Optical evaluation of immunocomplex of liquid crystal bound amyloid beta-42 levels associated with Alzheimer's disease

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Abstract

We present an optical biosensor in this study which is based on the LC orientation as a function of the peptide concentration to detect amyloid-beta-42 (Aβ42) antigen-antibody binding events. These binding of Aβ42 peptide to an Aβ42 antibody which is on a Dimethyloctadecyl[3-(trimethoxysilyl)propyl] ammonium chloride (DMOAP) occurs on an immobilized surface forming an immunocomplex. The disturbed orientation of LCs as a result of the binding of the formed immunocomplex observed using the polarized optical microscope. The microscopic and optical response of the LC-immunocomplex was determined as a function of decreasing Aβ42 peptide concentration. Aβ42 peptide concentrations as of 1000 pg/ml, 500 pg/ml, 200 pg/ml, 100 pg/ml, 50 pg/ml, 25 pg/ml, 20 pg/ml, 15 pg/ml, 10 pg/ml, 5 pg/ml, and 1 pg/ml, respectively were interacted with the optimum Aβ42 antibody concentration as of 25 μg/ml. There was a remarkable change in the reflection spectra of the samples with the decreasing Aβ42 peptide concentration. The concentration as low as 1 pg/ml of Aβ42 peptide was able to successfully detected in our system.

INTRODUCTION

Liquid crystals (LCs) are excellent detection materials in which the intrinsic properties of long-range orientational order and short-range molecule interactions can be used to convert biomolecular binding events into visible macroscopic optical signals (Brake, Daschner, Luk, & Abbott, 2003; Gupta, Skaife, Dubrovsky, & Abbott, 1998; Tan, Li, Liao, Yu, & Wu, 2014). These materials have unique properties such as self-orientation, dielectric, optical anisotropy and light-transmitting (Gupta et al., 1998; Kim, Kim, Kim, Oh, & Choi, 2005; S. Yang et al., 2012). Furthermore, the orientational changes of the LCs can be easily observed under a polarized optical microscope due to their unique birefringence (Artiga et al., 1998; Koenig Jr et al., 2009; Li, Li, Yang, Chen, & Xiong, 2015). Therefore, the LC-based biosensor system has been recently became a promising platform for chemical and biological detection, which can be used for real-time and unlabeled detection with high sensitivity and without any complex techniques (Li et al., 2015; Zapp, Westphal, Gallardo, de Souza, & Vieira, 2014). In 1998, Abbott and coworkers initiated a field of study using LCs as sensing elements in the detection of biomolecules (Gupta et al., 1998; S. Yang et al., 2012). In recent days, LC-based biosensors are widely used in organophosphate detection, enzymatic activity assays, bacteria, and viruses detection and to investigate protein-protein binding events (Cadwell et al., 2007; Chen & Zhong, 2013; Clare & Abbott, 2005; Li et al., 2015; Park & Abbott, 2008; K.-L. Yang, Cadwell, & Abbott, 2005; Zhong & Jang, 2014; Zhu, Shih, & Shih, 2013)

LC-based biosensors are widely used in the detection of neurodegenerative disorders, such as Parkinson's, Alzheimer's diseases. Alzheimer's disease (AD) is an age-related, progressive and unremitting form of neurodegenerative disease that is estimated to affect 1 in 85 people worldwide by 2050 (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007; C.-C. Liu, Kanekiyo, Xu, & Bu, 2013; Masters & Bateman, 2015). This disease induces severe loss of memory and cognitive decline, selective neuron death, and abnormal plaque

