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A sandwich immunoassay for detection of $A\beta_{1-42}$ based on quantum dots

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ABSTRACT

Alzheimer's disease (AD) is the primary cause of dementia over the age of 60, affecting more than 35 million people worldwide. Methods for early diagnosis of AD are critical for the development of effective treatments to combat this debilitating disease. It was confirmed that amyloid-beta peptide 1–42 ($A\beta_{1-42}$) is the biomarker of its early diagnosis. In this work, we present a new sandwich immunoassay method for the detection of $A\beta_{1-42}$ based on quantum dot (QDs) nanolabels and magnetic separation. In the presence of $A\beta_{1-42}$, QDs linked to magnetic beads (MB) via the formation of immune-sandwich complex and can be removed by a magnetic field. And as a result, fluorescence intensity from QDs in the supernatant decreased. Under the optimized conditions, there is a linear relationship between the fluorescence intensity of supernatant solution and the concentration of $A\beta_{1-42}$ from 0.50 to 8.0 nM with a limit detection of 0.2 nM (3σ). This immunoassay was applied to detect $A\beta_{1-42}$ in human cerebrospinal fluid (CSF) successfully.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative pathology causing dementia in humans [1]. With the aging of the population, the incidence of AD increased gradually, it affects more than 35 million people worldwide [2]. The clinical manifestations of AD included a decline in cognitive ability, alterations in behavior, irreversible memory loss, and language impairment [3]. Methods for early diagnosis are critical for the development of effective treatments to combat this debilitating disease [4]. Studies have confirmed that deposition of extracellular amyloid plaque is the main cause of AD [5], and the major component of amyloid plaque is the 42-residue-long peptide ($A\beta_{1-42}$), which has great tendency to misfold and form toxic aggregates. With regard to the close relationship between AD and $A\beta_{1-42}$, $A\beta_{1-42}$ is considered as a promising biomarker for AD [6].

In recent years, some attempts have been made to detect Aβ species in both aqueous solutions and cell derived samples, including enzyme-linked immunosorbent assay [7]; capillary electrophoresis [8]; immunoprecipitation-mass spectrometry [9]; flow cytometry-fluorescence resonance energy transfer assay [10]; surface plasma resonance-immunochip assay [11, 12]; resonance light scattering assay [13] and gold nanoparticle-based dot-blot immunoassay [14]. These methods, however, have some limitations. Some methods need expensive instruments, which limit their applications in routine testing. And the sensitivity of some methods is not very high. It has been found that $A\beta_{1-42}$ concentrations are 0.31 nM for healthy person and 0.16 nM for AD patients [12]. Although the results obtained by different methods are often different, the concentration of $A\beta_{1-42}$ in real samples is very low. It was found that the levels of β-amyloid in human cerebrospinal fluid (CSF) began to decline in the early stage of AD [6]. Therefore, it is highly desirable to develop a sensitive method for $A\beta_{1-42}$ without using expensive instruments.

Quantum dots (QDs) is a kind of excellent fluorescent nanomaterials which has many attractive features, including broad excitation, narrow and symmetric emission, and high fluorescence quantum yield [15–17]. Taking advantage of these properties, QDs have been used in biological applications for analytical determinations and imaging [18–20]. Herein, we develop a sandwich immunoassay for $A\beta_{1-42}$ detection using QDs as nanolabels. Firstly, biotinylated C-terminated antibody specific to $A\beta_{1-42}$ (C-Ab) bound onto streptavidin-coated magnetic beads (MB) via the strong bind between streptavidin and biotin; and then, samples, biotinylated N-terminated antibody (N-Ab), excessive streptavidin modified QDs were added successively. In the presence of $A\beta_{1-42}$, QDs linked with MB and can be removed by a magnetic field. As a result, the fluorescence intensity of the supernatant decreased

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with the increase of the $A\beta_{1-42}$ concentration. Thus, an immunoassay for $A\beta_{1-42}$, with a limit of detection of 0.2 nM was developed. And this assay can be applied for $A\beta_{1-42}$ detection in human cerebrospinal fluid (CSF).

2. Experimental methods

2.1. Chemicals and reagents

DAEFR HDSGY EVHHQ KLVFF AEDVG SNKGA IIGLM VGGVV IA ($A\beta_{1-42}$, purity > 95%) was purchased from China Peptides Co., Ltd. (Shanghai, China). Streptavidin modified magnetic beads (MB) with an average diameter of ca. 1 μm were purchased from Zhengzhou Innosep Biosciences Co., Ltd. (Zhengzhou, China). Biotinylated N-terminal antibody specific to N-terminal of monomeric human $A\beta_{1-42}$ (N-Ab) and Biotinylated C-terminal antibody specific to C-terminal of monomeric human $A\beta_{1-42}$ (C-Ab), human serum albumin (HSA), human IgA (HIgA), human IgM (HIgM) and human IgG (HIgG) were all obtained from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Glucose (Glc) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Lysine (K), Glutamic acid (E), Arginine (R), Tyrosine (Y), Valine (V), Leucine (L), Histidine (H), Phenylalanine (F) and Aspartic acid (D) were all ordered from Beijing Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Bovine serum albumin (BSA) was obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Streptavidin-modified QDs with a maximal emission at 525 nm was purchased from Wuhan Jiayuan Quantum Dots Co., Ltd. (Wuhan, China). Ultrapure water (18.2 MΩ) was prepared with a Milli-Q system (USA) and used in all experiments.

