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# Development of Chemically Signal Amplified Nano-Biosensor Mediated by Poly-L-Lysine

Min-Sik Kang[1], Kyu-Hyon Son[1], Tae-Hoon Lee[2], Sang-Mok Chang[3], Sung-Woong Han[1,*], and Hoon-Kyu Shin[1]

[1] *National Institute for Nanomaterials Technology, Pohang University of Science and Technology, 77, Cheongam-ro, Nam-gu, Pohang, Gyeongbuk, 37673, Korea*
[2] *Top Run R&D Center, Dong Yang Industrial Co. LTD., 46-14, Okge2gongdan-ro, Gumi, Gyeongbuk, 622-14, Korea*
[3] *Department of Chemical Engineering, Dong-A University, 840, Hadan-dong, Saha-gu, Busan, 604-714, Korea*

Amyloid $\beta$ (A$\beta$) is considered to be one of a potential biomarker to monitor Alzheimer's Disease (AD) not only for diagnostic purposes but for early detection. Here we describe a novel nano-biosensor for A$\beta$ mediated by poly-L-lysine (PLL) which was used for the amplification of detection signal for A$\beta$. The indirect enzyme-linked immunosorbent assay (ELISA) method was modified using PLL for the amplification of the A$\beta$ detection signal. A commercially available ELISA plate was modified by PLL using chemical agent and the amplified amino groups were activated by a chemical agent for the detection of A$\beta$. The detection was carried out by the traditional immunochemistry using primary antibody and fluorescence molecules conjugated secondary antibody. In the result, the fluorescence intensity was increased by the increasing treated A$\beta$ amount, and the sensitivity was approximately 2 times higher in the concentration of 2 ng/mL A$\beta$ treatment, and approximately 4 times higher in the concentration of 200 ng/mL A$\beta$ treatment compare with that of indirect ELISA detection method. We suggest our novel signal amplification method for the A$\beta$ early detection.



**Keywords:** Amyloid $\beta$ Detection, Nano-Biosensor, Alzheimer's Disease, Signal Amplification.

## 1. INTRODUCTION

Alzheimer's disease (AD) is an irreversible neurodegenerative disease caused by the lost of neural cell function in the brain. The disease is progressed by many complex pathways and molecules such as amyloid load, tauopathy, inflammation and oxidation damages.[1,2] Amyloid plaque is considered to be one of critical causative factor in the pathogenesis of AD. The plaque is produced by the accumulation of amyloid $\beta$ (A$\beta$) peptide which is secreated from the neural cell in the brain by the amyloid precursor protein (APP) processing. The processing is achieved by two secretases which are $\alpha$-secretase for the essential molecular signaling in neural cell proliferation, and $\beta$-secretase of the critical hallmark of A$\beta$ production.[3] The secreted A$\beta$ peptides are accumulated outside neural cell by it's hydrophopic rich residues of A$\beta$ and it results in $\beta$-sheet-rich amyloid fibrils accumulation interact with metal ions such as $Zn^{2+}$, $Cu^{2+}$, and $Fe^{3+}$, resulting in the formation of highly toxic amyloid plaque.[4]

The prediction of AD and early detection of A$\beta$ from human body could be one of a key issue in the clinical field because the amyloid plaque is irreversibly accumalated in the brain and currently used drugs for AD are not for the disassembling of the amyloid plaue but for the promoting the function of healthy neurons by acetylcholinesterase inhibitors. Moreover, although the drugs are used only for the mild cognitive impairment AD patients, but the therapeutic effect is not enough, and in many cases, the cognitive function is not improved at all.[5,6]

Because of the importance, many groups has been attempted to the detection of A$\beta$ in body, such as cerebrospinal fluid, urine, and plasma. To date, researches have been developing the sensor system of A$\beta$ detection in fluid.[7] The key issue of the early A$\beta$ detection system is how increase the sensitivity because the concentration of A$\beta$ in fluid is very low. Here we report a novel poly-L-lysine (PLL) mediated A$\beta$ sensor which is the detection sensitivity amplified by chemically modified PLL. The PLL molecule is used for the amplifing the functional groups to the target molecule. We compared the detection

*Author to whom correspondence should be addressed.

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sensitivity of the PLL A$\beta$ sensor and traditional indirect enzyme-linked immunosorbent assay (ELISA) method.

