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Surface-Directed Structural Transition of Amyloidogenic Aggregates and the Resulting Neurotoxicity

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ABSTRACT: The transition of amyloidogenic species into ordered structures (i.e., pre-bundling oligomers, proto-bundles, mature bundles, and amyloidogenic aggregates) is closely associated with many neurodegenerative disease pathologies. It is increasingly appreciated that the liquid–solid interface contributes to peptide aggregation under physiological conditions. However, much remains to be explored on the molecular mechanism of surface-directed amyloid formation. We herein demonstrate that physical environmental conditions (i.e., negatively charged surface) affect amyloid formation. Nontoxic amyloid aggregates quickly develop into intertwisting fibrils on a negatively charged mica surface. These fibrillar structures show significant cytotoxicity on both neuroblastoma cell-lines (SH-SY5Y) and primary neural stem cells. Our results suggest an alternative amyloid development pathway, following which Aβ peptides form large amyloidogenic aggregates upon stimulation, and later transit into neurotoxic fibrillar structures while being trapped and aligned by a negatively charged surface. Conceivably, the interplay between chemical and physical environmental conditions plays important roles in the development of neurodegenerative diseases.

INTRODUCTION

Among many, the most characteristic pathological feature of neurodegenerative diseases is the formation of amyloidogenic species originating from protein misfolding.1–22 Despite the biological and clinical significance, the underlying mechanisms of amyloid formation in vivo and its correlation with pathophysiological symptoms remain unclear. Numerous factors including elevated temperature, shearing force, low pH, salt concentration, liquid–substrate interfaces, and hydrophilic or hydrophobic surfaces were reported to affect amyloid formation.23–32 Moreover, the origin of cytotoxicity during amyloid development in vivo is still on debate. The belief in the cytotoxicity of both fibril (protofibril or mature fibril) and intermediate oligomeric amyloidogenic species is challenged.33–44

Increasing evidence indicates that complex physical and chemical environmental conditions (i.e., fluctuating levels of secretases, ligands, and mechanical cues) affect cell fate. It is conceivable that pathophysiological symptoms may be caused by interplay among varieties of contributors. Our earlier study reveal that both the secretion of the proinflammatory factor S100A9 and micelles originating from fatty acids trigger the formation of large amyloid aggregates, which may act as a sink for toxic species to mediate neuron damage.45,46 The presence of solid–liquid interface substrates provides templates for the misfolding and ordering of amyloidogenic proteins into fibrils, and eventually plaques.47,48 However, how physical environments facilitate the transition from large amyloid aggregates to ordered structures and affect the neuron system is not yet understood.47,49

Hen egg white lysozyme (HEWL) is highly homologous in sequence and structure to human lysozyme; therefore, it is commonly used as a model protein for the investigation of amyloid aggregation.50,51 Accumulated Aβ peptides are the main component of senile plaques and are derived from the proteolytic cleavage of a larger glycoprotein named amyloid precursor protein.52,53 In this study, HEWL and Aβ(1–40) amyloid aggregates were formed by agitation (continuous shaking), and later deposited on a freshly cleaved mica surface for further development. The effect of surface-directed amyloids on neural stem cells (NSCs) and SH-SYSY behavior and viability was observed in real time using fluorescence microscopy (FM). It is demonstrated that intertwisting fibrillar structures are produced from preformed amyloidogenic aggregates but not from freshly dissolved monomers. The so-developed amyloid fibrils induce significant toxicity in both NSCs and SH-SYSY cells. Our results suggest that the negatively charged surface interacts with the originally helpful amyloid aggregates (mediating OA and S100A9 toxicity), and triggers the formation of neurotoxic amyloid structures.

