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Development of liquid crystal biosensor for the detection of amyloid beta-42 levels associated with Alzheimer's disease

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This study represents the development of a biosensor which is based on the liquid crystal (LC) orientation as a function of the peptide concentration to detect an amyloid-beta-42 (Aβ42) antibody–antigen binding events. The Aβ42 peptide binds to the Aβ42 antibody forming an immunocomplex which is immobilized on the Dimethyloctadecyl[3-(trimethoxysilyl)propyl] ammonium chloride (DMOAP) coated surface. The disturbed orientation of LCs as a result of the binding of the formed immunocomplex was observed using the polarized optical microscope (POM) as a function of decreasing Aβ42 peptide concentration from 1000 to 1 pg/ml. The concentration, as low as 1 pg/ml of Aβ42 peptide was able to be successfully detected in our system. Apolipoprotein E4 (ApoE4), that specifically bound to the Aβ42 peptide, was added into the system and a remarkable change in reflection spectra of samples was observed with increasing Aβ42 peptide concentration. The concentration of ApoE4 protein was detected in the range of 0.1–30 nM by this system due to the interaction between the two proteins.

[**Key words:** Liquid crystal; DMOAP coating; Liquid crystal biosensor; Amyloid beta-42; Apolipoprotein E4]

Liquid crystals have recently become promising and fascinating materials for chemical and biological biosensing due to the sensitivity that produces a rapid, easily-visualized response (1). They have a great potential in the field of innovative biosensing applications as a sensing material based on their intrinsic properties such as self-orientation, long-range anchoring transitions, and short-range molecule interactions which can be used to convert biomolecular binding events into visible macroscopic optical signals (2–5). Biosensors based on the orientational changes of the LCs have been used in endotoxin (6), organophosphate detections (7–9), enzymatic activity assays (10,11), bacteria, and virus detection (6) as well as protein–protein and protein-ligand binding events (12,13). LC-based biosensor detections can be performed in ambient light in the absence of electrical energy or molecule labeling (14). Furthermore, optical signals can be readily monitored by the naked eye instead of complicated instruments (15). These substantial features of LC-based biosensors are suitable for easy detection, direct transmission, high speed and sensitivity operation, and low cost biological analysis (1,14).

Various LC-based biosensors have been reported for the detection of biological and chemical binding events for decades. An and Jang (15) demonstrated a new method for detecting cysteine in an aqueous interface by developing an LC biosensor. They observed lauroyl chloride and cysteine reaction at the aqueous/LC interface using lauroyl-doped LC as a cysteine indicator (15). Kim and Jang (1) developed an aptamer based biosensor using LC materials for interferon-γ detection in human blood and tuberculosis diagnosis. The binding of interferon-γ to the aptamer surface caused a change in LC orientation from a homeotropic to a random orientation (1). Su et al. (16) reported an improvement of an LC-based immunodetection system for the CA125 cancer biomarker. In this system, CA125 antibodies were immobilized on the ultraviolet modified DMOAP layer and CA125 antigen immunodetection on these layers showed a lower limit of detection (LOD) leading to an important change in optical texture of LC (16). These studies have paved the way for their use in the field of neurodegenerative disorders such as Parkinson's and Alzheimer's diseases (AD) (17–19). The neuropathological features of AD are the deposition of extracellular neuritic plaques involving the amyloid-beta peptide (Aβ) and intracellular neurofibrillary tangles (NFTs) (20–24) and for this accumulation ApoE4 protein is a primary risk factor. ApoE protein is an important serum protein involved in cholesterol storage, transport, and metabolism. There have been limited studies attempting to clarify the underlying mechanism of this increased risk and they reported that the development of competent methods to monitor the process from monomer to fibril accumulation of Aβ under physiological conditions based on the interaction of ApoE4 protein and Aβ42 peptide can make a significant contribution to the diagnosis of AD (25,26). However, many researchers focused on understanding the relationship between AD and Aβ42. Experimental evidence from both in vivo and in vitro studies have indicated that aggregation of Aβ induces pathogenic cascade, which