## 2. EXPERIMENTAL DETAILS

### 2.1. Chemical Modification of Poly-L-Lysine Medicated A$\beta$ Nano-Biosensor

To prepare the A$\beta$ nano-biosensor mediated by PLL, 96 well poly stylene ELISA plate (FEP-100-096, JET BIOFIL, Guangzhou, China) was washed with Milli-Q water (EMD Millipore Corporation, Billerica, MA, USA), then it was treated for 15 min with 2% (3-aminopropyl)trimethoxysilane (APTES; Sigma-Aldrich Co., LLC, St. Louis, MO, USA) in ethanol (99.5%). The ELISA plate was further treated with 1 mM succinimide-polyethylene glycol-N-hydroxysuccinimide (NHS-PEG-NHS; average M.W. 3.4 kDa; NANOCS Inc., New York, NY, USA) in phosphate-buffered saline (PBS) for 30 min as previously described.[8,9] Next, the succinimidyl substrate was treated with 1 mM PLL hidrobromide (M.W. 1 kDa–5 kDa, Sigma-Aldrich Co., LLC, St. Louis, MO, USA) in PBS for 1 h. The substrate was blocked with 1% bovine serum albumin (BSA; Sigma-Aldrich) for 30 min in order to block unbound NHS groups. The PLL modified substrate was treated with 1 mM succinimide-polyethylene glycol-N-hydroxysuccinimide again for activating the side chain amino groups as the same manner in above. Then, the A$\beta$ peptide 1–42 (human, implicated in Alzheimer's disease; abcam, Cambridge, MA, USA) was modified to the PLL mediated substrate in 5 different concentration (20 ng, 200 ng, 2 $\mu$g, 20 $\mu$g, and 200 $\mu$g/mL) for 2 hrs to yield an A$\beta$ nano-biosensor. The remaining NHS functional groups were blocked with 1% BSA again before the modification of antibodies. The A$\beta$ bound nano-biosensor was treated with 5 $\mu$g/mL anti-$\beta$ amyloid 1–42 antibody (rabbit polyclonal to $\beta$ amylolid 1–42, cross reacts with human, abcam) for overnight and then a secondaray antibody (Alexa Fluor 488 conjugated anti-rabbit Igg, Sigma-Aldrich) was treated for 1 hr.

### 2.2. Fluorescence Observation and Analysis of A$\beta$ Detection

The fluorescence observation was carried out using an inverted microscope (IX73; Olympus, Tokyo, Japan) and an EMCCD camera (iXon Life; Andor Technology LTD., Belfast, UK). A GFP filter (ex 470–490 nm/em 505 nm) was used to obtain the fluorescence image. The exposure times were controlled as the same condition in all image obtaining. The fluorescence intensity by the A$\beta$ detection was obtained by gray-scaled images using the highly sensitive EMCCD camera and the taken images were analyzed using a software Image Pro (Media Cybernetics, Bethesda, Maryland, USA). The number of white pixels on one image were counted, and all of the counted number in a single well were added for calculating the fluorescence intensity.

## 3. RESULTS AND DISCUSSION

Figure 1 shows the illustration of chemical modification for the development of chemically signal amplified nano-biosensor mediated by PLL. PLL was modified using a NHS-PEG-NHS on the ELISA plate and then the amplified amino groups were modified again using the NHS-PEG-NHS for the reaction of NHS groups and amino groups of A$\beta$. The PLL molecules were used for the amplification of the amount of functional groups to react with A$\beta$ in the sample solution.

Figure 2 shows the fluorescence images of the A$\beta$ detection by indirect ELISA sensor (Figs. 1(a–e)) and PLL nano-biosensor (f–j). The white pixels are the fluorescent