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RESULTS AND DISCUSSION

Surface-Directed HEWL and Aβ(1−40) Fibril Formation. Freshly dissolved solutions of 10 mg/mL HEWL and 0.1 mg/mL Aβ(1−40) were positioned under agitation at 800 rpm and elevated temperature as described in the Methods section for 24 h. Thioflavin-T (ThT) binding assay of HEWL shows a slow increase during the first 24 h, which is followed by a steep rise of ThT fluorescence intensity during our observation time until ca. 125 h (Figure S1). The absence of the lag phase in the ThT intensity curve indicates the rapid formation of HEWL fibrils. Figure 1. Fluorescence microscopy (FM) and atomic force microscopy (AFM) images of amyloid β(1−40) and HEWL fibril formation directed by the mica surface. (A) FM and (B,C) AFM images of HEWL and Aβ(1−40) oligomers and fibrils reveal that the negatively charged mica surface facilitates morphological transition of preformed oligomers to fibrillar structures. To form amyloidogenic oligomers, 10 mg/mL HEWL and 0.1 mg/mL Aβ(1−40) were subjected to continuous shaking at 800 rpm (57 °C for HEWL and 37 °C for Aβ(1−40)) for 24 h. The preformed oligomers are then deposited on the mica surface. Amyloid fibrils dominate the surface after incubation of entire mica pieces in deionized (DI) water for 24 h. For fluorescence imaging, all samples are stained by flushing with 100 mM ThT solution, followed by imaging using a 60× oil immersion objective lens. As ThT molecules bind specifically to the beta-sheet structures, the fluorescent images show the development of amyloidogenic structures. Scale bars denote 500 nm in all AFM images.

Figure 2. (A) AFM images show Aβ(1−40) structures during transition from pre-formed amyloid aggregates to fibrils on the mica surface with different ionic strengths. Following the deposition on the mica surface, amyloidogenic species are incubated in DI water for 0 h (top panel), 4 h (middle) and 16 h (bottom) before imaging. The scale bars denote 1000 nm in all figures. (B–D) Ratio between Aβ oligomers and fibrillar structures is estimated by measuring the surface coverage percentage. To perturb electrostatic interactions between the Aβ peptide and mica surface, (B) 0, (C) 20, and (D) 50 mM NaCl are added to the development solution. Our results demonstrate that with the emergence of fibrillar structures, there is a significant decrease in the quantities of oligomers, suggesting structural transition. The transition is affected by environmental ionic strength.
Control experiments: the cells were incubated in DMEM cell culture medium alone and the cell viability was equal to 100%. (2) Control surface-directed Aβ(1−40) HEWL amyloid aggregates obtained upon agitation for 3 d. In contrast, the fibrillar structures originating from HEWL amyloidogenic aggregates resemble: (1) control experiments: the cells were incubated in DMEM cell culture medium alone and the cell viability was equal to 100%; (2) HEWL amyloidogenic aggregates obtained upon agitation (continuous shaking at 800 rpm at 57 °C for HEWL and 37 °C for Aβ(1−40)) for 24 h; (3) Aβ(1−40) and HEWL amyloid aggregates obtained upon agitation for 3 d. In figure (C) and (D), various concentrations of preformed Aβ(1−40) and HEWL amyloid aggregates are obtained using centrifugal filtering tube, and incubated with SH-SYSY cells to assess viability, (1) 10 mg/ml HEWL and 0.1 mg/ml Aβ(1−40), respectively; (2) 2 × 10 mg/ml HEWL and 2 × 0.1 mg/ml Aβ(1−40); (3) 3 × 10 mg/ml HEWL, and 3 × 0.1 mg/ml Aβ(1−40); (4) 4 × 10 mg/ml HEWL, and 4 × 0.1 mg/ml Aβ(1−40); (5) 5 × 10 mg/ml HEWL and 5 × 0.1 mg/ml Aβ(1−40). In figure (E): (1) control experiments: the cells were incubated in DMEM cell culture medium alone and the cell viability was equal to 100%; (2) control experiments: the cells were incubated in DMEM cell culture medium on bare mica surface, (3) on mica surface-directed HEWL fibrils, (4) on surface-directed Aβ(1−40) fibrils.

