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Short communication

A novel device of array nanochannels integrated electrochemical detector for detection of amyloid β aggregation and inhibitor screening

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ABSTRACT

A device of array nanochannels integrated with sensitive electrochemical detector has been designed to detect amyloid β (Aβ) peptide aggregation and inhibitor screening. Owing to the highly amplification capacity of array nanochannels, the change in ionic current upon Aβ peptide aggregation and inhibitor disaggregation can be easily monitored at micro-ampere level.

1. Introduction

Amyloid β (Aβ) peptide is derived from the proteolytic cleavage of amyloid precursor protein by secretase enzymes. Aggregation of Aβ peptide is considered as a critical step in the pathogenesis of Alzheimer's disease (AD) [1,2]. Therefore, monitoring of Aβ aggregation kinetics could provide insights into the structural transition of Aβ peptides, and allow screening of efficient inhibitors for early diagnoses of AD and treatment. Up to now, considerable progress has been achieved in study of Aβ aggregation and toxicity [3,4]. For example, Chen et al. used conventional fluorescence spectroscopy and electrospray ionization mass spectrometry to investigate the effect of different inhibitors on metal ions induced Aβ aggregation [4]. These observations suggested novel agents for treatment of AD. However, these methods usually rely on ensemble averages from a large number of molecules and also a relatively expensive instrument is required. Simple, low-cost, and sensitive method for detection of Aβ aggregation and inhibitor screening is clearly needed for the clinical diagnosis and treatment of AD.

Nanopore/channel has been widely adopted in the construction of bioanalysis devices in recent years [5,6]. Among the particular attributes, nanopore/channel possesses exquisite ability of revealing the change in molecular volume by measurable ionic current, which is of paramount importance for studying peptide and protein folding [7]. Unfortunately, the amplitudes of ionic current are usually at the pico-ampere level with only several microseconds in the single nanopore/channel experiments, and it will be easily affected by the noises. To amplify every transient change in ionic current, expensive electronic detection equipment such as patch-clamp and other commercial devices are usually required in these single nanopore based detection devices [8,9].

As an alternative nanostructure, porous anodic alumina (PAA) membrane has high density array nanochannels, and can amplify the ionic current by several orders of magnitude compared to single nanopore [10,11]. Using the current change of an indicator molecule, Liu et al. developed a PAA membrane-based electrochemical method which could successfully detect potassium ions and adenosine triphosphate based on the conformational change of aptamer in the presence of target molecules [11]. Herein, we use PAA membrane coupled with electrochemical technique to monitor metal ions induced Aβ peptide aggregation. Using the changed ionic current, discovering of efficient inhibitors can also be performed on the present platform. This new strategy provides a novel and simple platform for label-free monitoring of Aβ peptide aggregation with high sensitivity, which is also promising in screening of viable Aβ-targeted drug therapies for AD.

2. Experimental section

2.1. Modification of PAA membrane with $A\beta_{40}$

The fabricated PAA membrane was first hydroxylated in boiled hydrogen peroxide (30% H_2O_2) for 0.5 h, then immersed in 10% of APTMS solution diluted in acetone for 12 h and heated at 120 °C for

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2 h to crosslink the silane layer. After that, the APTMS-grafted PAA membrane was treated with a 5% aqueous solution of glutaraldehyde overnight, followed by $A\beta_{40}$ (GL Biochem Ltd., Shanghai, China) drop-coating (20 μL, 1 mM) for 6 h [12]. Each modification step was followed by complete rinsing by water.

2.2. Electrochemical measurements

The as-prepared PAA membrane was clamped between two plastic poly(dimethylsiloxane) (PDMS) films and then placed between two 2 mL homemade half cells for electrochemical detection. For metal ion induced $A\beta_{40}$ aggregation experiments, 20 μL $A\beta_{40}$ (50 μM) was first dropped onto the PAA membrane for 1 h, allowing diffusion of free $A\beta_{40}$ into the array nanochannels. Then, different concentrations of Cu^{2+} solutions were added into the two half cells, incubating for 6 h at 37 °C. For inhibiting experiments, ethylene diamine tetraacetic acid (EDTA) was added to $A\beta_{40}$ aggregated nanochannels with the final concentration of 1 mM and incubated at 37 °C for 6 h. All the solutions were prepared using a phosphate buffer (PBS, 10 mM, pH 7.0). The electrochemical detection was performed on a CHI 660E electrochemical workstation (Chenhua, China) with two Ag/AgCl electrodes as the anode and cathode electrodes in 10 mM KCl solution.

3. Results and discussion

The strategy of Aβ peptides aggregation assay in array nanopores is illustrated in Fig. 1A. $A\beta_{40}$ is immobilized in the array nanochannels through chemical coupling. In the presence of Cu^{2+}, conformation of $A\beta_{40}$ changes, which induces aggregation of $A\beta_{40}$ into amyloid oligomers and then non-fibrillar aggregates [13] with increased volume. The free space for ions transport through nanochannels becomes smaller, resulting in a decreased ionic current. If inhibitor is added, the $A\beta_{40}$ aggregates will be partially reversed, leading to the increase of free transport space for ions and thus increase of ionic current (Fig. 1A). The change in ionic currents can be easily monitored at micro-ampere level in array nanochannels based device (Fig. 1B), which allows sensitive detection of Aβ aggregation and inhibitor screening in real-time (Fig. 1C). This simple device provides a new strategy for sensitive and label-free monitoring of Aβ peptide aggregation and inhibitor screening, which is promising in discovering of viable Aβ-targeted drug therapies for AD.

