Direct detection of Hyaluronan-binding protein (HABP) based on an Alumina sol-gel derived immunosensor with the capacitive measurement

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The use of sol-gel chemistry to produce the nano-materials to be applied in the immunosensors had attracted considerable interests for the low-temperature encapsulation of the protein and the preservation of the integrity and homogeneity of the protein surface microstructure[1]. Directly detection of the un-labeled immunochemical interaction based on the capacitance change had been reported because the type of immunoassay had a higher sensitivity, quick-response and the measurement had not any complicate instruments. However the electrochemical immunoassay was seldom used in the immunosensors formed by the sol-gel encapsulation for the difficulty in fabricating an insulting layer with the sol-gel materials. To overcome such a question, here a novel Al_2O_3 sol-gel with a special pore size was prepared and used to immobilize the antibody to form an immunosensor with a dense and insulting layer. The interaction in-between the un-labeled antibody-antigen was evaluated by the potentiostatic-step, a quick capacitive measurement performed in microseconds [2].

The Alumina sol-gel was prepared in our previous report [3]. The TEM image of the Al_2O_3 gel showed that Al_2O_3 clusters appeared as 50-60nm dark spots sticked together to form a porous nanoconstructure. The porous was 30-40nm in diameter, little larger than that of the antibody, which could be used to encapsulate the antibody.

Hyaluronan-binding protein (HABP), the important structural component of extracellular matrices, which served important structural and regulatory functions during development and in maintaining adult tissue homestats, was chosen as the target antigen. The immunosensor was fabricated by dripping the mixture of anti-HABP and Al_2O_3 sol on a bare Au electrode directly and then drying at 4℃. The Faradic current peaks of the bare Au electrode in 5mM$[Fe(CN)_6]^{3-/4-}$(figure 1a) disappeared when Al_2O_3-sol-antibody was modified on the electrode (figure 2c). Compared with the CVs of the electrode coated with only Al_2O_3 sol-gel (figure 2b), the current of the immunosensor was less. All these indicated the antibody was successfully immobilized on the electrode with Al_2O_3 sol-gel and the thin layer formed was insulting, which was suited to be fabricated a capacitive immunosensor.

The change in capacitance vs the logarithm of the concentration of the antigen was given in figure 3a. The detection range was from 1ng/mL to 500ng/mL. No capacitance change was observed when only Al_2O_3 sol was immobilized on the electrode (figure 3b). This suggested that the observed capacitance change caused by the interaction HABP antigen and antibody was not produced by an unspecific adsorption of the antigen to the electrode surface, which made the immunosensor be promise in clinical test.

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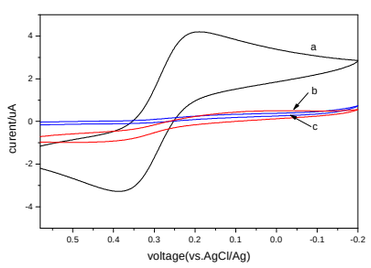

Figure 1: Cyclic voltamogramms recorded in a 5mM $[Fe(CN)_6]^{3-}$ (+0.1 M KCl) solution .The scan rate was 10mV/s. The

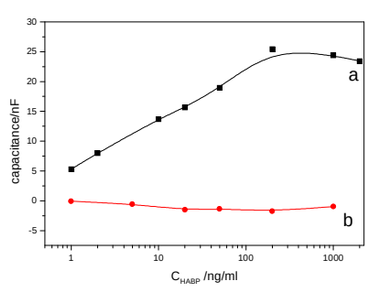

Figure2:(a) Capacitance change vs the logarithm of HABP concentration for the electrode (b)the nonspecific response for HABP immunosensor