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Surface Plasmon Resonance Analysis of Alzheimer's β-Amyloid Aggregation on a Solid Surface: From Monomers to Fully-Grown Fibrils

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We analyzed the aggregation of Alzheimer's β-amyloid (1-42) (Aβ42) peptides from fresh monomers to fully grown fibrils by using in situ surface plasmon resonance (SPR) spectrometry and ex situ atomic force microscopy (AFM). To immobilize Aβ42 peptide on an SPR chip surface, different carboxy-terminated surfaces were investigated: (1) self-assembled monolayer of 11-mercaptoundecanoic acid and (2) carboxylated dextran-modified surface. It was found that the carboxylated dextran surface was more appropriate due to a much lower degree of nonspecific binding. By using the carboxylated dextran surface, we further investigated effects of key environmental factors, such as the density of surface-bound Aβ42, the concentration of Aβ42 in solution phase, and the presence of Fe^{3+} ions on Aβ42 fibrillation. The increase in either the surface density of Aβ42 or its concentration in incubation solution highly accelerated the formation of amyloid fibrils on the chip surface. The presence of Fe^{3+} ions in the incubation solution induced significantly denser aggregates, resulting in a nearly 6-fold increase of SPR angle shift. This work shows that SPR analysis coupled with AFM can be effectively used for analyzing amyloid aggregation and deposition on a solid surface from the very beginning to fully grown fibrils.

Surface plasmon resonance (SPR) spectrometry is one of most sensitive analytical tools to detect a mass change above a metal surface.[1,2] A surface plasmon is an electromagnetic wave propagating along an interface between a metal and a dielectric material and can be resonant with an incident beam of light at a certain incident angle called an SPR angle.[3] This resonance leads to the extinction of reflected light at the SPR angle, even above the critical angle. In SPR spectrometry, a change of mass above the metal surface can be quantified in situ by monitoring a shift of SPR angle, which is very sensitive to environmental conditions, especially to a dielectric constant.[4,5] A modern SPR spectrometer can sense a mass change in a pg/mm^2 scale in real time without labeling analytes.[6,7] Because of those advantages, SPR spectrometry has become a popular and powerful tool for quantitative analysis of molecular interactions such as antigen−antibody, ligand−receptor, and host−guest molecular interactions.[7−11]

Amyloids are proteinaceous aggregates formed by a self-assembly of precursor monomers and typically have fibrillar morphology with cross β-sheet rich secondary structure.[12] The formation of amyloid is one of key characteristics in many neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease.[13,14] Despite the significance of amyloid formation, however, little research progress had been made due to the limited number of effective analytical methods. Although several methods, such as electron microscopy, turbidity, birefringent Congo red binding, thioflavin-T induced fluorescence, quasi-elastic light scattering, and atomic force microscopy (AFM) had been conventionally used to study amyloid self-assembly, each of them has its own limitations.[15−17] For example, the use of chemical dyes such as congo red or thioflavin T can affect the amyloid fibrillation process. It is not easy to study the amyloid

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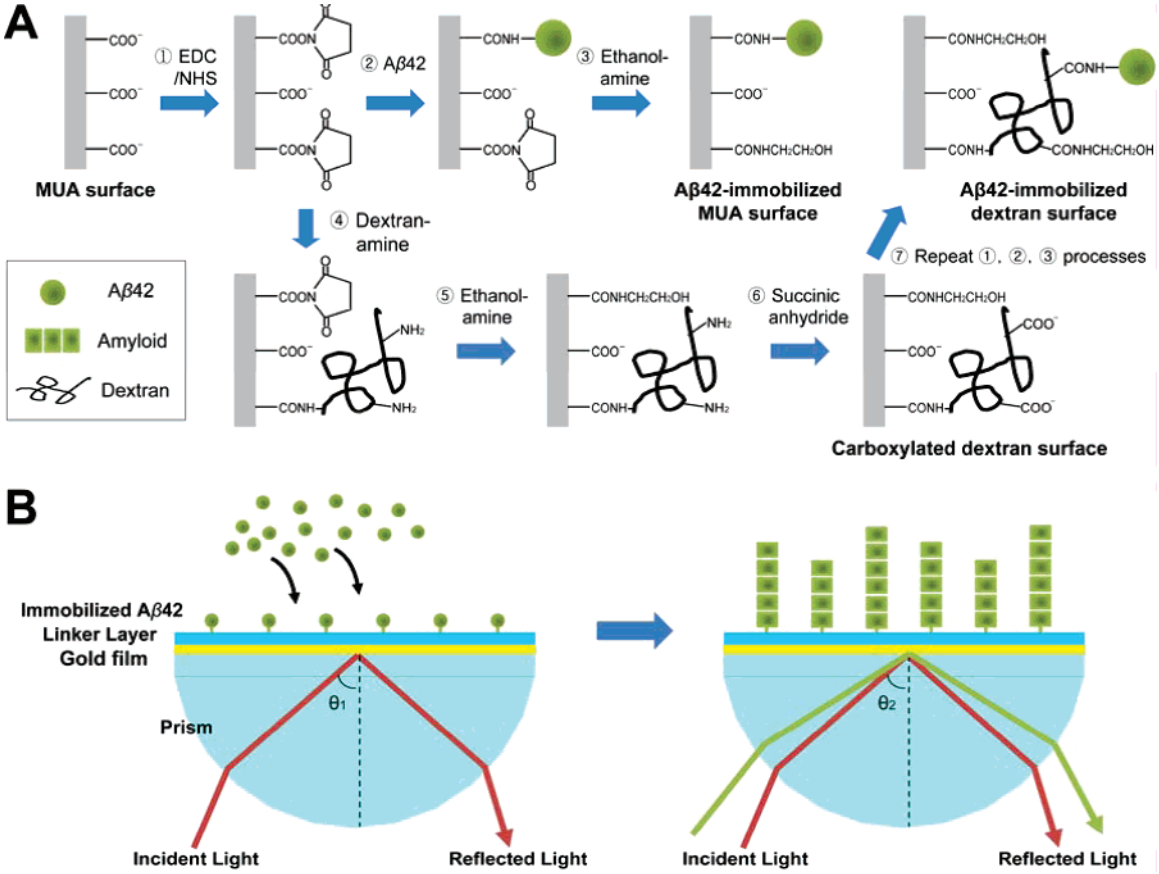
Scheme 1. (A) Surface Treatments for the SPR Analysis of Amyloid Formation on a Solid Surface[a] and (B) Experimental Scheme for the SPR Analysis of Aβ42 Fibrillation on a Solid Surface[b]