Figure 3. Measurements of SH-SYSY cell line viability by WST-1 assay in the presence of Aβ(1−40) and HEWL samples. The red, green and yellow colors represent 1 day, 2 days and 3 days coincubation with Aβ(1−40) and HEWL, respectively. In figure (A) and (B), the columns represent: (1) control experiments: the cells were incubated in DMEM cell culture medium alone and the cell viability was equal to 100%; (2) Aβ(1−40) and HEWL dissolving buffer; (3) freshly dissolved Aβ(1−40) peptide and HEWL, respectively; (4) Aβ(1−40) and HEWL amyloid aggregates obtained upon agitation (continuous shaking at 800 rpm at 57 °C for HEWL and 37 °C for Aβ(1−40)) for 24 h; (5) Aβ(1−40) and HEWL amyloid aggregates obtained upon agitation for 3 d. In figure (C) and (D), various concentrations of preformed Aβ(1−40) and HEWL amyloid aggregates are obtained using centrifugal filtering tube, and incubated with SH-SYSY cells to assess viability, (1) 10 mg/ml HEWL and 0.1 mg/ml Aβ(1−40), respectively; (2) 2 × 10 mg/ml HEWL and 2 × 0.1 mg/ml Aβ(1−40); (3) 3 × 10 mg/ml HEWL, and 3 × 0.1 mg/ml Aβ(1−40); (4) 4 × 10 mg/ml HEWL, and 4 × 0.1 mg/ml Aβ(1−40); (5) 5 × 10 mg/ml HEWL and 5 × 0.1 mg/ml Aβ(1−40). In figure (E): (1) control experiments: the cells were incubated in DMEM cell culture medium alone and the cell viability was equal to 100%; (2) control experiments: the cells were incubated in DMEM cell culture medium on bare mica surface, (3) on mica surface-directed HEWL fibrils, (4) on surface-directed Aβ(1−40) fibrils.

The distinction may reflect different packing arrangements of proteinaceous material within the corresponding fibrils.

Compelling evidence suggests that Aβ(1−40) very rapidly and efficiently forms spherical amyloid aggregates at neutral pH (Figure S3A–F). In contrast, the conversion from oligomers to amyloid fibrils is slow, following a nucleated conversion mechanism (Figures S3 and S4).55,56 The acidic pH and continuous shaking expedite the process and lead to the formation of mature fibrils (Figure S5).57 Aβ(1−40) fibrils, which were produced after continuous shaking for 2 weeks, are ca. 1 nm in height and 30 nm in width measured by AFM and resemble these surface-directed amyloid structures, indicating a similar packing arrangement. We suspect that the amyloid fibril morphology varies depending on the association mechanism of the round-shaped amyloid aggregates (Figures 1B and S4). As the fibrils on the mica surface were formed by incubation of preformed amyloidogenic aggregates (individual or associated), it is plausible that the fibrillar transition on the mica surface follows a nucleated conformational conversion mechanism similar to the bulk solution.

Conceivably, after being “trapped”, the electrostatic interactions between the negatively charged mica surface and positively charged regions of beta-sheet contents help align the otherwise randomly packed amyloidogenic aggregates and thus facilitate the formation of fibrillar structures.58,59 Consistently, when the electrostatic interactions are perturbed by the addition of excessive salt, smaller amounts of fibrillar structures emerge on the mica surface (Figure 2A). For example, quantities of fibrillar structures with 50 mM NaCl diminished down to ~40% of the ones after 24 h development in DI water. The transition from round-shaped oligomers to intertwined fibrils is hindered by the increase in ionic strength, and the
effect depends on the added salt concentration (Figure 2B–D). Additionally, with increasing salt concentration, the preformed amyloidogenic aggregates seem to be able to further accumulate, which may be caused by mediated electrostatic interactions.

**Effect of Surface-Directed Amyloids on SH-SY5Y and NSCs.** The effect of Aβ(1–40) and HEWL amyloids on the viability of SH-SY5Y neuroblastoma cells was assessed by WST-1 assay. SH-SY5Y neuroblastoma cells were pre-incubated with the amyloid and control samples from 1 to 3 d, followed by measuring the cell viability (Figure 3). Freshly dissolved Aβ(1–40) and its amyloid aggregates formed after 24 h shaking at neutral pH did not induce a significant decrease in cell viability. The level of cell survival dropped to ca. 80% and even to ca. 60% upon incubation with Aβ(1–40) fibrils formed after incubation at acidic pH for 2 and 3 d, respectively (Figure 3A). In contrast, all HEWL samples did not induce observable changes in cell viability under similar experimental conditions (Figure 3B). Considering the fact that 5 μL of protein solution was diluted 20 times after mixing with 100 μL of cell solution before WST-1 assay, we further increase protein concentration by concentrating HEWL and Aβ(1–40) amyloid aggregates solutions. A decrease in the cell survival rate down to 50% for Aβ(1–40) and 60% in the presence of HEWL amyloid aggregates was observed when the protein concentration was increased by 5 fold (Figure 3C,D).