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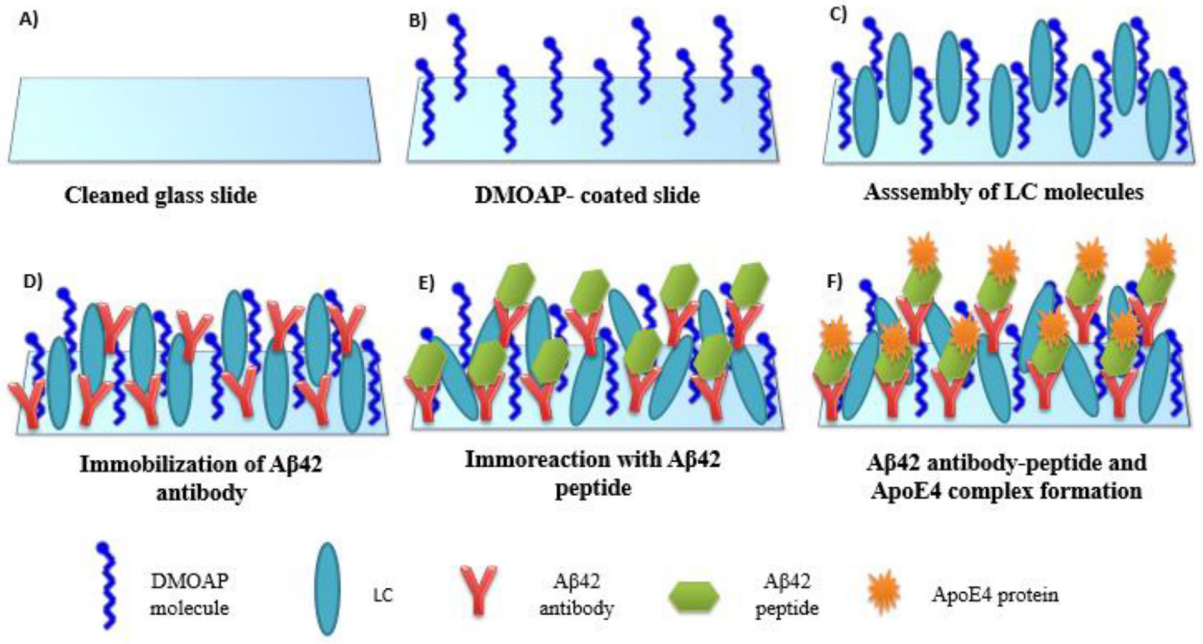

FIG. 1. Sample preparation procedure of liquid crystal (LC)-based immunodetection biosensor. (A) Cleaning the glass slides, (B) coating of glass slides with DMOAP alignment layer, (C) assembly of the LC molecules on the DMOAP coated glass slide, (D) immobilization of Aβ42 antibody on the assembled LC molecules, (E) immunoreaction with the Aβ42 antibody and peptide, (F) Aβ42 antibody-peptide and ApoE4 complex formation.

causes neuron loss and dementia at the onset of AD (19,20,27). Aβ fragment is a normal product of metabolism of amyloid precursor protein (19), which is a transmembrane protein and that is located in the brain's neuronal and glial cells (28). Therefore, the Aβ42 monomer and its aggregates are considered promisingly important biomarkers in plasma and cerebral spinal fluid for the diagnosis and prognosis of AD (29–33). 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 cerebral spinal fluid is 652 ± 235 pg/ml (34,35). Currently, the detection of Aβ42 is performed using different techniques such as mass spectrometry, surface plasmon resonance, electrophoresis and the enzyme-linked immunosorbent assay (ELISA), electrochemical method (33,36–40). ELISA technique is commonly used for Alzheimer's detection due to its high sensitivity, selectivity and reliability features. However, this method is costly, time-consuming, labor-intensive, elaborate, and requires highly skilled personnel (20–36). The current techniques used for the detection of Aβ, both physiologically and pathologically, have many disadvantages that encourage the researchers to develop a biosensor which is a faster, more sensitive, selective, economic way and easily applicable detection on the patient.

In this study, we have developed a new LC-based biosensor for the highly sensitive detection of Aβ42. In the first stage of our study, DMOAP was coated on microscope glass slides surfaces in order to induce the homeotropic/perpendicular alignment of LC molecules. Then an LC film was formed by dropping LC molecules onto DMOAP-coated microscope glass slides. In the second stage of our study, the different concentrations of Aβ42 antibody, Aβ42 peptide and ApoE4 protein were prepared. Afterward, ApoE4 protein was bound to the Aβ42 peptide forming the Aβ42 antibody–antigen-ApoE4 complex and this complex was immobilized on the LC film surface. The changes in the optical textures of LC orientations due to the binding of complex on the LC surface was observed by using polarized optical microscope (POM), spectrometric analysis was performed via Ocean Optics spectrometer (Ocean Optics, Dunedin, FL, USA) and the LOD of the biosensor was determined.