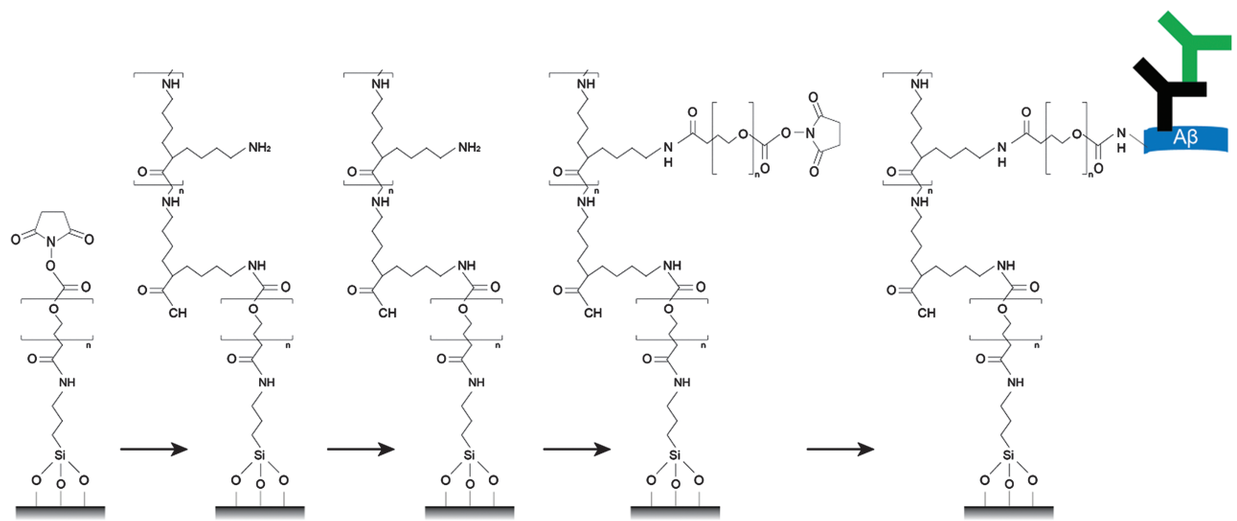


**Figure 1.** Illustration of chemical modification of A$\beta$ nano-biosensor using PLL. The amount of functional groups for reaction with A$\beta$ was amplified using NHS ester activated PLL. The detection of A$\beta$ signal was carried out by a traditional immunochemistry method using a primary antibody to A$\beta$ and a fluorescence molecule conjugated secondary antibody to the primary antibody.

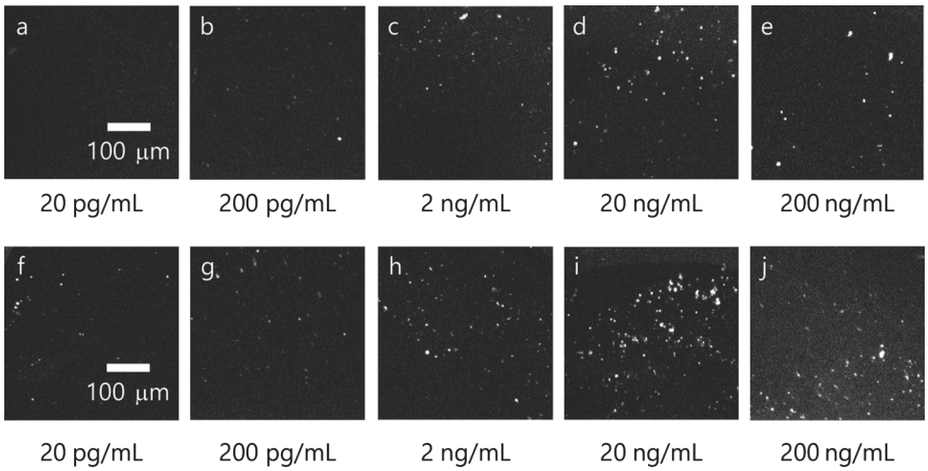


**Figure 2.** Typical fluorescence images of A$\beta$ detection using PLL A$\beta$ nano-biosensor. The fluorescence intensity was gradually increased by increasing the amount of treated A$\beta$. Figure (a–e) are the images of the indirect ELISA A$\beta$ sensor and (f–j) are images for PLL A$\beta$ nano-biosensor. The treated amount of A$\beta$ was put in the below of the images respectively.

of secondary antibodies bound to primary antibodies react with A$\beta$ on the two kinds of sensor. The fluorescence signal was gradually increased by increasing the amount of A$\beta$ in the sample solution in both sensors. In the case of PLL nano-biosensor, the fluorescence signal was significantly increased by increasing the amount of A$\beta$ compare with that of indirect ELISA sensor.