The effect of surface-directed Aβ(1–40) and HEWL amyloid fibrils on the SH-SY5Y cell line was measured by WST-1 upon incubation of cells on the amyloid-coated mica surface (Figure S6). It has been reported that a hydrophilic and negatively charged surface is suitable for cell growth. Although mica possesses both characters, cell viability on the mica surface decreased by ca. 10% during 1–2 d of incubation and by ca. 15% upon incubation (Figure 3E). In the presence of surface-directed Aβ(1–40) amyloids, the cell survival rate decreased by ca. 20% on the 2 d of incubation and by ca. 50% on 3 d of incubation, which are statistically significant changes. The viability of cells co-incubated with HEWL fibrils formed on the mica surface did not change on the first day of co-incubation and decreased by ca. 20% during the second day. There was a significant decrease by ca. 35% on the third day. These results suggest that the originally non-toxic Aβ and HEWL aggregates can transit into toxic amyloidogenic structures with the assistance of the negatively charged surface.

The effect of surface-directed amyloids on NSCs was recorded in real-time using fluorescence imaging. When being plated on the mica surface decorated with these amyloid fibrils, NSC death was observed within 20 h of incubation (Figure 4A,B and Video S1). It is demonstrated that NSCs with a high Dcx level die early (Figure 4C), which is observed as disappeared fluorescence signals (Figure S9), whereas, the number of Hes5-positive NSCs maintains within 20 h incubation. It is reasonable to conclude that differentiated NSCs (represented by the high Dcx level) are more sensitive to stimulations induced by surface-directed amyloid fibrils. Experiments performed using NSC spheres show similar results. The originally round-shaped and dense morphology of NSC spheres can hardly be maintained when in contact with the mica surface decorated with amyloid fibrils. During the first few hours, NSCs detach themselves from the peripheral region of NSC spheres and adopt a round-shaped morphology. In the meantime, Hes5 fluorescence disappears, suggesting cell death. In bright field images, we observed that organization of NSC spheres is destructed after 20 h. Fluorescence images show that the core of the Hes5-positive cells remains, although with decreased volume (Figure 4D). These results together indicate that NSC death is mostly induced by direct contact with amyloid fibrils, which is consistent with Harte’s report that amyloid aggregates are more toxic to differentiated pheochromocytoma (PC12) cells.

Studies on solid model surfaces such as hydrophobic Teflon surfaces, highly ordered pyrolytic graphite surfaces, hydrophilic silica surfaces, and hydrophilic mica surfaces can provide mechanistic details on how model surfaces alter aggregation, elongation and senile plaque formation, which verifies the role of the negatively charged surface in the development of AD symptoms. The catalysis...
effect of surfaces (i.e., solid and membrane) is also verified by simulation studies, showing that the membrane can promote the formation of \(\beta\)-sheets.\(^{68-72}\) Indeed, single stranded and rope-like fibrillar structures have been observed on a chemically modified mica surface.\(^{73}\) Twisting of protofilaments was rarely observed, possibly due to the electrostatic trapping force provided by the mica surface. In this study, neurotoxic \(\alpha\beta(1-40)\) and HEWL amyloid fibrils are produced by incubating preformed amyloid aggregates on the mica surface in DI water. The difference lies in the fact that we deposit preformed amyloid aggregates on the mica surface instead of a freshly dissolved peptide. In bulk solution and on the surface, fibril growth is accomplished by attaching peptide molecules to the active ends of various fibrillar structures. The proteinaceous material can be protein molecules attached to the mica surface or freely diffused molecules in solution. It is likely that \(\alpha\beta\) monomers and protofilaments bind tightly to the surface, and thus these trapped single-stranded protofilaments cannot twist around one another, as might readily occur in solution.\(^{73}\) With preformed amyloid aggregates, amyloid fibrils form by association and rearrangement of the \(\beta\)-sheet content during incubation in DI water. The volume of the aggregates provides separation from the surface and allows the protofilaments to twist around one another. Moreover, amyloid aggregates are composed of numerous \(\beta\)-sheet fragments, which are electrostatically polar structures.\(^{73}\) The study using all-atom explicit solvent replica exchange molecular dynamics simulations reveals that \(\alpha\beta\) amyloid formation involves the electrostatic interaction among His, Glu and Asp residues.\(^{74}\) The negatively charged mica surface may provide alignment and facilitate the association of these amyloid fragments and monomers in the larger amyloid structures. As the twisted conformation is shared by various \(\beta\)-amyloids and often was toxic, the development of protofilaments from amyloid aggregates on a charged surface may be pathologically related to AD.\(^{75-78}\)

