Au nanochannels technique and its application in immunoassay

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**Abstract**

Au nanotubules (channels) can be electrolessly plated within the pores of polycarbonate microporous filtration membranes. When an electric field was applied on the cell consisting of the membrane and Pt electrodes, the response current decreased due to a baffle effect from big molecules when the electrolyte ion and big molecules passed through the channels modified with chemical groups. Based on this principle, a nanotubules-based sensing technique has been developed. This method can be applied to the determination of human IgG with a detection limit of 0.34 ng/mL.

**Keywords:** Au nanotubules, baffle effect, sensing, immunoassay.

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Nanochannels techniques for separating or detecting the organic and biomolecules are developing and many papers on the nanochannel have been issued. Having been exploring molecular-size, chemistry and charge selectivity of the nanotubule, Martin et al. separated two enantiomers from a chiral drug. A lot of papers about analysis of inorganic and organic substances based on single nanochannel have been published. For example, the \( \alpha \)-hemolysin channel with 1.5–2.6 nm diameter assembled in nature or artificial double fat layer served to detect avidin and bromodeoxyuridine antibody, DNA sequences and single chain DNA. However, this kind of channel has poor machine stability and small assembly efficiency. Li et al. prepared Si-Na membrane containing a single MWNT channel similar to the \( \alpha \)-hemolysin channel. This channel has high machine stability, but it is complex to fabricate and difficult to modify. Although this stochastic sensing by single channels is sensitive and rapid, many varieties of molecules cannot be detected because its size is not in the range of the channel diameter. In addition, the sensing signal was not easily analyzed and the signal current was small to pA level where requires very sensitive apparatus.

In our work, Au nanotubules (channels) can be electrolessly plated within the pores of polycarbonate microporous filtration membranes. We originally studied the IgG transport behavior of antibody through the nanochannels modified with chemical groups. With the existence of an electric field, a response current \( I_1 \) can be recorded when the electrolyte ion and big molecules such as sheet anti-human IgG (SA-HIgG) moved through the channels. After HIgG was added to the feed cell, the SAIgG-HIgG complex formed due to immunoreaction in the solution made intensive baffle event and the current decreased to current \( I_2 \). According to the current difference between \( I_1 \) and \( I_2 \), we can determine HIgG with a detection limit of 0.34 ng/mL. Compared with single nanochannel, nanochannels produced stronger current signal. The method provides a new convenient, rapid and sensitive bioanalysis such as the analysis of DNA, enzyme, virus and antigen-antibody.

1 Experimental

(i) Apparatus and reagents. CHI660 Electrochemistry Work Laboratory (Shanghai Chenghua Instrument Company) produced U-tube permeation cell with Pt foils as electrodes. Polycarbonate microporous filtration membranes with pore diameters of 100 nm (Millipore Company) were used to prepare Au nanochannels. HIgG and SAIgG (Dingguo Company), milli Q 18-Ω water, PBS buffer (pH = 7.5) were used throughout the experiments. Other reagents were of analytical reagent grade.

The membrane containing nanochannels was mounted between two halves of a U-tube permeation cell. Two halves are feed cell and permeation cell, respectively, the middle of which has a pore of 0.5 cm in diameter connecting two cells. Electrochemistry Work Laboratory produced U-tube permeation cell with Pt foil electrode to get current signals.

(ii) Procedure. (1) Preparation and modification of nanochannels. Au channels were prepared and modified with Cl⁻ according to the method. The membrane was immersed in a solution containing 0.026 mol/L of SnCl₂ and 0.07 mol/L of trifluoroactic acid for 45 min. The membrane was activated by immersing in an aqueous solution of ammoniacal AgNO₃ (0.9 mol/L) for 5 min in N₂ after being rinsed with three 100-mL portions of methanol. Then, the membrane was rinsed in water 4 times and immersed in methanol. After being rinsed in water 4 times, the Ag-coated membrane was immersed in an Au plating bath (pH = 10.87) at ca. 5°C. The membrane was rinsed with water 4 times and then immersed in HNO₃ (25%, volume percent) for 12 h. As a result, the fine and uniform Au nanochannels can be obtained. To modify Cl⁻ onto the channels, the Au membrane was immersed in KCl (0.1 mol/mL) solution for 12 h. (2) Experimental condition. The membrane containing 55 ± 2 nm pore served as experimental material. The electrolyte solution was 0.15 mol/L of KCl (pH = 7.5). The solvent of all samples was PBS buffer (pH = 7.5). All measurements were made under 1.7 V at room temperature. (3) Amperometric measurement. After electrolyte solution (6 mL) and PBS
buffer (2 mL) were dropped in two cells, $10^{-3}$ mg/mL in SAHlgG (2 mL) was added to the feed cell and responsive current was measured. When different concentrations of HlgG were added to the feed cell, the response current was recorded after immunoreaction lasting 10 min for each addition of HlgG. On the other hand, the control experiment was done under the same condition with sheet IgG, bovine IgG and rabbit IgG, respectively.

2 Result and discussion

(i) Image of nanochannels surface. Au channels were electrolessly plated within the pores of polycarbonate microporous filtration membranes and the inside diameter of Au nanochannels was a function of plating time. The longer the time lasts, the smaller the pore diameter is. Fig. 1 shows the SEM image of membranes of 100 nm in diameter after plating for 4 and 6 h, respectively. The diameter of the latter is smaller obviously. The black dots in the pictures represent the channels and the white ones may be the Au nanoparticles. In addition, pH value of Au plating solution partly determined the Au deposition. The high pH made the pore bottle-like and Au layer rough. On the other hand, the too low pH made the plating difficulty and even there was no plating layer. In our work, the better plating pH was 10.87.

(ii) The principle of sensing based on nanochannels. Fig. 2 shows the principle of sensing based on nanochannels. The size of electrolyte ion is 0.01—0.1 nm. Under an electric field, the ions freely transport through nanochannels and produce transmembrane current. When big size molecules entered into the channels, the baffle effect decreased the current. For a single pore in the membrane the blockage event was perhaps stochastic. But the statistic result produced by the channels array was that the membrane impedance increased and the current decreased. Based on this principle, the HlgG was detected using SAHlgG as signal molecules. After the nanochannels are modified with CI−, IgG molecules can smoothly transport through the channels. Under the electric field, K+ and Cl− as electrolyte ions moved through the channels together with SAHlgG and a current $I_1$ (μA) can be measured. When HlgG was added to solution, SAHlgG-HlgG complexes with bigger size made more intensive blockage event and the second current $I_2$ can be recorded. HlgG in the sample can be detected by the difference of currents $\Delta I$ ($I_1 - I_2$). Compared with HlgG with small size (20—25 nm), the diameter of larger size SAHlgG-HlgG (51 nm) is little smaller than that of channel (55 nm). The existence of SAHlgG-HlgG produced intensive blockage event resulting in a visual current change.

(iii) Sensing of HlgG based on nanomultichannels. The relationship of response current and concentration of HlgG is shown in Fig. 3. Fig. 3 shows that the increase of concentration of HlgG results in the decrease of current because of more blockage event from big SAHlgG-HlgG complexes, i.e. $\Delta I$ ($I_1 - I_2$) would increase due to the increase of HlgG concentration. Under the selected conditions, the detection limit was 0.34 ng/mL.

![Fig. 2. Schematic description of nanochannels sensing.](image)

![Fig. 3. Transmembrane current observed at different concentrations (mg/mL) of human IgG.](image)