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Near-Field Detection of Aβ Proteins Using Micro Beads

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Abstract

In this paper, we present the possibility of quantification analysis for Aβ captured by micro beads using Near-filed detection. In order to evaluate detection efficiency, detected signals were compared with different sizes of micro beads and a varied number of micro beads. Also, Aβ deposits and Aβ binding to micro beads were measured, therefore, we observed the Aβ deposit and light scattering around the surface of micro beads induced by attached Aβ. This method can be used for quantitative analysis for not only the number of Aβ, but also the binding ratio of Aβ to micro beads.

Keywords : Beta amyloid, Azheimer's disease, Near-filed, Quantitative analysis

1. INTRODUCTION

Alzheimer's disease (AD) is a critical social problem as the number of older aged individuals is increasing in society. It is classified by a common cause of dementia, which is a loss of cognitive function and a behavior disorder. Also, AD is characterized by two types of structures in patient's brains: senile plaques and neurofibrillary tangles [1]. Senile plaques are deposits of Aβ protein in the central nervous system, and neurofibrillary tangles are an accumulation of hyperphosphorylated tau protein in the neuron [2,3]. Many senile plaques in the brain or especially detected in Alzheimer's patients, accelerating nerve cell death. An early diagnosis for AD is the pathologically progressed quantitative detection of Beta-amyloid (Aβ) deposits. The main isoforms of Aβ found in AD patient`s brain are Aβ 40 and Aβ 42 peptides of amino acids. The concentration of Aβ 42 is approximately 10% of that regarding Aβ 40 and the ratio of Aβ 42 to Aβ 40 may be discriminating patients with AD [4].

The detection and real-time monitoring of Aβ, in vivo, is widely used Thioflavin-T (Th-T) [5]. The dye Th-T can be easily binded to Aβ fibril deposits that are 4-10 nm in diameter. Then, it fluoresces strongly with emission at 490 nm. However, Th-T ineffectively passes the blood–brain barrier (BBB) and improved Th-T based compounds have been reported [6]. Meanwhile, detection and capture of Aβ outside the central nervous system has been under study. For example, Aβ deposits are observed within the blood, olfactory bulb, and saliva [7-9]. The results of those studies represent that, in vitro diagnosis of AD is possible. Quantitative detection of Aβ has been attempted to Enzyme-linked immunosorbent assay (ELISA) [10].

In this paper, we propose the possibility of quantification analysis for Aβ using Near-filed detection. Aβ are selectively captured using treated micro beads to form antigen-antibody complexes. Near-field generates the evanescent field upon the surface from the prism with the incident angle of total internal reflection. Micro particles, which are on the evanescent field, generate the illumination through the internal reflections inside the particle. Therefore, micro bead or Aβ deposit does not require any tagging materials for detection in this system. This method can be used for not only quantitative analysis for the number of Aβ, but the binding ratio of Aβ to micro beads as well.

2. DESIGN

2.1 Preparation of Samples

Two different sizes of magnetic micro beads were prepared comparing the signal intensities on particle sizes. 20 μl of magnetic micro beads, diameter of 1 μm (Chemicell® SiMAG-Carboxyl) and 2.8 μm (Dynabead® M-270 Carboxyl), were washed twice with 250 μl 2-Morpholinoethanesulphonic acid (MES , pH 5.7; Sigma®).

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For the reaction with an antibody, micro beads were soaked in 250 μl EDC solution for 5 min at room temperature. EDC was dissolved in 5 μg of N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (Sigma® E6383) in an MES buffer. After washing twice, 5 μl of antibody (Covance® Beta-Amyloid 17-24 Monoclonal Antibody) was mixed in the solution. The mixtures were incubated for 2 hours at 4℃ and residues were removed by washing three times with the MES solution. To block the nonspecific binding, micro beads were incubated in a blocking solution containing 0.5% Bovine serum albumin (BSA) for 2 hours at 4℃. Samples in the blocking solution were washed with pH.7 phosphate buffered saline (PBS) containing tween 20%.

A soluble form of Aβ 40 (Sigma® Amyloid β protein Fragment 1-40) dissolved in a PBS buffer solution. In this study, various amounts of Aβ were mixed with micro beads: 0.03 μg, 0.05 μg, 0.07 μg and 0.1 μg. Also, 10,000 micro beads with a diameter of 2.8 μm were used for easy observation. The process of antigen-antibody reaction carried out for 2 hours at 4℃.