MATERIALS AND METHODS

Materials and reagents All the chemicals were commercially available materials. The nematic LC of 4′-pentyl-4-biphenylcarbonitrile (5CB) was supplied from Sigma Aldrich (St. Louis, MO, USA). Microscope glass slides were in the dimensions of 15 mm × 25 mm (Sail Brand, China). Dimethyloctadecyl[3-(trimethoxysilyl)propyl]ammonium chloride (DMOAP) used as the aligning agent dissolved in methanol was purchased from Sigma–Aldrich. Dimethyl sulfoxide (DMSO) was received from Merck (Kenilworth, NJ, USA). Anti-beta Amyloid-42 antibody (ab10148) was obtained from Abcam (Cambridge, UK), whereas Amyloid-β Protein Fragment 1–42 (A9810) and Apolipoprotein E4 human (A3234) were purchased from Sigma–Aldrich.

Instruments Optical observations were performed on Nikon CIPOL-50X-1000X EPI-DIA polarized optical microscope (Nikon, Tokyo, Japan). Reflection analysis were performed via Ocean Optics Spectrometer USB2000+ (Ocean Optics).

DMOAP coating of glass slides Glass slides were cleaned with distilled water (deionized), 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, the slides were immersed in an aqueous solution of 1% (v/v) DMOAP for 90 min and then rinsed with distilled 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 were 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 which was already coated with DMOAP. 5CB (2 μl) was dropped on the glass slide to observe the alignment of LC molecules, which are intended to provide a homeotropic alignment on the glass slide surface coated with DMOAP.

Formation of Aβ42 antibody–antigen-ApoE4 complex The samples containing Aβ42 antibody and Aβ42 peptide were prepared at different concentrations. In the first step, aqueous solutions of the Aβ42 antibody were diluted with distilled water (16) and 1 μl droplet of the diluted Aβ42 antibody solutions at the concentrations of 10 μg/ml, 25 μg/ml, 50 μg/ml, and 100 μg/ml were immobilized on the formed LC films. These antibodies were incubated overnight at room temperature. The POM images prove that 25 μg/ml Aβ42 antibody concentration can be selected as the optimum antibody concentration. Afterward, Aβ42 peptide was first dissolved in the solvent of DMSO at a concentration of 1 mg/ml. The particular Aβ42 peptide concentration was prepared by diluting in 10 mM sodium phosphate buffer (Na-PB at pH 7.5). Then, the diluted Aβ42 peptide solutions at the concentrations of 1 pg/ml, 5 pg/ml, 10 pg/ml, 25 pg/ml, 50 pg/ml, 100 pg/ml, 500 pg/ml, and 1000 pg/ml were reacted with the optimum antibody concentrations for 90 min in order to determine the LOD of biosensor. The formation of the antibody–antigen immunocomplex leads to a change in the orientation LCs when it was observed under the POM. Moreover, ApoE4 protein concentration is important to understand the degree of specific binding of ApoE4

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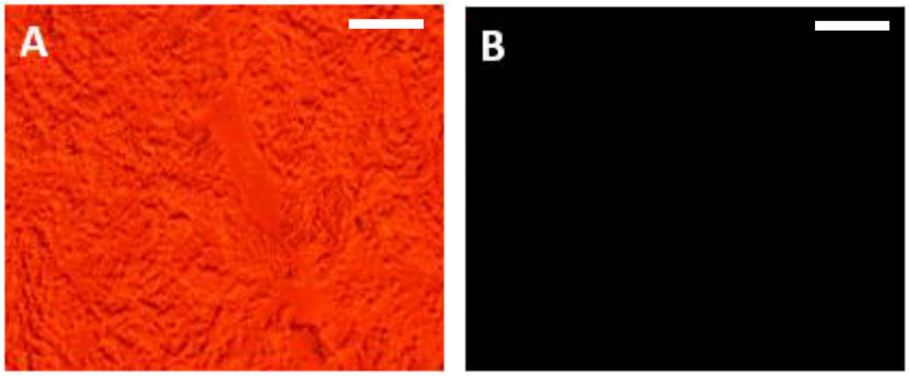

FIG. 2. The POM images of LC on the non-coated DMOAP glass slide (A) and on the DMOAP- coated glass slide (B). Scale bar: 10 μm.