The effect of surface-directed amyloids on the neural system is investigated using the neuron-origin SHSY5Y cell-line and NSCs. For SHSY5Y, HEWL and \(\alpha\beta(1-40)\) were toxic in two circumstances: developed on the mica surface and large quantities of amyloidogenic aggregates (non-mature fibrils and gels). As mature HEWL and \(\alpha\beta(1-40)\) fibrillar aggregates are not toxic, it is conceivable that surface-directed \(\alpha\beta(1-40)\) and HEWL fibrils were the toxic amyloid structures, and the presence of the negatively charged surface in vivo may help convert the non-toxic amyloidogenic aggregates into toxic amyloidogenic structures. Previous studies reveal that formation of amyloidogenic species can directly affect NSCs during the development of neurodegenerative diseases.\(^{79}\) Evidence suggests that \(\alpha\beta\) amyloids lead to NSC death and differentiation,\(^{80}\) which may exhaust the stem cell pool and hinder the healing process of the neural system. In this study, NSC differentiation and self-maintenance are assessed at the single cell level by tracking Hes5-GFP expression (indicative of self-renewing NSCs) and Dcx-RFP to label neuroblasts (indicating progress towards differentiation).\(^{81-86}\) The NSC growth rate is another key feature we quantified to statistically assess the self-renewal of NSCs. By quantitatively analyzing real-time NSC imaging on the mica surface decorated with \(\alpha\beta(1-40)\) amyloids, we demonstrate that NSCs with high expression of Dcx are more vulnerable to toxic \(\alpha\beta\) amyloidogenic structures. However, the effect is diminished when NSCs are maintained as neural spheres, where NSCs are protected from direct contact with \(\alpha\beta\) amyloids. These results suggest that surface-directed amyloids in-vivo may directly cause neuron cell death, but exert less effect on NSCs.

### CONCLUSIONS

Overall, our results, in combination with previous studies, emphasize the importance of physical environmental conditions during AD development. The negatively charged surface could not only induce the formation of intertwined protofilaments, but also provide a “dish” filled with \(\beta\)-sheet structures. These \(\beta\)-sheet structures may act as “seeds” for further amyloid formation and finally senile plaques.

### EXPERIMENTAL SECTION

#### Sample Preparation.

All chemicals including HEWL were purchased from Sigma-Aldrich, unless mentioned differently. \(\alpha\beta(1-40)\) was produced by Alexotech (Umea, Sweden) in 0.5 mg weighed aliquots. Experiments were performed by using \(\alpha\beta\) peptide concentrations of 0.1 mg/mL and HEWL 10 mg/mL, respectively, determined by weight and optical density. HEWL was dissolved in 20 mM glycine buffer, pH 2.3. The \(\alpha\beta(1-40)\) peptide was dissolved at low temperature (on ice) following a protocol released previously.\(^{77}\) The chilled \(\alpha\beta\) peptide powder was dissolved in 10 mM NaOH at a concentration above 1 mg/mL, followed by sonication in an ice bath for 1 min. Then, a trace amount of 1 M NaH2PO4 buffer was added to adjust the sample solution to either pH 7.4 or 3.0. The stock solution of the freely diffused \(\alpha\beta\) peptide was diluted to desired concentrations before experiments with neutral pH.