2.2 Sample Setup

The area where particles were illuminated by an evanescent field covered 1.4 mm × 0.4 mm of the surface. To congregate the micro beads at the center of the detection region, a neodymium magnet was settled beneath the cover glass. Samples with diluted water (DI water) were dropped to the glass cover placed above the magnet shaped 1 mm in diameter of the cylinder, then micro beads were smoothly moved to center of the magnet surface. Fig. 1 shows the photograph of congregated micro beads above the glass cover.

In this work, two types of samples were prepared for evaluation. First, detected signals were compared with the different size of micro beads and the varied number of micro beads. This test used micro beads without any treatment process. Two diameter sizes of micro beads, 1 μm and 2.8 μm, were prepared and each number of micro beads was varied from 10,000 to 1,000,000.

Next, Aβ binding to micro beads was tested. 2.8 μm diameter micro beads were used for binding to Aβ. A number of Aβ, which were bound to micro beads were prepared at 0.03 μg, 0.05 μg, 0.07 μg and 0.1 μg. Each Aβ was dropped in 100 μl DI water where 3,333,333 micro beads were contained. Thereby, 0.3 μl of those samples included 10,000 microbeads. After the binding process, each sample was washed three times with PBS to remove the residues of Aβ deposits in solution and DI water was filled.

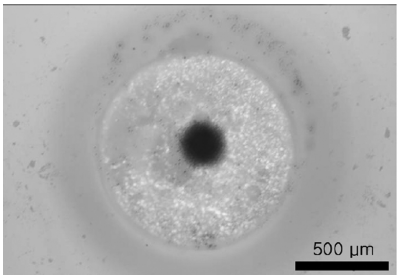

Fig. 1. Photograph of congregated of micro beads by top of cylinder shape magnet.

2.3 Measurement Setup

The schematic of the optical setup is shown in Fig. 2. A conventional microscope (BX-53, Olympus) was used for observations of samples. Laser beam source, a wavelength of 632.8 He-Ne laser (MELLES GRIOT), with normal powers of 10 mW was settled beside the dove prism made by BK7. The prism was optically coupled to the cover glass using the immersion oil (Type-F Olympus). Then, the evanescent field was generated up to 150 nm above the surface of the cover glass. Light that was illuminated from samples reached the CCD image sensor of the microscope and spectrometer (SV2100, KMAC) through the dual port. The spectrometer selectively counted a 632.8 nm wavelength of illumination, which was generated by the evanescent field.

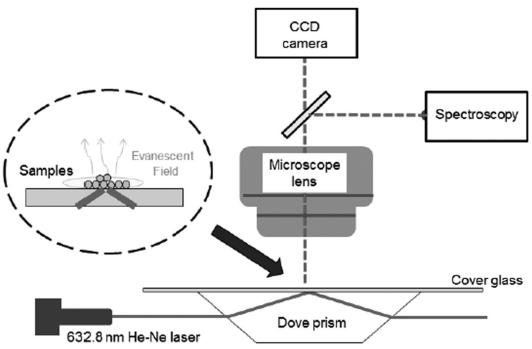

Fig. 2. Schematic of optical setup.