formation in the cerebral cortex (Hardy & Selkoe, 2002; Y. Liu et al., 2014; Yu et al., 2015; Zhao, Wu, Xie, Ke, & Yung, 2010). However, thus far, no effective treatment for Alzheimer's disease has been found and developed (Yu et al., 2015). The neuropathological hallmarks of AD are the deposition of extracellular neuritic plaques containing amyloid-beta peptide (Aβ) and intracellular neurofibrillary tangles (NFTs) (L. Liu et al., 2013; Rauk, 2009; Rolinski, Amaro, & Birch, 2010; Yu et al., 2015). Experimental evidence from both in vitro and in vivo studies have shown that amyloid-beta (Aβ) aggregation induces pathogenic cascade that leads to neuronal loss and dementia at the onset of Alzheimer's disease (Glenner & Wong, 1984; Masters & Bateman, 2015; Yu et al., 2015). Aβ fragment is a normal product of Amyloid Precursor Protein (APP) metabolism (Masters & Bateman, 2015). APP is a transmembrane protein located in neuronal and glial cells of the brain (Müller & Zheng, 2012). Under physiological conditions, the APP sequentially cleaved by α secretase and β secretase enzymes, resulting in the formation of extracellular non-toxic soluble fragment and APP intracellular domain (AICD) (Overk et al., 2014; Schott et al., 2016). Under pathological conditions, the APP protein is cleaved by β secretase (BACE) and γ secretase enzymes and extracellular amyloid- beta-42 (Aβ42) fragment monomers are formed (Cole & Vassar, 2008). These Aβ42 monomers then draw closer to form β-sheets structure (Breydo et al., 2016). The sheets ultimately form ordered fibrils known as toxic Aβ42 plaques (Roeters et al., 2017). The formed Aβ42 plaques accumulate in the synapses of the brain, leading to loss of signal transmission between the connecting neurons (Haass & Selkoe, 2007; Madav et al., 2019). Therefore, the Aβ42 monomer and its aggregates are considered promising important biomarkers in plasma and cerebral spinal fluid (CSF) for the diagnosis and prognosis of AD (Ammar et al., 2013; Choi, Islam, Lee, Song, & Oh, 2011; Doong, Lee, & Anitha, 2010; Golde, Eckman, & Younkin, 2000; Haes, Chang, Klein, & Van Duyne, 2005; Li et al., 2015; L. Liu et al., 2013). According to recent clinical studies, it is known that the Aβ42 level in the plasma of Alzheimer's patients is 36-140 pg/ml, and the Aβ42 level in the CSF is 652 ± 235 pg/ml (Fagan et al., 2009; Roher et al., 2009). Currently, the detection of Aβ42 is performed using different techniques, such as mass spectrometry (MS), scanning tunneling microscopy (STM), surface plasmon resonance (SPR), electrophoresis and the enzyme-linked immunosorbent assay (ELISA) (Dai, Molazemhosseini, & Liu, 2017; Haes et al., 2005; Hestekin, Kurtz, & Lutz-Rechtin, 2014; Kang, Lee, Oh, & Choi, 2009; Picou, Moses, Wellman, Kheterpal, & Gilman, 2010; Wang, Sweeney, Gandy, & Sisodia, 1996). ELISA technique is commonly used for Alzheimer's detection due to its high sensitivity, selectivity and reliability features. However, this method is expensive, time-consuming, labor-intensive, elaborate and requires a skilled operator (Dai et al., 2017; Yu et al., 2015). The fact that the current techniques used for the detection of Aβ, both physiologically and pathologically, has many disadvantages that encourage the researchers to develop a biosensor which is faster, more sensitive, selective and easily applicable detection on the patient.

In this study, we have established a new LC-based approach for the highly sensitive detection of Aβ42. In the first stage of our study, Dimethyloctadecyl[3-(trimethoxysilyl)propyl] ammonium chloride (DMOAP) was coated on a glass surface of microscope slides in order to obtain the vertical alignment of LC molecules. The LC molecules were then dropped onto DMOAP coated glass slides and a LC film was formed. The different concentrations of Aβ42 antibody and Aβ42 peptide were prepared. Afterward, the Aβ42 antibody immunocomplex was formed immobilizing the Aβ42 antibody and Aβ42 peptide on the LC film surface. The binding of immunocomplex to LC molecules leads to a change in the orientation of the LC which can be observed via a polarized optical microscope (POM). After observation of the optical textures by using POM, spectrometric analysis was performed between 200nm and 900 nm via Ocean Optics spectrometer and the detection limit of our biosensor was determined.

MATERIALS AND METHODS

Materials. Microscope glass slides (15 mm × 20 mm) were received from Mesostate (Taiwan). Dimethyloctadecyl[3-(trimethoxysilyl)propyl]ammonium chloride (DMOAP) as the aligning agent dissolved in methanol was purchased from Sigma–Aldrich. Dimethyl sulfoxide (DMSO) and 4-Cyano-4'-pentylbiphenyl (5CB) were received from Merck. Anti-beta Amyloid - 42 antibody (ab10148) was obtained from Abcam. Amyloid β Protein Fragment 1-42 (A9810) was purchased from Sigma-Aldrich.

Instruments. Optical observations were performed on Nikon CIPOL-50X-1000X EPI-DIA Polarized Optical Microscope (USA). Reflection analysis were performed on Ocean Optics Spectrometer USB2000+(UK).