[a] The Aβ42-immobilzed "MUA" surface was prepared by the standard amine coupling chemistry using EDC/NHS (①). The surface activated by EDC/NHS was exposed to a fresh Aβ42 solution to form a peptide bond between the amine group on the peptide and the carboxylic group on the MUA surface (②). The deactivation of unreacted sites was achieved by blocking NHS-activations with ethanolamine (③). For the preparation of the Aβ42-immobilized "dextran" surface, dextranamine molecules were first covalently anchored to the MUA surface by EDC/NHS chemistry (① and ④), followed by the blocking of remaining NHS-activations (⑤). Then, the dextranamine-treated MUA surface was incubated in a succinic anhydride solution to carboxylate the remaining free amine groups on the dextran (⑥). By repeating the ①, ②, and ③ processes, Aβ42 could be immobilized to the carboxylated dextran surface. [b] The MUA surface or dextran-treated MUA surface with surface-bound monomeric Aβ42 peptides were incubated in a freshly prepared Aβ42 solution. The self-assembly and fibrillation of Aβ42 were studied by monitoring the SPR angle shift in situ.

formation with electron microscopy because it can change the morphology of aggregates. In the present study, we developed a SPR-based method coupled with ex situ AFM analysis for the quantitative analysis of Alzheimer's β-amyloid (Aβ) aggregation from a very early stage of aggregation to fully grown fibrils on a solid template surface. Considering that the aggregation and deposition of amyloid peptides are significantly affected by the presence of solid surface, such as the cell membrane,[18,19] it is reasonable to investigate the self-assembly of amyloid peptides on a solid surface. In this regard, we recently developed a synthetic amyloid-template using *N*-hydroxysuccinimide-modified glass substrate for the effective in vitro analysis of Aβ deposition and fibrillation.[20,21] We found that the solid template system can efficiently simulate the in vivo aggregation and deposition of Aβ even though the quantification of amyloid formation was indirectly achieved by ex situ AFM.

To study the self-assembly of Aβ peptides in situ on a full-time scale on a solid surface, we evaluated the interactions between Aβ peptides on a surface and in solution. After immobilizing monomeric Aβ peptides onto a carboxy-terminated self-assembled monolayer or a carboxylated dextran surface, we studied the self-assembly of Aβ42 peptides on a solid surface using SPR and ex situ AFM as illustrated in Scheme 1. As a control experiment, surfaces without surface-bound Aβ42 peptides were also tested. Our results obtained by SPR and ex situ AFM show that a dextran-treated surface is much more effective to observe the self-assembly of Aβ42 peptides, since nonspecific interactions between the surface and the peptides are very small. Using the dextran-treated surface, we further tested the effect of several key environmental factors, such as surface density of immobilized Aβ42 peptides, fresh Aβ42 concentration in the solution phase, and the presence of Fe^{3+} ions.

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EXPERIMENTAL SECTION

Materials. Human β-amyloid (Aβ) 42 peptide was obtained from rPeptide Co. (Athens, GA). 1,1,1,3,3,3,-Hexafluoro-2-propanol (HFIP), dimethyl sulfoxide (Me_2SO), *N*,*N*-dimethyl formamide (DMF), 11-mercaptoundecanoic acid (MUA), 11-mercaptoundecanol (MUOH), 1-undecanethiol (UDT), 209 kDa poly L-lysine (PLL), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), succinic anhydride, ethanolamine, and iron (III) chloride were purchased from Sigma-Aldrich (St. Louis, MO). Gold-coated SPR chips were obtained from K-MAC (Daejeon, Republic of Korea). 10 and 500 kDa dextranamines were obtained from Invitrogen (Carlsbad, CA). According to the information given by the manufacturer, the number of amine groups per 10 and 500 kDa dextrans was 3.2 and 89, respectively.

Preparation of Fresh Aβ Solution. Uniform and nonaggregated monomeric Aβ42 peptides were prepared as described before.[20,22] Briefly, pre-existing Aβ42 peptide aggregates were disintegrated into fresh monomers by dissolving the peptides in HFIP at 2.5 mg/mL and then sonicating the solution in a water bath for 3 min. Aβ42 peptide film was formed on the Eppendorf tube wall by aliquoting the solution into the tubes and fully evaporating volatile HFIP in a vacuum desiccator. Then peptide films were stored at −20 °C. Immediately prior to use, the peptide was dissolved in Me_2SO and diluted with phosphate-buffered saline (PBS, pH 7.4), a 10 mM phosphate buffer (PB, pH 7.0), or a 20 mM Tris-HCl solution (pH 7.4 at 37 °C) to a desired concentration. The Me_2SO content in the Aβ solution was kept at a concentration of 5 vol %. For the analysis of Fe^{3+}-induced fibrillation, 20 mM Tris-HCl buffer solution containing 100 μM iron (III) chloride and 5% Me_2SO was used.

Formation of 11-Mercaptoundecanoic Acid Self-Assembled Monolayer (SAM). Gold-coated SPR chips were treated with a piranha solution of 70% H_2SO_4−30% H_2O_2 (7:3, v/v) for 15 min at 60 °C, rigorously rinsed with deionized water until neutralized and dried in a stream of nitrogen (N_2) gas. (Caution: piranha solution is very corrosive and can explode when mixed with a large amount of organics.) Then, the SPR chips were treated with a 10 mM MUA solution in ethanol for more than 12 h, sonicated in a water bath for 10 min, rinsed with ethanol, and dried by N_2 gas.