protein to Aβ42 peptide. ApoE4 protein was dissolved in distilled water and then diluted in 10 mM sodium phosphate buffer before adding in the immunocomplex system. ApoE4 protein solutions (1 μl) prepared at five different concentrations of 30 nM, 20 nM, 10 nM, 3 nM, and 0.1 nM were added in the antibody–antigen immunocomplex. The formation of the complex consisting of the ApoE4 protein solution that specifically binds to the Aβ42 antibody–antigen is illustrated in Fig. 1. Fig. 1B shows DMOAP molecules bind chemically to the clean glass slide surface and produce a uniform monolayer coating. DMOAP includes 18 units long alkyl chains which extend perpendicularly from the surface. Therefore, DMOAP forms two-dimensional lattice on the glass slide surface. Alkyl chains have a lot of empty space between them. The molecules of the biphenylcarbonitrile LC penetrate between the DMOAP alkyl chains and are aligned parallel to them, as shown in Fig. 1C. The antibody was then added to the immunodetection system. Antibodies adsorb to the DMOAP monolayer, thus triggering a slight transition in the LC optical texture (Fig. 1D). As indicated in Fig. 1E, the mechanism of this LC-based system is based on a significant orientational change of the LCs that is caused by the binding of the Aβ42 peptide to an Aβ42 antibody bound surface. The Aβ42 antibody-Aβ42 peptide binding occurs between the epitope in the antigen and the paratope in the antibody to form an immunocomplex. The form of Aβ42 antibody–antigen immunocomplex leads to a change in the significant orientation of the LC. In order to determine ApoE4 protein concentration by using this immunodetection system, ApoE4 protein that specifically binds to the Aβ42 peptide was added to the immunodetection system (Fig. 1F).

Optical analysis Optical textures of the formed immunocomplex were determined via a POM as a function of the changes in LC orientation resulting from antibody–antigen binding at the interface. All changes in the orientation of LC were captured by a complementary metal-oxide-semiconductor (CMOS) camera attached to the microscope using a 10× objective lens (Nikon). The reflectance spectra of all immunocomplex samples were determined using an Ocean Optics spectrometer at right angle (90°). Spectral experiments were carried out at room temperature in the range of 200–900 nm of wavelength. The experimental results showed that all the immunocomplex samples have a maximum reflectance peak at 547 nm.

RESULTS AND DISCUSSION

Determination of optimum Aβ42 antibody and Aβ42 peptide concentrations In the first step of the study, the assembly of the LC molecules on the DMOAP coated glass slide was determined via POM to understand the Aβ42 peptide and antibody concentration effect on the LC orientation. For this purpose, LC was dropped onto DMOAP-coated microscope glass slide and the textures of DMOAP coated and non-coated glass slides were compared with each other to recognize the DMOAP coating effect. Fig. 2A shows the LC texture on the non-coated DMOAP glass slide, whereas Fig. 2B shows LC texture on DMOAP-coated glass slide. The texture in Fig. 2B shows dark since LC molecules have homeotropic orientation through the DMOAP coating.

The determination of Aβ42 antibody concentration is important to understand the required concentration to maintain the homeotropic orientation of the LCs. After the DMOAP coating of glass slides, the optimum antibody concentration was determined. In order to determine the optimum antibody concentration, one drop of four different samples left on the DMOAP glass slides and the changes in the LC orientations were observed as shown in Fig. 3. All of the bright leakage in the images are the regions where the immunocomplex was formed. These regions were seen in a round shape. No leakage was observed in the region where immunocomplex was not formed. As shown in Fig. 3A and B, LC cell shows a uniformly dark optical image at low antibody concentration. When the concentration of the antibody solution further increased to 50 μg/ml, the LC cell appeared bright as shown in Fig. 3C. It was seen that an excessive amount of antibody immobilized on the glass slide disrupted the homeotropic orientation of LCs. Inspection of Fig. 3C and D reveals that the higher the concentration of antibody is, the brighter the LC cell is, as more antibody was immobilized on the surface and destroyed the LC alignment. It is ideal to observe dark images under the POM if the antibody was immobilized on the surface of LC in the cell. Therefore, the optimum concentration of the Aβ42 antibody solution was selected as 25 μg/ml.