PAA membranes were fabricated via a two-step aluminum oxidation process [14]. The morphology of the formed PAA membrane was characterized by scanning electron microscopy (SEM, S-4800 Hitachi, Japan). As shown in Fig. 2A, the PAA membrane has regular array nanochannels with open channels diameters of ~50 nm and ~80 nm for the up and bottom sides, respectively. The whole thickness was about 30 μm. In principle, the open channel size of top and bottom sides should be the same. However, a relatively smaller diameter in bottom layer is found, which might be due to the incomplete removal of the barrier layer. For immobilization $A\beta_{40}$ in nanochannels, Al–OH groups of PAA surface was first coupled with 3-aminopropyltrimethoxy-silane (APTMS) and glutaraldehyde, which then transformed into reactive aldehyde groups for the covalent binding of $A\beta_{40}$. The presence of APTMS in the PAA membrane was characterized using an X-ray photoelectron spectroscopy (XPS, K-Alpha, Thermo Fisher Scientific, USA). As shown in Fig. 2B, the PAA membrane did not show the Si 2P peak (red line), while a clear peak of Si 2P appeared (black line) after PAA was treated with APTMS, revealing the immobilization of APTMS in nanochannels. FTIR was used to probe the immobilization of $A\beta_{40}$ by monitoring the absorption of amide I and II vibrations using pure PAA membrane as the reference. As shown in Fig. 2C, the amide I band (1631 cm^{-1}) was attributed to the C=O stretching vibration of the peptide linkage in the peptide background. The amide II band (1491 cm^{-1}) was due to the N–H bending and C–N stretching. This result demonstrates the successful immobilization of $A\beta_{40}$ in nanochannels. In addition, the I–V properties before and after $A\beta_{40}$ immobilization were recorded (Fig. 2D). Owing to the immobilization of $A\beta_{40}$, the free space for ions transport in array nanochannels becomes smaller, resulting in a decreased ionic current upon $A\beta_{40}$ immobilization as revealed in Fig. 2D.

The Aβ peptides have high affinity to metal ions, which has been reported to be a risk factor for AD developing owing to Aβ aggregation. Here, $A\beta_{40}$ aggregation induced by Cu^{2+} was electrochemically

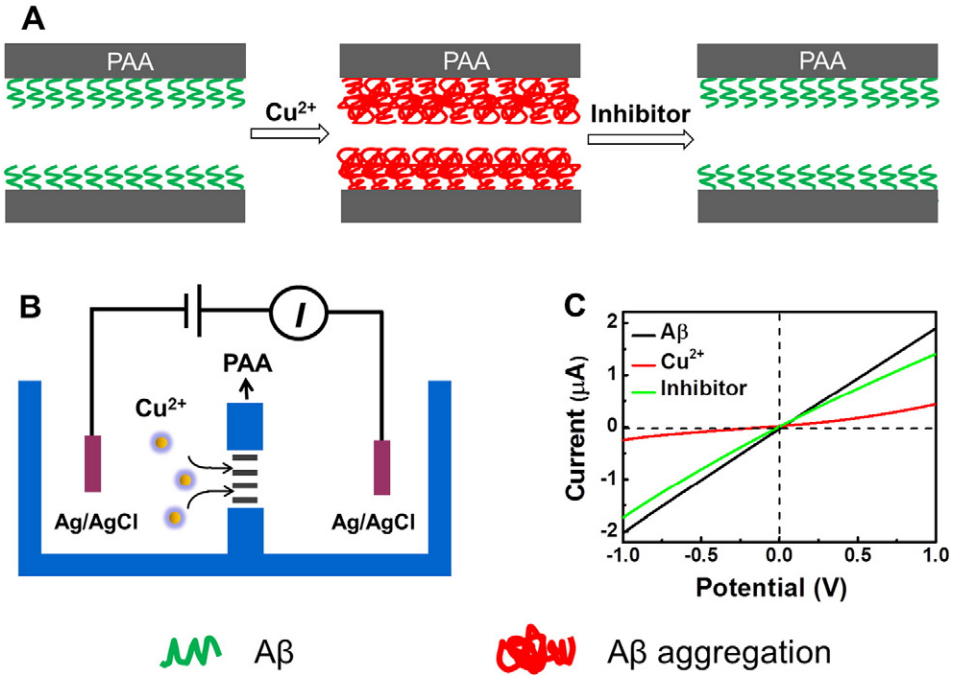

Fig. 1. The strategy of Aβ aggregation and inhibitor screening assay. (A) Illustration of detection of Aβ aggregation and inhibitor screening in array nanochannels. (B) Schematic diagram of I–V measurement setup. (C) The I–V properties of array nanochannels under different conditions with a scan rate of 100 mV/s. Black line: Aβ immobilized array nanochannels; Red line: metal ions induced Aβ aggregation in array nanochannels; Green line: addition of inhibitor in array nanochannels.