Formation of Carboxylated Dextran Surface. Immobilization of dextranamine was achieved by standard amine coupling chemistry (Scheme 1A).[9] Briefly, the carboxy-terminated MUA/gold surface was activated by treating with an aqueous mixture of 0.1 M EDC and 0.025 M NHS (1:1) for 10 min, exposed to 0.1 mg/mL dextranamine solution in 10 mM PB (pH 7.0) for 1 h, and deactivated by 1 M ethanolamine solution (pH 8.5) for 15 min. To carboxylate remaining amine groups on the dextran, the SPR chips were then incubated in a 0.1 M succinic anhydride solution in DMF under gentle stirring for 4 h, washed with deionized water, and dried by N_2 gas.

SPR Analysis of β-Amyloid Fibrillation. Aβ42 was immobilized on the carboxy-terminated surfaces by the amine coupling method as shown in Scheme 1A. The MUA-formed gold chip was treated with a mixture of 0.1 M EDC and 0.025 M NHS (1:1) for 7 min, and the caboxylated dextran surface was treated with a mixture of 0.4 M EDC and 0.1 M NHS (1:1). Then, a freshly prepared Aβ42 solution in 10 mM PB (pH 7.0) at a desired concentration was reacted with an NHS-activated surface for 15 min. The remaining NHS-sites were blocked with a 1 M ethanolamine solution for 5 min. Then, SPR chips were incubated in a freshly prepared Aβ42 solution with or without an addition of additives at 37 °C. To prevent evaporation of buffer solution during incubation, the opening of the SPR cuvette was tightly sealed by Parafilm. All of the reaction processes described above were monitored by a double channel SPR, Autolab Esprit (Echo Chemie, The Netherlands), equipped with a homemade water heating system. Injection of solutions into the cuvettes was manually done. The running buffer solution for the SPR-angle measurement consists of 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), 4 mM EDTA, 150 mM NaCl, pH 7.4, filtered over 0.2 μm and supplemented with 0.005% Tween-20.

Visualization of Aβ42 Deposits Formed on a Solid Surface by ex Situ Atomic Force Microscopy (AFM). After SPR analysis, SPR chips were washed with a SPR running buffer and further with deionized water and dried with N_2. Morphological analysis of Aβ42 deposits was achieved by ex situ AFM. The sample surface was imaged using a Multimode AFM equipped with a Nanoscope IIID controller (Digital Instruments Inc.) under ambient conditions. AFM analysis was carried out in the tapping mode in air under the following conditions: scan rate, 1−1.5 Hz; an "E" scanner; a NCHR silicon cantilever (Nanosensors Inc.); a resonant frequency range of AFM cantilevers, 250−350 kHz; number of pixels, 512 × 512. Representative images in each case were obtained by scanning different samples and at least five spots over the entire surface area.

RESULTS AND DISCUSSION

Aβ42 Fibrillation on a Synthetic Template with MUA Surface. For the quantitative analysis of Aβ42 deposition and fibrillation using SPR, it is essential to minimize a nonspecific binding of Aβ42 peptides onto a solid surface.[6,7,23] We tested a self-assembled monolayer surface of MUA molecules, which are one of the ω-substituted alkanethiolates and can readily form a carboxy-terminated self-assembled monolayer on a gold surface.[24] MUA surface is known to be similar to phospholipid bilayers in cell membranes, consisting of hydrophilic head groups and hydrophobic hydrocarbon chains.[25,26] To evaluate the nonspecific interaction between the Aβ42 peptides and the MUA surface, we incubated a MUA layer-coated SPR-chip with or without (i.e., bare) surface-bound monomeric Aβ42 peptides. After further incubation in a fresh 30 μM Aβ42 solution for 11 h at 37 °C, significant amounts of amyloid fibrils were observed on both the bare MUA surface and the Aβ42-immobilized MUA surface according to the ex situ AFM analysis (Figure 1). SPR angle shifts after incubation

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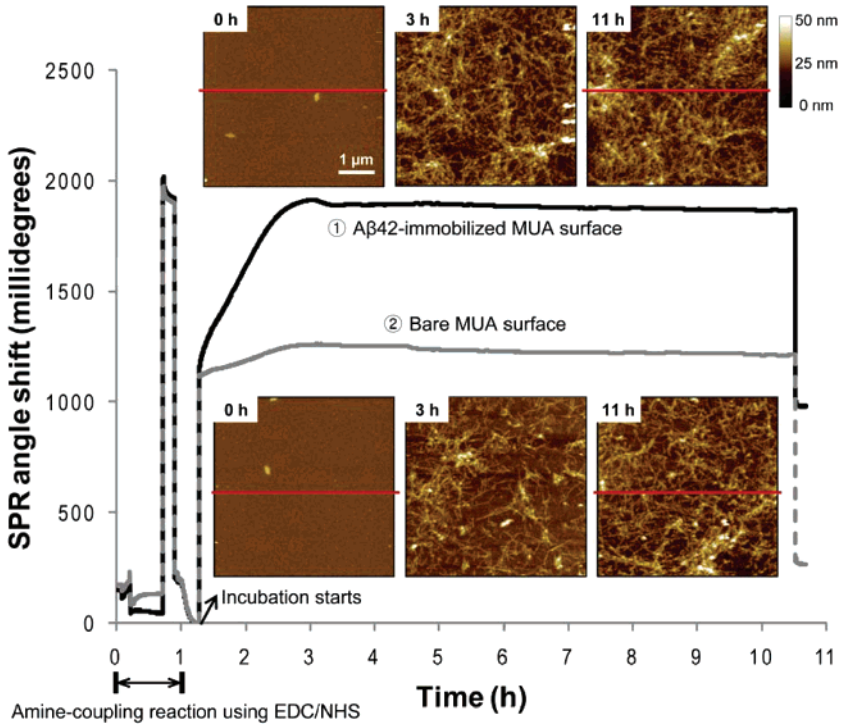

Figure 1. In situ SPR sensorgram and ex situ AFM images for the aggregation of Aβ42 on a MUA surface. SPR chips with or without (i.e., bare) immobilized Aβ42 peptides were incubated in a freshly prepared 30 μM Aβ42 solution in PBS containing 5% Me_2SO at 37 °C. During the incubation, the change of the SPR angle was monitored in situ, and the surface of the SPR chip was scanned by ex situ AFM. The size of each AFM image is 5 × 5 μm^2.