In the following step, the selected optimum antibody concentration of 25 μg/ml was interacted with different Aβ42 peptide concentrations from 1 pg/ml to 1000 pg/ml to determine the LOD of

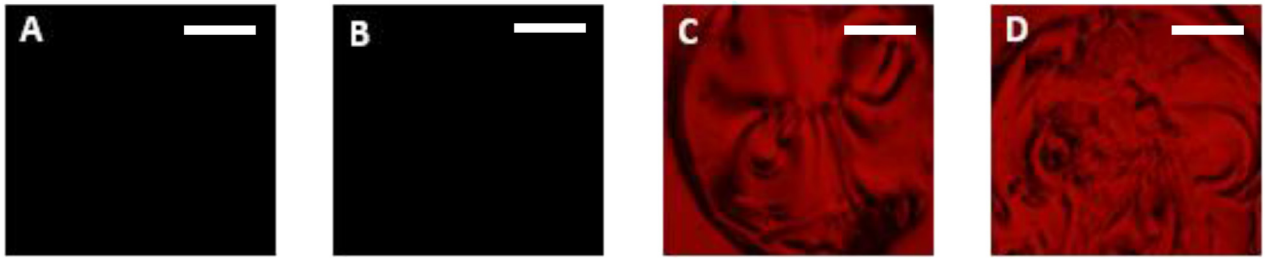

FIG. 3. POM images of samples including antibody concentration of (A) 10 μg/ml, (B) 25 μg/ml, (C) 50 μg/ml, and (D) 100 μg/ml. Scale bar: 20 μm.

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the biosensor. Aβ42 antibody (25 mg/ml) was immobilized on the LC film assembled on the DMOAP coated glass slide. Before Aβ42 antibodies interacted with Aβ42 peptides, DMSO was dropped onto the Aβ42 antibody surface to understand whether DMSO has an effect on the LC alignment or not. No effect of DMSO on the orientation of LCs had been observed. Optimum antibody concentration was interacted with various peptide solution concentrations in the range of 1 pg/ml to 1000 pg/ml at room temperature. After Aβ42 peptide solution addition to the Aβ42 antibody immobilized surface, binding of the Aβ42 peptide to the surface was determined by monitoring the optical response of the LCs. Therefore, the minimum concentration of Aβ42 peptide solution that could induce an orientational change by the LCs was detected to determine the LOD of this LC-based biosensor. The low Aβ42 peptide concentration did not cause a strong orientational transition of LCs because the amount of Aβ42 peptide captured by the Aβ42 antibody was too low as shown in Fig. 4B–D. As the Aβ42 peptide concentration was gradually increased, much more Aβ42 peptide was captured by the Aβ42 antibody. Fig. 4E–I proves that there was an increase in the number of antibody–antigen immunocomplexes, and a significant orientational transition in LCs alignment. POM images indicated that the minimum concentration of 1 pg/ml Aβ42 peptide is required to produce a change in the LC orientation on the surface from homeotropic to random. Based on the above results, the LOD of this LC-based optical biosensor was determined as 1 pg/ml Aβ42 peptide concentration which is lower than those of the other biosensors in the literature. According to the studies in the literature, Dai et al. (36) fabricated that simple in vitro biosensor to detect β-amyloid 42 in phosphate-buffered saline and human serum. They notified that they detected β-amyloid 42 concentration was from 0.0675 μg/ml to 0.5 μg/ml. Additionally, Yu et al. (20) have been developed a gelsolin-bound-Aβ1–40/1–42 detection system for detecting Aβ1–40/1–42 level associated with AD, and the detection limit was determined as 28 pM in the study in question.

Then Aβ42 antibody–antigen–ApoE4 protein was formed with the addition of ApoE4 protein in the system to understand the interaction between ApoE4 protein and Aβ42 peptide. The change in the LC orientation caused by this complex was determined via POM and spectrometer. When ApoE4 protein was added only on the Aβ42 antibody, it was observed that there was not any change in LC orientation, since ApoE4 protein could not bind to the Aβ42 antibody. When ApoE4 protein was added to the antibody–antigen immunocomplexes in the concentration range of 0–30 nM, there was a change in LC orientation as shown in Fig. 5. The antibody–antigen immunocomplexes for the binding of ApoE4 protein were prepared with the peptide concentrations of 100 pg/ml, 50 pg/ml, 25 pg/ml, 10 pg/ml and 25 μg/ml on the antibody. POM images show that the change in LC orientation decreased with