3. RESULTS AND DISCUSSIONS

3.1 Measurement of Varied Number of Micro Beads

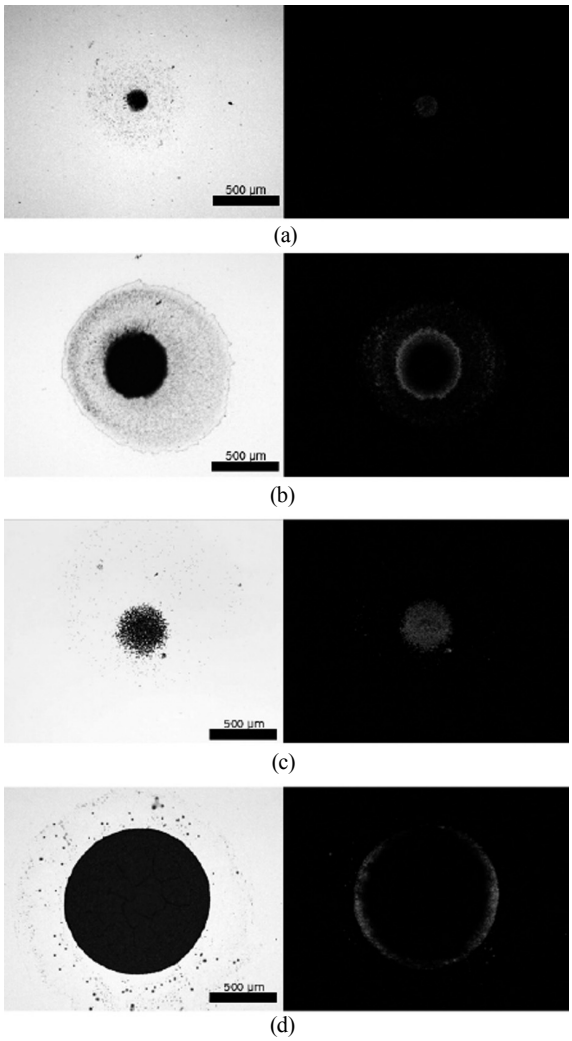

Fig. 3. Photographs of congregated micro beads and their luminescence by the evanescent field. (a) 10,000 1 μm micro beads, (b) 1,000,000 1 μm micro beads, (c) 10,000 2.8 μm micro beads, (d) 1,000,000 2.8 μm micro beads.

Fig. 3 shows the photographs of congregated micro beads and their luminescence by the evanescent field. 2.8 μm diameter micro beads were relatively more congregated than the 1 μm diameter ones. Since magnetic micro beads were worked by magnetic force and surface tension, heavy particles were more affected by magnetic force and easily congregated above the magnet. Previously, micro beads of 100 nm size were detected by illumination lights by this system. On the other hand, it was difficult to congregate using the magnet. Illumination images show that the center region of congregated micro beads was gradually weak with increasing the number of micro beads. Micro beads were overlapped vertically as the number of micro beads increased and illuminations from the bottom of the micro beads were blocked out by the upper ones. According to computation, if micro beads accumulated a body-centered cubic disposition, 10,000 2.8 μm diameter micro beads were mostly placed without overlap, while, 1,000,000 of those were overlapped beyond 17 layers. Also, 1 μm diameter micro beads were assumed to overlap approximately 12 layers when 1,000,000 micro beads were dropped. Fig. 4 shows the signal intensities for different numbers of micro beads. Signal intensity indicated the light intensity at 632.8 nm wavelength detected by spectrometer. The difference of signal intensities between 2.8 μm and 1 μm was due to the scattering cross section. The signal intensities for each size were linearly increased when a small number of micro beads were detected; on the other hand, the increasing ratio was gradually decreased and converged with increasing the sample amounts.

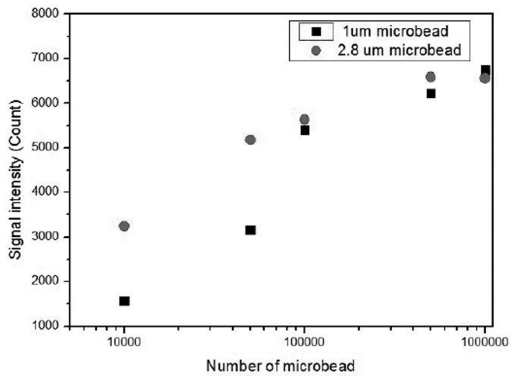

Fig. 4. Measured signal intensity with 1 μm and 2.8 μm micro beads.

3.2 Measurement of Aβ Binding to Microbeads

Before evaluating the signal intensity of Aβ binding to micro beads, varied numbers of Aβ were induced by the evanescent wave. Illuminations of Aβ deposits, approximately a 4 nm sized structure, were successfully observed and the measurement results with a varied number of Aβ were shown in Fig. 5. Each result was the average value from 5 times repetition. Signal intensity measured from micro beads illumination was increased when the number of micro beads was increased.