with or without surface-bound Aβ42 were 982.2 and 265.8 mdeg, respectively. We attempted to correct the bulk-effect by calculating the differential SPR signal between the surfaces in Figure 1. However, as shown in Figure S1A (in Supporting Information), it was difficult to remove the bulk effect from the SPR signal due to the saturation of SPR signal as well as the high level of nonspecific interactions between Aβ42 and the MUA surface. We found that the ratio of SPR signals for both surfaces was only 3.75 in this case (Figure S1B). Note that a change of 0.1 deg in SPR angle shift or 1 000 RU corresponds to a mass change of ~1.0 ng/mm^2 near the surface.[6]

According to the SPR sensorgrams in Figure 1, a negligible lag phase in the formation of amyloid fibrils was observed on both surfaces during incubation. In addition, the growth of amyloid fibrils was saturated within 2 h regardless of the presence of surface-immobilized Aβ42 peptides. We supposed that this was mainly due to the nonspecific interaction between the MUA surface and Aβ42 peptides dissolved in the solution phase. The fast saturation of amyloid growth in the SPR sensorgram was attributed to the detection limit of the SPR spectrometer. Since SPR spectrometers use an evanescent wave of which intensity decays exponentially with distance from the surface of light incidence for the detection of environmental change near the metal surface, the reliable detection limit of SPR is about 300 nm from the metal surface.[4,27] This is very narrow for fully grown amyloid fibrils, which are several micrometers long according to our AFM observations. Thus, the adjustment of variables (e.g., time span, Aβ42 concentration) and the minimization of nonspecific binding are needed for monitoring the effects of other stimulants on Aβ fibrillation.

In order to investigate the nonspecific binding of Aβ42 to solid surface, we tested six different surfaces that include COOH-terminated MUA, OH-terminated MUOH, CH_3-terminated UDT, and MUA surfaces modified with carboxylated dextran (10kDa, 500 kDa), or PLL (209 kDa). As shown in Figure S2, all the tested surfaces except for the 500 kDa carboxylated dextran surface exhibited significant amounts of nonspecific binding. The morphology of amyloid aggregates was also different depending on the surface chemistry. While MUA, MUOH, and PLL surfaces resulted in the formation of fibrillar aggregates, UDT surface resulted in amorphous aggregates. Differential effect between the surfaces was thought to be originated from different hydrophobicity,[25,26] net electrical charge,[19,28–30] and molecular conformation of surfaces.[28,31] Since the 500 kDa carboxylated dextran exhibited the lowest nonspecific binding of Aβ42, we used the 500 kDa dextran surface for further study.

Aβ42 Fibrillation on a Synthetic Template with Carboxylated Dextran Surface. Dextran, a polysaccharide hydrogel, is known to resist the protein adsorption because of its high hydrophilicity, electrical neutrality, and conformational flexibility.[28]

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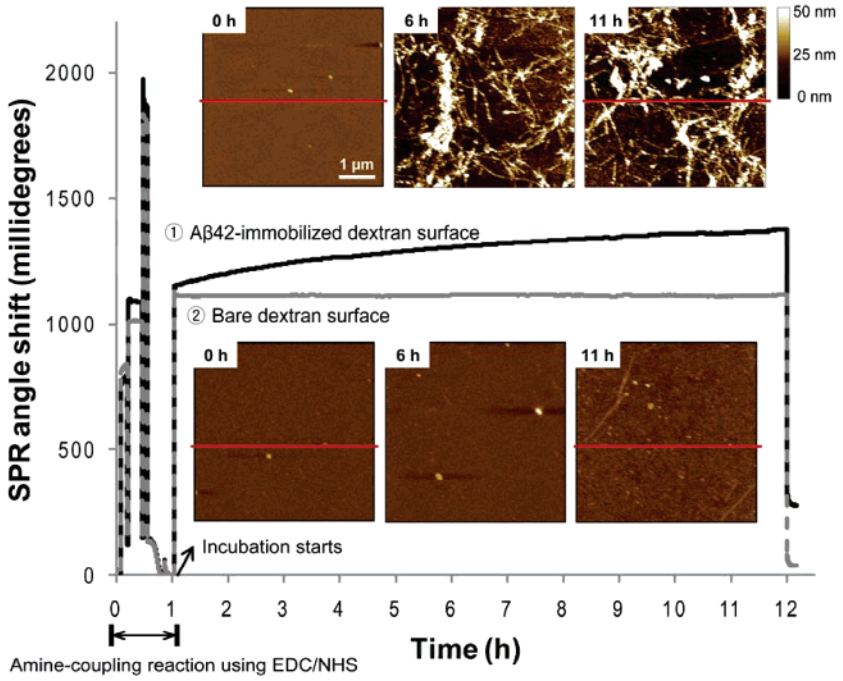

Figure 2. In situ SPR sensorgram and ex situ AFM images for the aggregation of Aβ42 on a dextran-treated MUA surface. The condition for sample preparation and the AFM analysis was same as in Figure 1. The size of each AFM image is 5 $\times$ 5 μm^2.

Here the dextran-modified SPR chip with or without (i.e., bare) surface-bound Aβ42 was incubated in a fresh 30 μM Aβ42 solution for 11 h at 37 °C. According to our analysis by SPR and ex situ AFM, a large amount of fibrillar amyloid aggregates were observed when the Aβ-monomers-immobilized dextran surface was incubated. In contrast, minimal amounts of amyloid aggregates were observed on the bare dextran surface (Figure 2). Cross-sectional AFM images of Aβ42 aggregates formed on a SPR-chip surface also show that carboxylated dextran (500 kDa) surface exhibited a much lower degree of nonspecific binding than MUA surface (Figure S3). In addition, we could correct the bulk effect by calculating differential SPR signals between surfaces as shown in Figure S1A, and the ratio of SPR signals between bare and Aβ-immobilized surfaces increased to 7.26-fold (Figure S1B).