Preparation of DMOAP-Coated Microscope Glass Slide and Formation of the LC Film. Glass slides were cleaned in distilled water (deionized), IPA (Isopropyl alcohol) and ethanol by ultrasonication for 15 min at room temperature. The glass slides were dried under the stream nitrogen and then baked at 80°C for 15 min. After the cleaning process was completed, the slides were immersed in an aqueous solution of 1% (v/v) DMOAP for 15, 30 and 60 minutes followed by rinsing with DI water for removing the excess DMOAP. Subsequently, the glass slides were dried again with nitrogen and baked at 80°C for 15 min. The slides left in the oven during the day to cool down to room temperature. The purpose of this step is to provide the homeotropic alignment of LC molecules on the glass slide. The next step is the formation of LC films. Prior to the addition of the Aβ42 antibody on the DMOAP-coated glass slides, 2 µl 5CB was dropped on these glass slides to observe the alignment of LC molecules which are intended to provide a homeotropic alignment on the glass surface with the help of DMOAP. Thus, a LC film was successfully formed on DMOAP coated glass slide.

Ιμμοβιλιζατιον οφ Αμψλοιδ βετα-42(Αβ42) Αντιβοδιες ανδ Φορματιον οφ Αμψλοιδ βετα-42 (Αβ42) Αντιβοδψ-Αντιγεν Ιμμυνοζομπλεξ. Aβ42 antibody and Aβ42 peptide of specific concentrations were prepared. In the first step, aqueous solutions of the Aβ42 antibody were diluted with DI water. The diluted Aβ42 antibody solution was immobilized by dispensing 1 µl droplet of Aβ42 antibody (at 100 µg/ml, 50 µg/ml, 25 µg/ml, 20 µg/ml, 10 µg/ml, 1 µg/ml) on the formed LC films. These antibodies were incubated overnight at room temperature. Afterward, Aβ42 peptide was firstly dissolved in dimethyl sulfur oxide (DMSO) at a concentration of 1mg/ml. The particular Aβ42 peptide concentration was prepared by diluting in 10 mM sodium phosphate buffer (Na-PB)(pH 7.5). Then, the diluted Aβ42 peptide solution (25 pg/ml, 50 pg/ml, 100 pg/ml) was reacted with the determined antibody concentrations for 30 minutes, 60 minutes and 90 minutes in order to determine the optimum antibody concentration. The optimum time was determined as 1.5 hours. As a result of these steps, an Aβ42 antibody-antigen immunocomplex was formed as seen in Figure 1. After the optimum antibody concentration was selected, different Aβ42 peptide concentrations of 1000 pg/ml, 500 pg/ml, 200 pg/ml, 100 pg/ml, 50 pg/ml, 25 pg/ml, 20 pg/ml, 15 pg/ml, 10 pg/ml, 5 pg/ml, 1 pg/ml were reacted with the optimum antibody concentrations, respectively.

Optical Analysis. Optical textures were determined via a polarizing optical microscope and changes in LC orientation resulting from antigen-antibody binding were observed using an optically polarizing light microscope. All changes in the structure of LC were captured by a CMOS (complementary metal-oxide-semiconductor) camera attached to the microscope using a 10X objective lens (Nikon). The reflectance spectra of all samples were determined using an Ocean Optics spectrometer. Spectral experiments were carried out at room temperature in the range of 200-900 nm of wavelength. The experimental results showed that all the samples have a maximum reflectance peak at 547 nm.

RESULTS AND DISCUSSIONMicroscopic Analysis

Investigation of DMOAP coated Glasses

To observe the success of DMOAP coatings, 2 µl of 5CB liquid crystal was dropped onto DMOAP- coated microscope glass slide. Figure. 2 a. shows the LC structure can be seen on the non-coated DMOAP glass slide. Figure 2 b, c, d show the LC structure cannot be seen on the DMOAP coated glass slides, since LC molecules have homeotropic orientation through the DMOAP coating.

Δετερμινατιον οφ Οπτιμυμ Αντι-αμψλοιδ βετα-42 (Αβ42) Αντιβοδψ ὃνζεντρατιον

In order to determine the optimum antibody concentration, six different samples, which are shown in Table 1, were prepared and then each sample was dropped onto the LC film surface. Before Aβ42 antibodies interacted with Aβ42 peptides, DMSO was dropped onto the Aβ42 antibody surface. No effect of DMSO on the orientation of liquid crystals has been observed. Subsequently, antibodies at these six different

concentrations were interacted with three Aβ42 peptide concentrations of 100 pg/ml, 50 pg/ml and 25 pg/ml, respectively.

The formed antibody-antigen immune complex leads to a change in the orientation LCs from homeotropic to a random one. The changes corresponding to the random orientation of the LC molecules were observed by a polarized optical microscope (POM). The POM images showed that a notable change when the samples including the Aβ42 peptide concentrations of 100 pg/ml, 50 pg/ml, 25 pg/ml interacted with 100 μg/ml, 50 μg/ml and 25 μg/ml Aβ42 antibody concentrations as seen in Figure. 3, Figure. 4, Figure 5. The POM images prove that 25 μg/ml Aβ42 antibody concentration can be selected (Sample 3) as the optimum antibody concentration.