Figure 3 shows the result of monitoring the fluorescence intensity change by changing the amount of A$\beta$ and the intensity data was shown in Table I. The fluorescence intensity of indirect ELISA method was 1.20, 1.26, 1.32, 1.42, and 1.38 (a.u.) in the A$\beta$ concentration of 0.02, 0.2, 2, 20, and 200 ng/mL, respectively. The result shows



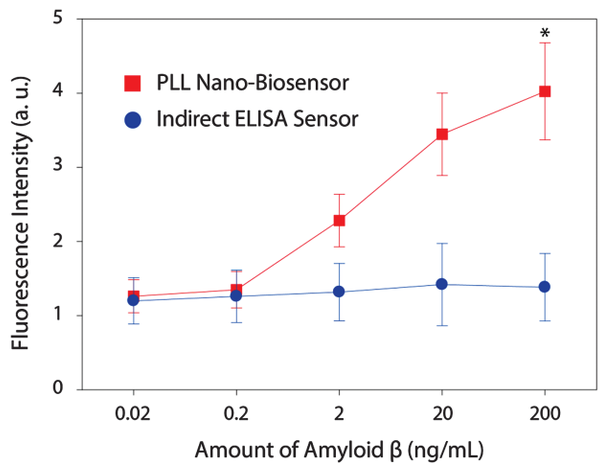


**Figure 3.** The comparison of A$\beta$ sensing performance of indirect ELISA sensor and PLL nano-biosensor. The fluorescence intensity of A$\beta$ detection by indirect ELISA (blue circle) was gradually increased by increasing the treated amount A$\beta$ molecules. The fluorescence intensity of A$\beta$ detection by PLL nano-biosenosr (red square), the signal was significantly increased compare with that of indirect ELISA sensor, and the sensitivity was approximately 3 times higher than that of indirect ELISA sensor in the 200 ng/mL A$\beta$ treatment ($p < 0.001$, one-way ANOVA).

a tendency to increase, but not in a significant manner. On the other hand, in the case of PLL nano-biosensor, the fluorescence intensity was 1.26, 1.34, 2.82, 3.45, and 4.02 (a.u.) in the same treatment condition with indirect ELISA sensor. The fluorescence intensity was significantly increased by increasing the treatment amount of A$\beta$. In the range of 0.02–0.2 ng/mL A$\beta$ treatment condition, the fluorescence intensity was almost no difference in both sensors. However, the intensity was shown a drastic increase in the 2 ng/mL A$\beta$ treatment condition, and the fluorescence intensity of PLL nano-biosensor was approximately 2 times higher than that of indirect ELISA sensor. The difference was greater by increasing the A$\beta$ treatment and the fluorescence intensity of PLL nano-biosensor was approximately 4 times higher than that of indirect ELISA in the the 200 ng/mL A$\beta$ treatment condition.

The fluorescence intensity of A$\beta$ detection by indirect ELISA method was not shown a drastic detection performance because in the design of binding chemistry, NHS ester/amino group binding was selected, and the A$\beta$ peptide has only 3 amono groups in the side chain in the whole peptide residues (Arg 5, Lys 16, Lys 28).[10–12] Moreover, other frequently used binding chemistry such as maleimide/Cys couldn't selected because A$\beta$ is a highly hidrophobic peptide so no cysteine contained in the residues.[13, 14] On the other hand, the PLL A$\beta$ nano-biosensor showed 2–4 times higher A$\beta$ sensing performance because the the A$\beta$ binding was amplified by PLL which has a role to enhance the amount of A$\beta$ binding.

**Table I.** Comparison of fluorescence intensity of indirect ELISA and PLL A$\beta$ sensor.

| Amount of A$\beta$ (ng/mL) | 0.02 | 0.2 | 2 | 20 | 200 |
|---|---|---|---|---|---|
| Indirect ELISA | 1.20 | 1.34 | 1.32 | 1.42 | 1.38 |
| PLL Sensor | 1.26 | 1.59 | 2.82 | 3.45 | 4.02 |

## 4. CONCLUSION

In this study, we demonstrated a novel signal amplified A$\beta$ nano-biosensor mediated by PLL and succeeded in the enhancing of A$\beta$ detection. The sensitivity of A$\beta$ detection by PLL nano-biosensor showed approximately 2–4 times higher than that of traditional indirect ELISA sensor. The present sensor could be more optimized by controlling the PLL length and protocols. We believe that our novel method would be applied for the A$\beta$ detection sensor in human fluid.

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