Fig. 6 shows the measurement of signal intensities that 10,000 2.8 μm diameter micro beads were treated with a different number of Aβ. Signal intensities of each sample were relatively high compared with the signal intensity of micro beads without Aβ. From this result, derived signal intensities composed the illumination of micro beads and Aβ by the evanescent field. However, signal intensity was

not increased when a greater number of Aβ was treated. It seems that the number of Aβ was too great for binding to micro beads. As all micro beads have to bind to a maximum number of Aβ, the signal intensity were measured a constant value shown in Fig. 6. According to the measurement result of Aβ detection shown in Fig. 5, binding ratio of a 10,000 number of 2.8 μm micro beads and Aβ was saturated from 0.01 μg Aβ.

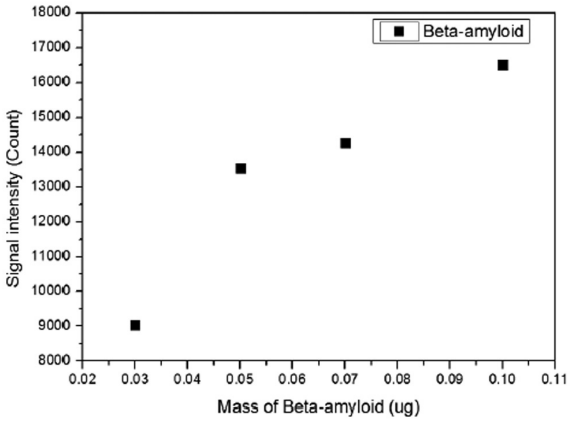

Fig. 5. Measurement of signal intensities with increasing number of Aβ.

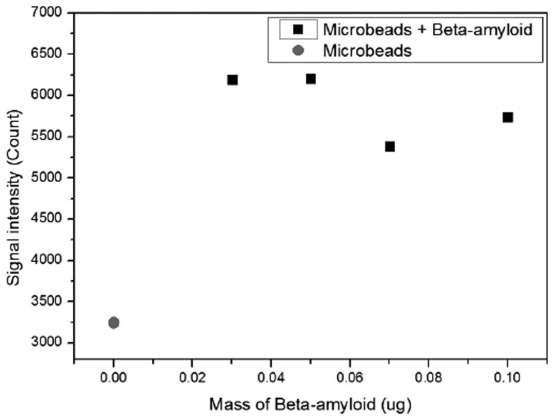

Fig. 6. Measurement of signal intensities for 2.8 μm diameter micro beads with EDC treatment and micro beads after binding to a different number of Aβ .

Fig. 7 shows the photographs of micro bead samples. Fig. 7 (a) were the micro beads with EDC treatment and its illumination image is shown in Fig. 7 (b). On the other hand, Fig. 7 (c) shows the micro beads after being treated with Aβ. Also, Fig. 7e (d) shows its illumination image and differing from Fig. 7 (b), the light scattering around the surface of micro beads were appeared. It means that some interactions occurred on the surface of micro beads.

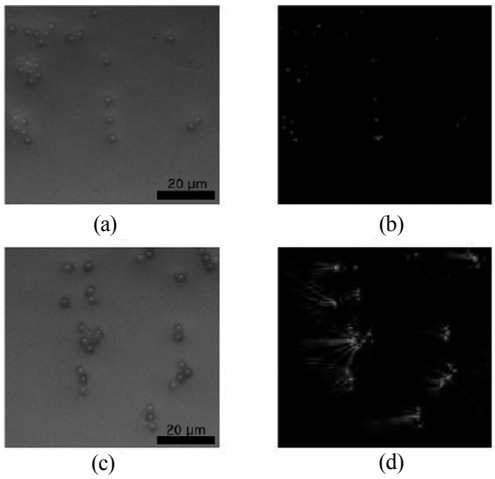

Fig. 7. Photograph of samples. (a) Micro beads only, (b) Illumination of micro beads, (c) Micro beads with Aβ, (d) Illumination of micro beads with Aβ.

4. CONCLUSIONS

In this work, we have investigated the analysis of Aβ using the Near-field detection for the accurate in vitro quantification of the AD pathogenic protein. Micro beads were used to capture Aβ and luminescence by the evanescent field detection. Comparing the detection signals of Aβ deposits and micro beads binding to Aβ, we found the constraint of the binding ratio concerning micro beads and Aβ. It supposed that detecting Aβ binding to various sizes of micro beads can help the numerical analysis of Aβ binding on the surface of micro beads.

Based on the above techniques such as the antigen-antibody reaction and Near-field detection, this system can be applied to the microfluidic system.

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