According to the SPR sensorgram, the length of the lag phase was observed to be over 6 h, which was much longer compared to that on the MUA surface, and the saturation phase in the SPR sensorgram disappeared in the case of the dextran surface. The disappearance of the saturation phase should be caused by the reduced nonspecific binding of Aβ42 to the dextran surface, which might be due to a decreased electrostatic interaction between the dextran surface and amyloid aggregates. The dextran surface has much lower net negative charges compared to the MUA surface, while both Aβ42 monomers and aggregates are known to be negatively charged at pH 7.4.[32,33] The electrostatic interaction alone, however, may not explain the disappeared saturation in SPR sensorgrams, since electrostatic interactions decrease in a buffer solution with high salt concentration. We speculated that the saturation in SPR sensorgrams could also be influenced by a combinatorial effect of amyloid density on the solid surface, fibril conformation, and SPR detection limit. According to the previous report,[34] the conformation of polymers grafted to a surface depends on the density of grafting, and the polymers adopted brushlike structure due to repulsive interactions between them at high grafting concentration. The MUA surface used in this study should contain much higher density of grafting points for amyloid according to our result (see Figure 1), which should cause much earlier saturation than dextran surface that has a more flexible 3D structure and lower density of linker groups (i.e., carboxyl group). However, the dextran-based chip system may also result in the saturation of SPR signal as in the MUA surface under certain conditions, such as very high Aβ42 concentration or elevated temperature. We believe that our system can be versatilely used for monitoring the effect of stimulants on amyloid formation by adjusting the variables.

Effect of Surface Density of Immobilized Aβ42 Peptides. We investigated the effect of surface-immobilized Aβ42 density considering that surface-bound Aβ42 peptides may induce a conformational change of amyloid peptides dissolved in the solution phase and accelerate the amyloid aggregation on the surface.[20,26,35] We controlled the surface density of immobilized fresh Aβ42 peptides by varying the Aβ42 concentration in solution (0, 3, 15, 30 μM) used in the immobilization process before the start of incubation. The solution phase for the incubation of thus-prepared solid amyloid template initially contained 30 μM of fresh Aβ42. According to our result, the degree of fibrillation on the surface increased with increasing surface density of immobilized Aβ42 (Figure 3). SPR angle shifts after incubation of the amyloid templates treated with 0, 3, 15, and 30 μM fresh Aβ42 solutions were 40.66, 76.70, 120.24, and 283.64 mdeg, respectively. Ex situ AFM analysis of the template surface also supports the result of

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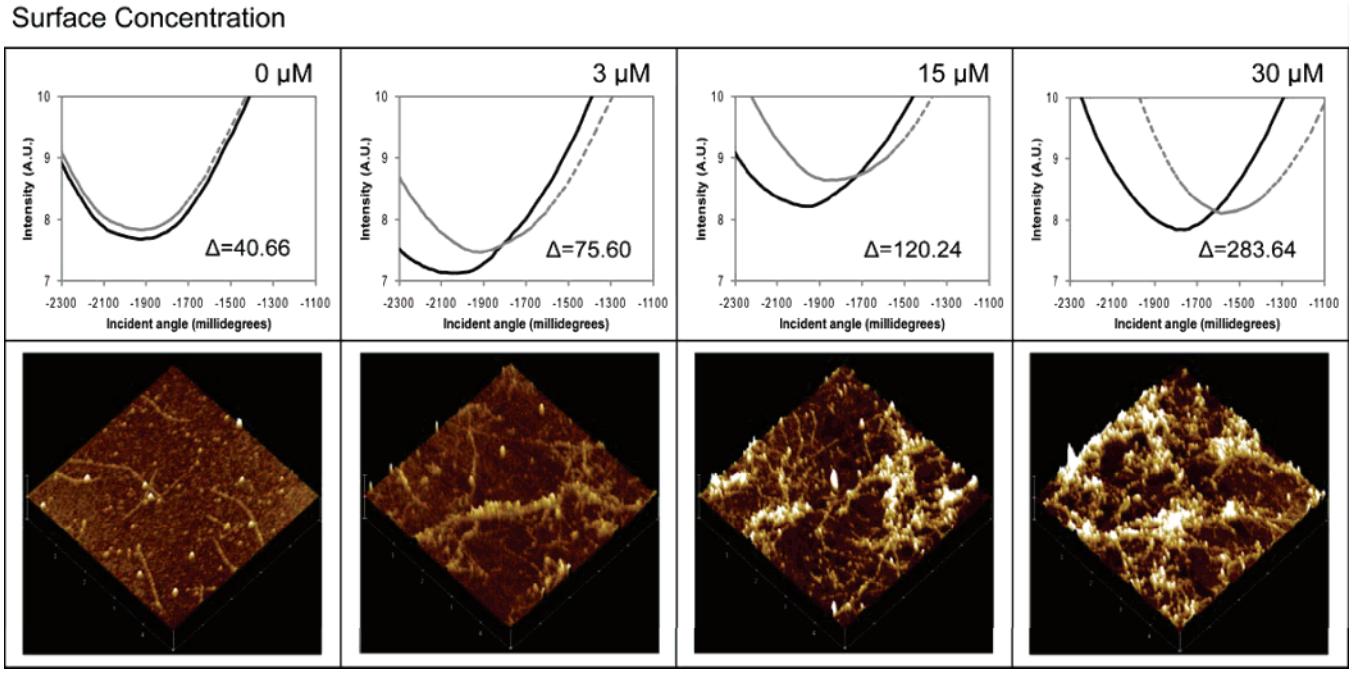

Figure 3. Effect of surface density of immobilized Aβ42 on the deposition of Aβ42 on the dextran surface. Controlling the surface density of Aβ42 was indirectly achieved by varying Aβ42 concentration in solution (0, 3, 15, and 30 μM) used in the immobilization process. The comparison of SPR angle profiles before and after the incubation for 11 h shows that the catalytic effect promoting Aβ42 aggregation of the peptides increases with its surface density.