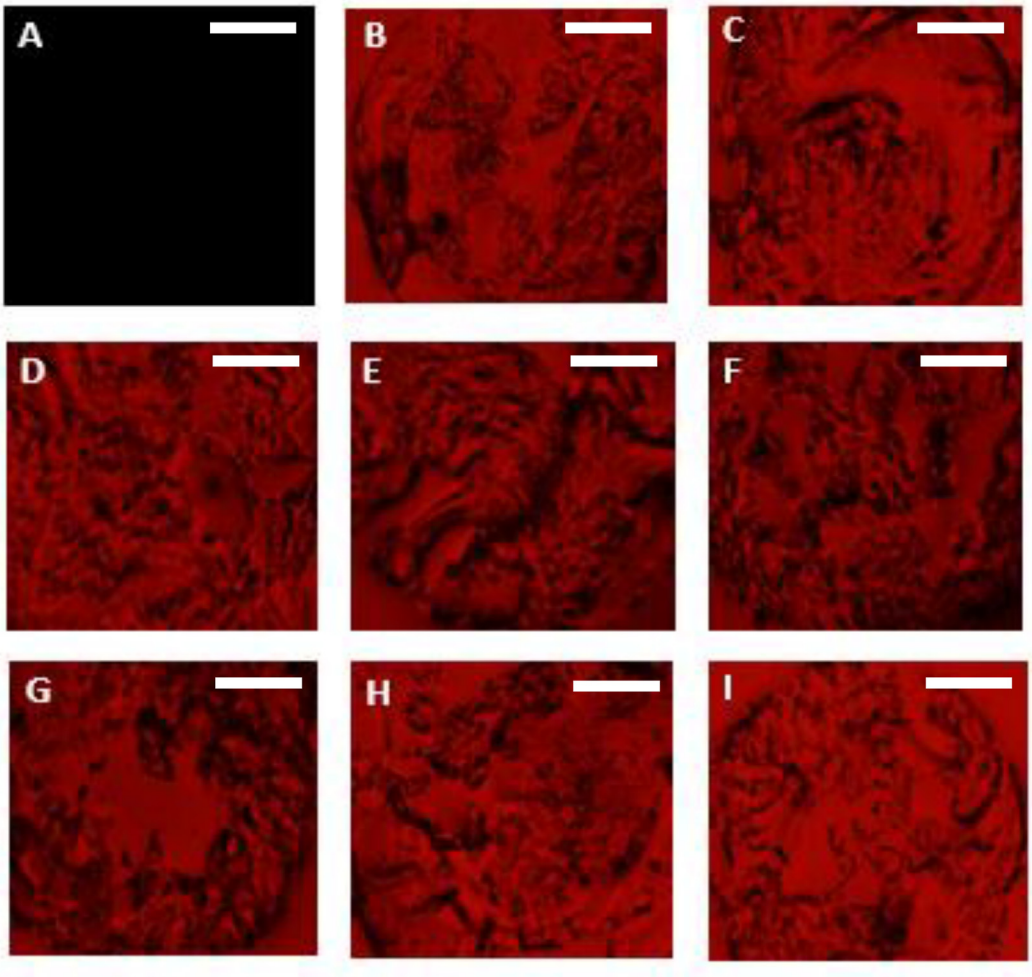

FIG. 4. POM images of the samples of (A) including the optimum Aβ42 antibody concentration without adding Aβ42 peptide, and after adding the Aβ42 peptide at the concentration of (B) 1 pg/ml, (C) 5 pg/ml, (D) 10 pg/ml, (E) 25 pg/ml, (F) 50 pg/ml, (G) 100 pg/ml, (H) 500 pg/ml, and (I) 1000 pg/ml. Scale bar: 20 μm.