Then the selected optimum antibody concentration was interacted with different Aβ42 peptide concentrations as of 1000 pg/ml, 500 pg/ml, 200 pg/ml, 100 pg/ml, 50 pg/ml, 25 pg/ml, 20 pg/ml, 15 pg/ml, 10 pg/ml, 5 pg/ml, and 1 pg/ml, which are listed in Table 2, to determine the detection limit of the biosensor.

Δετεςτιον οφ Αμψλοιδ βετα-42 (Αβ42) Πεπτιδε ὃνςεντρατιον

1 μl of 25 μg/ml anti- Aβ42 antibody was immobilized on the glass with DMOAP alignment layer and then interacted with various concentrations of Aβ42 peptide for 1.5 hours at room temperature. Figure 6 and Figure 7 show that the changes in the orientation of LC direction determined via a POM. In the absence of peptides or antibodies, the images as seen in Figure 6a-e & Figure 7f-k were black because the alignment of nematic LCs was perpendicular to the glass surface with the DMOAP monolayer. Figure. 6a1-e1 & Figure 7f1-k1 show that the immobilized Aβ42 antibodies were insufficient to change the homeotropic alignment of LCs. As the Aβ42 peptide concentration was gradually increased, the number of antigen-antibody immunocomplexes also increased, which caused significant orientational changes in LCs alignment due to their larger size of the immunocomplex (Figure 6a2-e2 & Figure 7f2-k2). Therefore, the optical textures of LCs have changed significantly to reflect the amount of available immunocomplexes. This LC - based optical biosensor was examined to determine the minimum concentration of Aβ42 peptide that could induce an orientational change of LCs. The biosensor was incubated with Aβ42 peptide concentrations in the range of 1000 pg/ml - 1 pg/ml. The change in LC optical textures was considerable when the biosensor was incubated with all the concentrations of Aβ42 peptide as shown in Fig. 6 and Fig. 7. Moreover, these results indicate that a minimum concentration of 1 pg/ml Aβ42 peptide was required to change of LCs orientational on the surface. Therefore, the LOD of this LC-based optical biosensor was determined as 1 pg/ml Aβ42 peptide. Dai and co-workers fabricated that simple in vitro biosensor for the detection of β-amyloid 42 in phosphate-buffered saline (PBS) and undiluted human serum. The concentration range of β-amyloid 42 in this study was from 0.0675 μg/ml to 0.5 μg/ml. Yu and co-workers have been developed gelsolin-bound-Aβ1–40/1–42 detection assay for detecting level variations of Aβ1–40/1–42 associated with AD progress. Compared to other studies, a very low detection limit was achieved in our optical sensor.

Spectroscopic Analysis

Reflection spectra of all the samples including LCs bonded antigen-antibody immunocomplexes were determined at right angles from the samples with an Ocean Optics spectrometer (USB2000+). These reflection spectra of all samples were determined as a function of the Aβ42 peptide concentration.

With the addition of Aβ42 peptide concentrations on the 25 μg/ml Aβ42 antibody, the reflectance percentages of the samples were gradually increased as shown in Figure 8 Reflection value in the presence of 1000 pg/ml Aβ42 peptide was obtained as 59.68%, when the Aβ42 peptide concentration was decreased up to 1 pg/ml it was shown that the reflection value was obtained as 9.48%.

CONCLUSION

In summary, a LC-based biosensor for the detection of a biomarker of neurodegenerative disease, Aβ42 peptide, has been developed in this study. The optimum antibody concentration to be used for the detection of the Aβ42 peptide was determined at 25 μg/ml and it was immobilized on the glass with DMOAP alignment

layer. Subsequently, optimum antibody concentration was interacted with various Aβ42 peptide concentrations between 1000 pg/ml and 1 pg/ml. The changes in the orientation of LC direction induced by the antigen-antibody binding events was observed via a POM. Optical measurements of these LC samples were performed by using Ocean Optics spectrometer. According to all these results, the lowest detectable Aβ42 peptide concentration was determined as 1 pg/ml. The optical results showed that there was a 50% reduction in the reflection samples with the decreasing Aβ42 peptide concentration from 1000 pg/ml to 1 pg/ml. The decrement in the Aβ42 peptide leads a less immunocomplex formation. As a result of the studies, an optical biosensor with high sensitivity and selectivity has been developed.

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