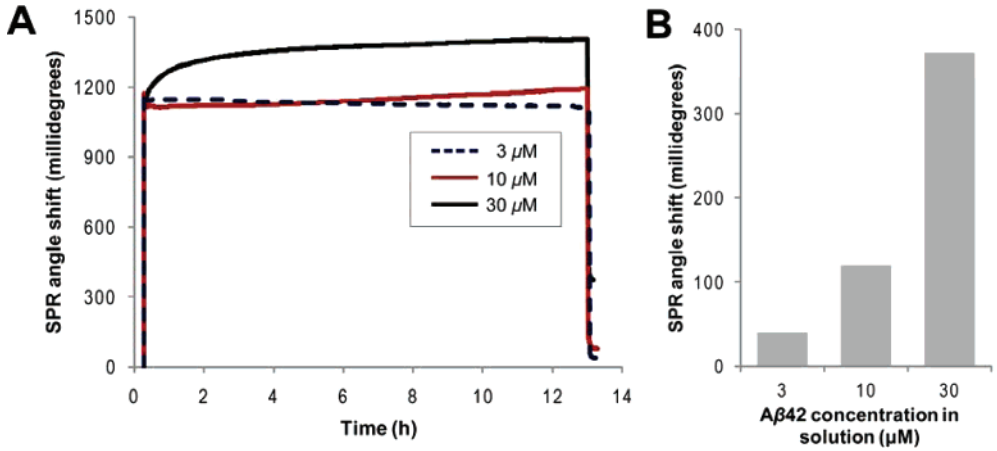

Figure 4. Effect of Aβ42 concentration in the solution phase: (A) SPR binding curves and (B) resultant SPR angle shift after incubation in a solution with different concentrations of Aβ42. Incubation was carried out in a freshly prepared Aβ42 solution with concentrations of 3, 10, and 30 μM.

the SPR angle shift. When the Aβ42 peptide was not immobilized onto the surface, only small amorphous aggregates and few short fibrils were observed, while both the number and the length of fibrils substantially increased with the higher surface density of immobilized Aβ42. The accelerated rate of amyloid fibril formation with surface-immobilized Aβ42 was attributed to the high effective-concentration of Aβ42 on the solid surface (i.e., crowding) rather than to the electrostatic interaction between the dextran matrix and Aβ42 peptides. Aβ42 peptides immobilized on the surface should contribute to the increase of the effective concentration of Aβ42 that can act as the nucleation site for fibrillation.

Effect of Aβ Concentration in the Solution Phase. We further tested the effect of the fresh Aβ42 concentration in the incubation solution on Aβ42 fibrillation on a dextran surface. Figure 4A shows the result of the SPR angle shift obtained after the incubation of a template in a freshly prepared Aβ42 solution with different concentrations of 3, 10, or 30 μM, respectively. In those cases, the concentration of Aβ42 in a solution used in the immobilization onto the dextran surface was kept at 30 μM. According to our result (Figure 3), the concentration of 30 μM seemed to be more appropriate for the analysis of amyloid deposition. SPR angle shifts after incubation in a 3, 10, and 30 μM Aβ42 solution with immobilization of Aβ42 were found to be 40.09, 122.90, and 373.14 mdeg, respectively (Figure 4B). When we incubated a bare dextran template (i.e., without immobilized Aβ42 on the surface) in a 30 μM Aβ42 solution, the SPR angle shift was 40.66 mdeg, which was nearly the same as the above case of a template with immobilized Aβ42 on the surface incubated in a 3 μM Aβ42 solution.

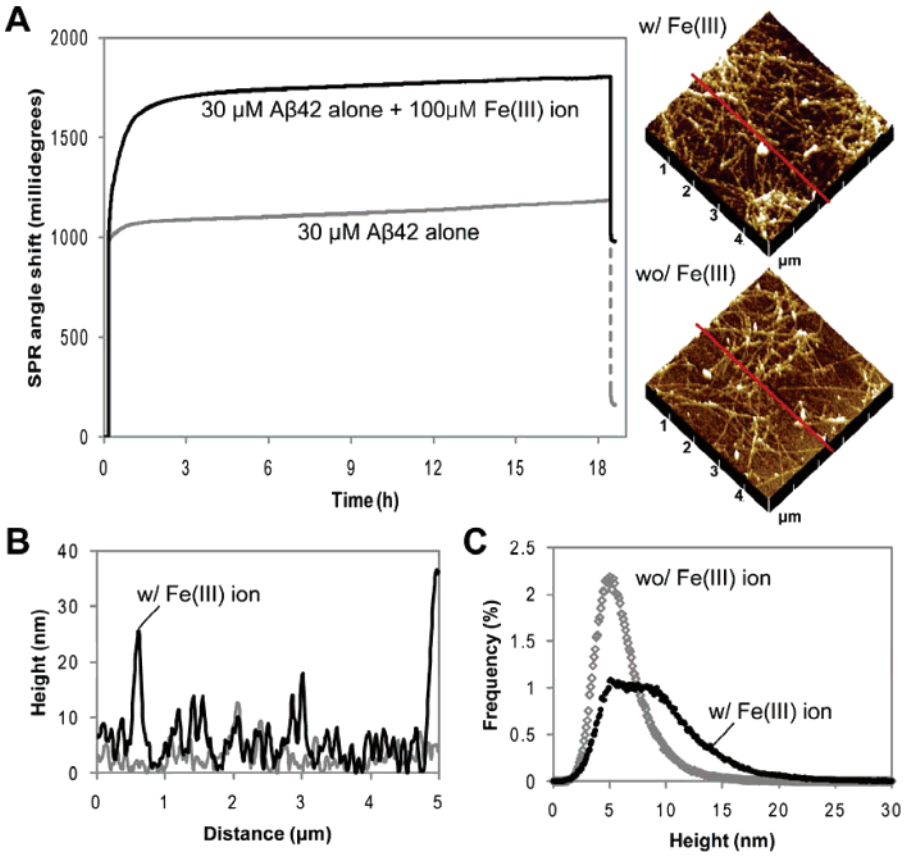

Figure 5. (A) In situ SPR sensorgram and representative ex situ AFM micrographs for the formation of fibrillar amyloids from Aβ42 peptides in the absence or presence of Fe^{3+} ions (100 μM). The size of each AFM image is 5 $\times$ 5 μm^2. AFM micrographs were processed by Nanoscope software to obtain the (B) cross-sectional view along the red line and (C) height−frequency distribution for quantitative analysis.