2.2. Preparation of $A\beta_{1-42}$ solution and cerebrospinal fluid samples

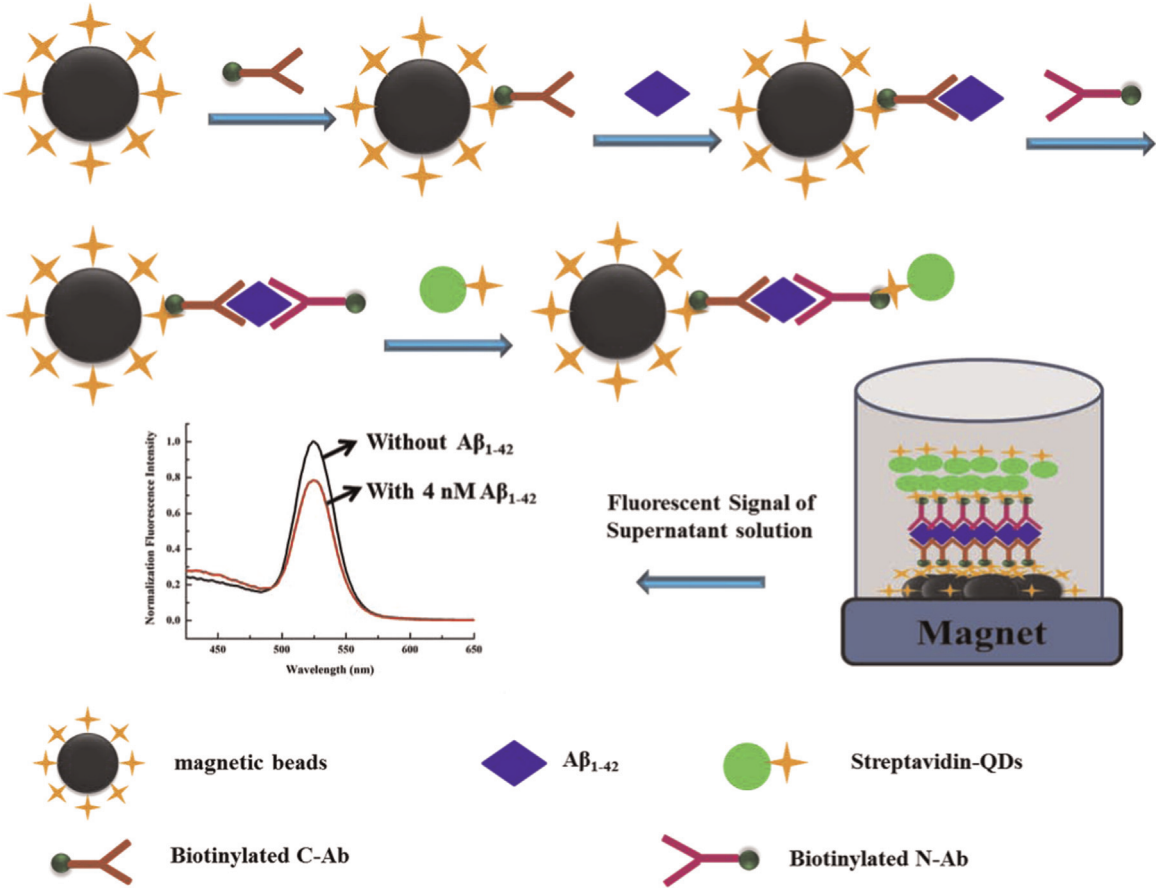
The $A\beta_{1-42}$ monomer solution was prepared immediately before use according to the previous reports [21,22]. Briefly, 1 mg lyophilized $A\beta_{1-42}$ were dissolved in freshly prepared 1 mL NaOH (10 mM) solution, then the solution was centrifuged at 22,000 g for 60 min to remove any insoluble particles. The exact concentration of $A\beta_{1-42}$ monomer was determined using an UV-2450 spectrophotometer (Shimadzu, Japan), according to the maximum absorbance at 276 nm ($\varepsilon = 1410\ M^{-1}\ cm^{-1}$).

The cerebrospinal fluid (CSF) samples were obtained from the Second Affiliated Hospital of Chongqing Medical University and stored at −80 °C until thawed for the sandwich immunoassay. After thawed, the CSF sample was centrifuged at 22,000 g for 60 min to remove the potential aggregates and any impurities. And the patients' consent and approval from the Institutional Research Ethics Committee of Chongqing Medical University were obtained for research purposes.

2.3. Procedure of $A\beta_{1-42}$ detection

The assay was performed in a 48-well microtiter plate. The schematic diagram of the sandwich immunoassay was shown in Scheme 1.

Step 1. Firstly, blocking buffer, *i.e.*, 0.01 M phosphate buffered saline (PBS, pH 7.4, containing 1% BSA) was added into each well of a 48-well plate (1000 μL/well) and incubating overnight at 4 °C. After removing the blocking buffer, 50 μL of 10 mg mL^{-1} (the final concentration is 1.25 mg mL^{-1}) MB were added into each well and washed three times with immune buffer (0.01 M PBS, pH 7.4, containing 0.05% (v/v) Tween 20, 350 μL/well) to remove sodium



Scheme 1. Schematic diagram of the sandwich immunoassay for $A\beta_{1-42}$.