H_2O_2), thoroughly rinsed with water, ethanol and dried in a stream of nitrogen gas. The glass side of this gold disk was adhered to the prism via a matching oil with the same refractive index (n = 1.518) and placed inside the holding block of the SPR equipment (AutoLab Spirit®, Eco Chemie B.V., Netherlands) with the gold surface facing upward. A custom built flow cell was screwed into place, on top of the gold disk, leaving a space of 10 μl (ϕ = 3 mm) where solution can pass through the gold surface. Immobilization steps were monitored by SPR detection, controlled by Autolab SPR version 4.2.1 software. The freshly cleaned disk was incubated in 150 mM 11-mercaptoundecanoic acid solution for 5 h. During this time self-assembled monolayer was formed on the gold surface. These optimum values of concentration and incubation time were obtained from prior tests when the angle shift of the self-assembled layer of 11-mercaptoundecanoic acid on gold disk surface reach a steady value. For the coupling of anti-CA 125, the surface was activated with 0.2 M EDC/0.05 M NHS for 50 min, the optimum activation time. Then, 30 μg ml^{-1} of anti-CA 125 in 10 mM Tris–HCl pH 7.50 was incubated on this surface and then with ethanolamine pH 8.50 for 7 min to block the remaining reactive sites. Finally any pinholes on the surface were blocked with 1% BSA for 1 h.

2.2.2. SPR system

All SPR experiments were performed using SPR AutoLab. Solution was delivered using a syringe pump (Kd Scientific, USA) into the SPR flow injection system, equipped with a custom build flow cell. Standard CA 125, serum sample, and regeneration solution were loaded with a sample injector (Valco, USA). The flow cell was maintained at 25 °C by a circulating water bath (Grant Instrument, UK). A monochromatic p-polarized laser (λ = 670 nm) was directed through prism onto the gold disk. The interaction between immobilized anti-CA 125 on the gold disk and CA 125 antigen was measured as the SPR angle shift by a photodiode detector among a dynamic range of 4000 millidegree.

2.3. Capacitive immunosensor

2.3.1. Anti-CA 125 immobilization

Gold rod electrode (ϕ = 3 mm, 99.99% purity) was cleaned and treated followed the steps described by Limbut et al. (2006a,b). Immobilization of capacitive immunosensor followed the same steps as the immobilization of SPR immunosensor except the time allowed for self-assembled monolayer and the chemical used in the blocking step. The gold rod electrode was immersed in 150 mM of 11-mercaptoundecanoic acid solution for 15 min to form monolayer. This optimum value was obtained by determining percentage of surface coverage of 11-mercaptoundecanoic acid formation on electrode surface (Limbut et al., 2006a). For the blocking step, 10 mM of 1-dodeccanethiol ethanolic acid was applied for 20 min. The electrochemical behavior of each immobilization step was studied by cyclic voltammetry (Eco Chemie μ-autolab B.V., Netherland, and software package GPES 4.7). A reduction of redox peaks after each immobilization step indicated the existence of an additional layer on the electrode surface. After the final blocking step the redox peaks disappeared.

2.3.2. Capacitive system

The reaction cell (10 μl) of the capacitive flow system consisted of an anti-CA 125 modified gold working electrode, a custom made Ag/AgCl reference electrode and a stainless steel tube auxiliary electrode. They were connected to a potentiostat (ML 160, AD Instruments, Australia). Carrier buffer was flowed through the system using a peristaltic pump (Gilson, France). Standard CA 125, serum sample, and regeneration solution were injected into the flow cell by a sample injector (Valco, USA). The capacitance was measured using a potentiostatic step method (Berggren et al., 1998). The current responses obtained from the application of 50 mV potential pulses were used to calculate the capacitance followed the procedures described by Limbut et al. (2006a,b). In brief the logarithm of the current response was plotted *versus* time, the capacitance of the layer covering the electrode was obtained from the slope and plotted as a function of time.

2.4. Optimization of the flow injection SPR and capacitive immunosensors

Parameters affecting the performances of both SPR and capacitive immunosensors (Table 1) were optimized. Initial conditions were 300 μl of 15 U ml^{-1} of CA 125 with 10 mM phosphate buffer pH 7.20 at a flow rate of 10 μl min^{-1} for SPR and 200 μl of 5 U ml^{-1} of CA 125 with 10 mM Tris–HCl pH 7.20 at a flow rate of 100 μl min^{-1} for the capacitive system (Wu et al., 2006). The response was the average of three injections. The optimization was performed by changing a single parameter while keeping other parameters constant. The optimum operating condition was considered by balancing between the signal and analysis time for one analysis.