SPR and ex Situ AFM Analysis of Fe^{3+}-Induced Fibrillation of Aβ42 Peptides on the Surface. We investigated the effect of Fe^{3+} ions on the aggregation and deposition of Aβ42 on a solid surface using ex situ AFM micrographs and SPR sensorgrams. Metal ions such as Fe^{3+}, Cu^{2+}, and Zn^{2+} are known to significantly affect the rate of aggregation and its morphology, affecting the progression of Alzheimer's disease.[21,36−39] In particular, the role of Fe^{3+} ions on the aggregation of Aβ42 is interesting, since Fe^{3+} ions had been reported to catalyze the formation of fibrillar amyloid aggregates by binding to the amino acid residues of Aβ such as Tyr, Glu, and Asp.[40] However, the detailed mechanism underlying the accelerated fibrillation of Aβ42 by Fe^{3+} ions still remains controversial.[41] According to our result shown in Figure 5, the initial rate of amyloid fibrillation was highly accelerated in the presence of Fe^{3+} ions by nearly 6-fold according to SPR analysis. SPR angle shifts after incubation in the presence or in the absence of Fe^{3+} ions were found to be 979.7 and 161.8 mdeg, respectively. Ex situ AFM analysis of the SPR chip also shows the enhanced fibrillation in the presence of Fe^{3+} ions. For the quantitative analysis, we processed AFM micrographs to show the cross-sectional view (Figure 5B) and height−frequency distribution (Figure 5C). Both results confirmed the result obtained by SPR sensograms. This result shows that our SPR-based system can be used for the effective analysis of Aβ42 aggregation under different environmental conditions.

CONCLUSIONS

We developed a SPR-based analytical system that could be applied for monitoring Aβ42 fibrillation from fresh monomers under various conditions. Considering that the initial step of amyloid aggregate formation has a significant effect on further fibril growth and deposition of Aβ42,[42] the analysis of Aβ42 aggregation from the beginning step of the self-assembly process should be important for understanding the mechanism underlying amyloid fibrillation. While fluorescence- or absorbance change-based methods had been widely used for the study of Aβ42 fibrillation,[16] they suffered from the limitation of requiring additional dyes, such as Congo red and thioflavin T or S, that can interfere with the amyloid formation process. Here, without additives or a further labeling step, we could directly analyze the fibrillation of Aβ42 from the initial monomeric Aβ42 on a solid surface by using SPR. The current work is expected to be further

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applied to the development of a high-throughput screening method based on a SPR imaging system.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Supporting Information

Surface Plasmon Resonance Analysis of Alzheimer's β-Amyloid Aggregation on a Solid Surface: From Monomers to Fully-Grown Fibrils

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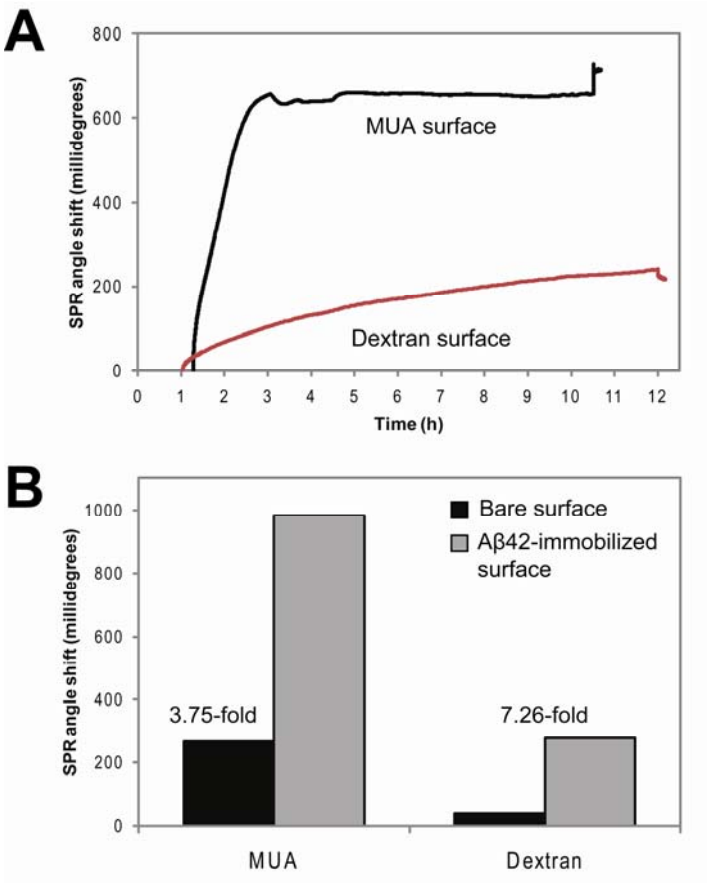

FIGURE S1. For convenient comparison between MUA (Figure 1) and dextran (Figure 2) surface, we plotted (A) *In situ* differential SPR signal (①−② in Figure 1 and Figure 2) between bare and Aβ42-immobilized surface and (B) resultant SPR angle shifts occurred by the deposition of Aβ42.