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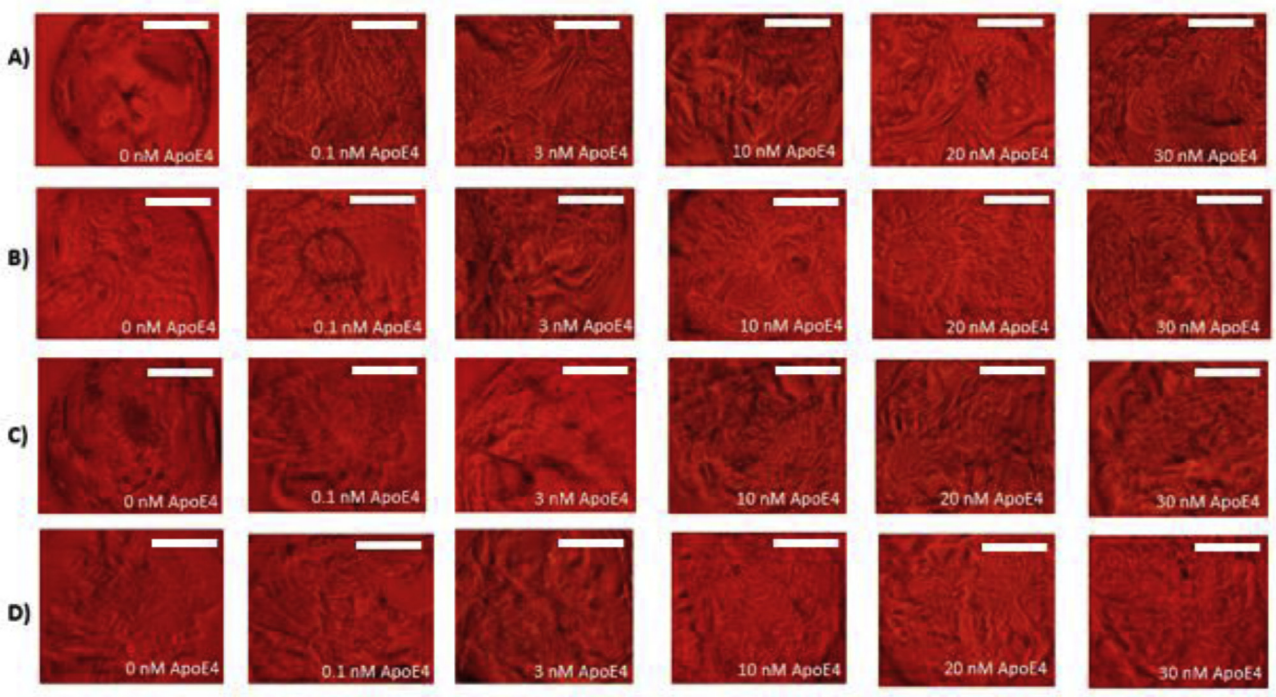

FIG. 5. POM images of the immunocomplex including (A) 100 pg/ml Aβ42 peptide concentration, (B) 50 pg/ml, (C) 25 pg/ml, and (D)10 pg/ml. Scale bar: 20 μm.

the decreasing ApoE4 protein bounded to the peptide to form complex. The antibody–antigen immunocomplexes induce a more significant change in the optical texture of LC than ApoE4-–antibody–antigen complexes. This is because of the Aβ42 antibody–antigen immunocomplex exhibiting a stronger binding affinity compared to the ApoE4 protein and Aβ42 peptide binding in the immunocomplex.

Subsequently, reflectance changes induced by the LC orientational change depending on the binding immunocomplex amount on LC molecules were determined via an Ocean Optics spectrometer (USB2000+) at right angle (90°). These reflection spectra were obtained as a function of the increasing Aβ42 peptide concentration as shown in Fig. 6.

Fig. 6 shows the changes in the reflectance percentage as a function of increasing Aβ42 peptide concentration from 1 pg/ml to 1000 pg/ml. Aβ42 peptide concentrations were plotted at a logarithmic scale. The reflection value in the presence of 1 pg/ml Aβ42 peptide was obtained as 9.5%. When the Aβ42 peptide concentration was increased up to 1000 pg/ml it was shown that the reflection value increased up to 59.7%. As the concentration of Aβ42 peptide was increased, optical texture significantly changed depending on the formation more and more antibody–antigen immunocomplexes as shown in Fig. 4 and this change in the LC orientation gave rise to a significant change in the reflectance of the sample as shown in Fig. 6.

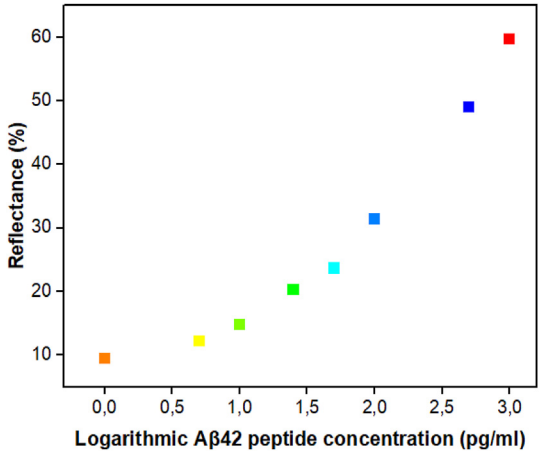

FIG. 6. Reflection spectra of antibody–antigen immunocomplex as the function of the increasing peptide concentration.