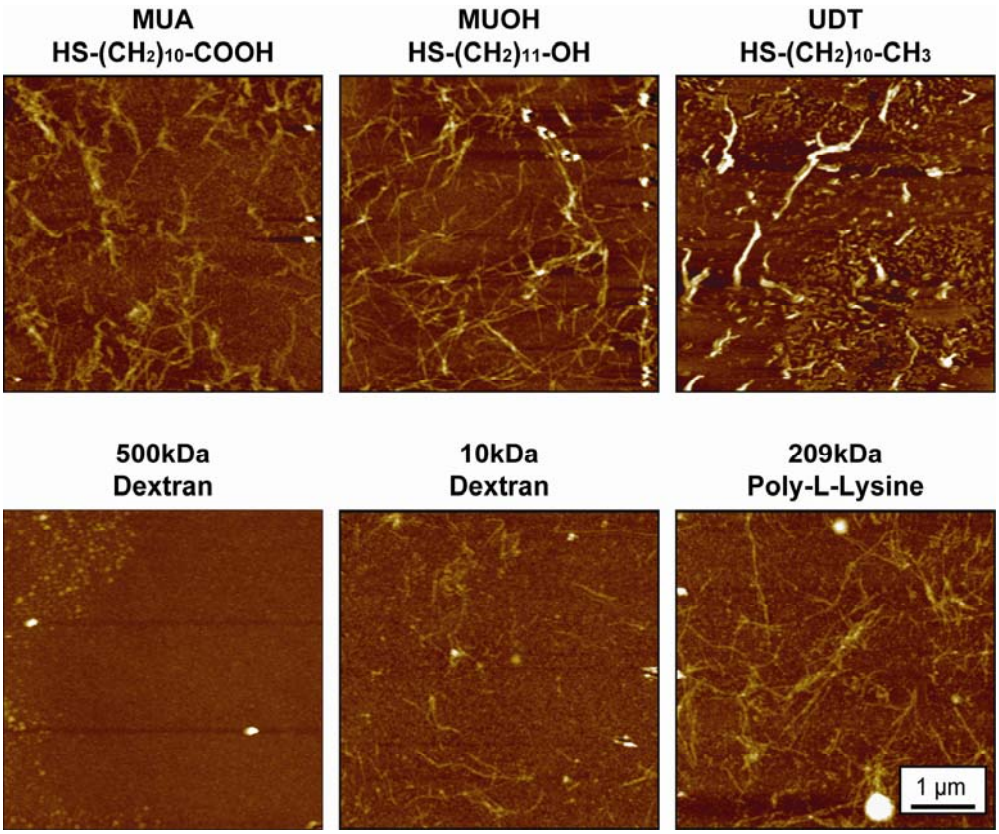

FIGURE S2. Non-specific interaction of Aβ42 to six different surfaces: three kinds of SAM surfaces such as COOH-terminated MUA, OH-terminated MUOH, and CH_3-terminated UDT and three kinds of biopolymer surfaces such as 10 kDa dextran, 500 kDa dextran, and 209 kDa PLL. SPR chips were incubated with a 30 μM Aβ42 solution at 37 °C for 12 h and then scanned by *ex situ* AFM. The size of each AFM image is 5 × 5 μm^2.

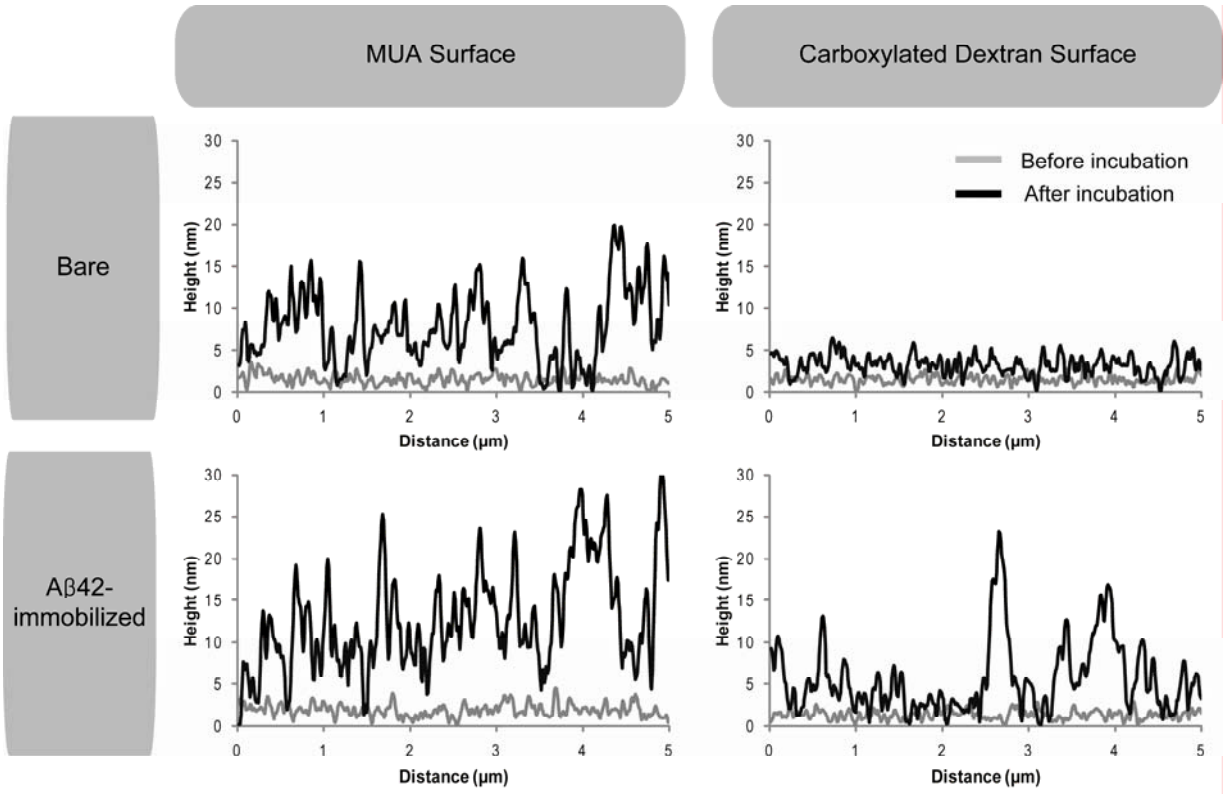

FIGURE S3. Cross-sectional analysis of Aβ42 aggregates formed on a SPR-chip surface before (grey line) and after (black line) incubation. Cross-sectional analysis of AFM images was processed using Nanoscope software provided by the AFM manufacturer (Digital Instruments Inc.). Cross-sectional plots were obtained along the red lines drawn on AFM images in Figure 1 and Figure 2.