After adding the ApoE4 protein solution to the immunocomplex, the reflection spectra of the LCs bonded complexes were also determined. Fig. 7 shows the reflection spectra of these complexes including different Aβ42 peptide concentrations from 10 pg/ml to 100 pg/ml versus increasing ApoE4 protein concentrations from 0.1 nM to 30 nM.

The reflection value in the presence of 0.1 nM ApoE4 protein and 100 pg/ml Aβ42 peptide concentration was obtained as 37%. When the ApoE4 protein concentration was increased to 30 nM, the reflection value was obtained as 51.2% in the complex including 100 pg/ml of Aβ42 peptide (Fig. 7, line A). When the Aβ42 peptide concentration was attenuated from 100 pg/ml to 50 pg/ml, reflection values were obtained as 34.1% and 46.9% for the lowest ApoE4 protein concentration of 0.1 nM and the highest ApoE4 protein concentration of 30 nM, respectively (Fig. 7, line B). With the decreasing peptide concentration up to 25 pg/ml, the reflection values were obtained as 33% and 45.2% for the 0.1 nM and 30 nM ApoE4 protein concentrations, respectively (Fig. 7, line C). When the complex was formed with the result of the reaction between 10 pg/ml Aβ42 peptide, the reflection value was obtained as 31% for 0.1 nM ApoE4 protein concentration whereas this value was obtained as 42% for 30 nM ApoE4 protein concentration (Fig. 7, line D). As a result, the highest reflection percentage value was observed in the complex, which occurred in the presence of the highest ApoE4 protein and Aβ42 peptide concentrations. The reflection spectra show that an increase in the number of complexes depending on the increasing reflectance values with the increasing Aβ42 peptide and ApoE4 protein concentrations.

In summary, an LC-based biosensor for the detection of a biomarker of neurodegenerative disease, Aβ42 peptide, has been

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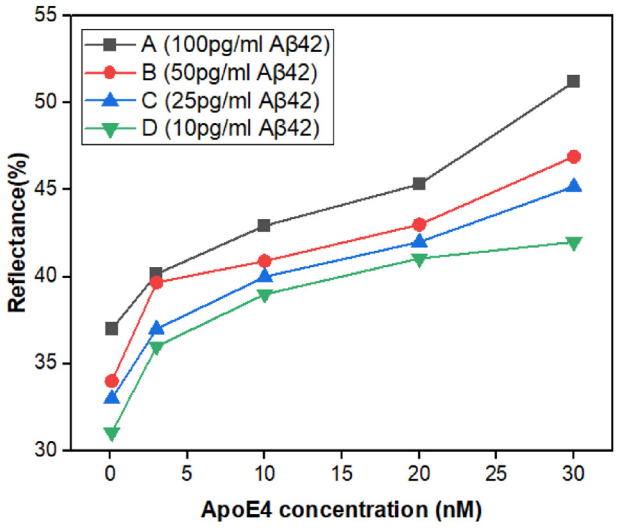

FIG. 7. Reflection spectra of antibody-peptide-ApoE4 immunocomplex including 100 pg/ml Aβ42 peptide (line A), 50 pg/ml Aβ42 peptide (line B), 25 pg/ml Aβ42 peptide (line C), and 10 pg/ml Aβ42 peptide (line D) as the function of the increasing ApoE4 concentration.

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 DMOAP coated glass slide. Subsequently, optimum antibody concentration was interacted with various Aβ42 peptide concentrations between 1 pg/ml and 1000 pg/ml. The changes in the orientation of LC direction induced by the antibody–antigen binding events were realized through POM and Ocean Optics spectrometer. According to all of 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 of samples with the decreasing Aβ42 peptide concentration from 1000 pg/ml to 1 pg/ml. The decrement in the Aβ42 peptide concentration leads a less immunocomplex formation. In addition, ApoE4 protein was detected with this biosensor in the range of 0.1–30 nM due to the interaction between the ApoE4 protein and Aβ42 peptide. The experimental results showed that an LC-based optical biosensor with high sensitivity and selectivity has been developed.

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