Amyloidogenic structures (i.e., oligomers, large aggregates and mature fibrils) were produced following different operational protocols. To form densely packed amyloid fibrils, the HEWL solution was subjected to shaking at 800 rpm at 57 °C for a week. An \(\alpha\beta(1-40)\) amyloid gel was formed by incubation at pH 3.0 and 37 °C for a week with no agitation. Prior to cytotoxicity experiments, \(\alpha\beta(1-40)\) and HEWL amyloid fibrils were dialyzed to pH 7.4 at 4 °C. To produce amyloid aggregates, HEWL (57 °C and pH 2.3) and \(\alpha\beta(1-40)\) (37 °C and pH 7.4) were subjected to agitation at 800 rpm for 24 h. The surface directed protofilaments formed by deposition of amyloid aggregates on a mica surface. Firstly, \(\alpha\beta(1-40)\) and HEWL amyloid aggregate solutions were kept on the mica surface for 30 and 5 min, respectively. Different deposition times were needed due to the difference in concentrations. Mica pieces of ~1 cm by 1 cm in size carrying amyloid aggregates were then thoroughly washed in running water and incubated in a Petri dish holding a large volume of DI water (~10 mL) at room temperature for 24 h before AFM, FM imaging, and cell viability tests.

#### ThT Assay.

A ThT stock solution was prepared by dissolving 2.5 mM ThT (Merck Schuchardt) in phosphate buffer (10 mM phosphate, 150 mM NaCl, pH 7.4) and filtered before use. This stock solution was diluted 50-fold in the phosphate buffer to produce a working solution. 10 μL of protein aliquots were collected and added to 300 μL of the working solution. The mixture was incubated in the dark for 1 min to allow ThT binding. The ThT fluorescence intensity was measured using a FluoroMax-2 spectrophuorometer (JobinYvon/Pxel Instruments). The excitation and emission wavelengths are 440 and 485 nm, respectively. The slit width is 5 nm. The ThT fluorescence intensities were normalized to the fluorescence intensity of the free dye in solution.
Atomic Force Microscopy. All imaging experiments were carried out at room temperature in air with a Dimensions 3000, Veeco, Woodbury, NY, and a PicoPlus, Agilent atomic force microscope. Images were acquired in the tapping mode with silicon (Si) cantilevers (spring constant of 20–100 N/m) and operated below their resonance frequency (typically 230–410 kHz). The images were flattened. The contrast and brightness were adjusted for optimum viewing conditions. Amyloid samples produced in solution were deposited on the surface of freshly cleaved mica (Good Fellow) for 5 min (HEWL) and 30 min (Aβ(1–40)). The mica pieces were washed 3 times with 200 μL of DI water and dried in a flow of N2 gas at room temperature. The samples with surface-directed fibrils were taken out from DI water and dried with a flow of N2 gas prior to imaging.

Fluorescence Imaging. Aβ(1–40) and HEWL amyloid structures were deposited on mica. To visualize the structures, mica pieces were flushed with a 100 μM ThT solution, followed by washing with DI water3 times. The fluorescence of the stained amyloids was visualized with an Olympus IX71 inverted fluorescence microscope equipped with a 100 W mercury lamp, a UV filter set, and a 60× oil immersion objective. A UV light shutter controlled the exposure time of ThT fluorescence. Images were recorded using a charge coupled device camera (Olympus DP 70).

Cell Culture. SH-SYSY neuroblastoma cells (ATCC CRL-2266) were routinely cultured in the Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum. NSCs were isolated and cultured from rat embryos of day 16.5. Disassociated cortical hemispheres were cut into 1 mm3 pieces in ice-cold phosphate-buffered saline (PBS, pH 7.2) with 10 mM d-glucose. Small pieces of cortical tissue were collected and enzymatically dissociated by using a Papain Dissociation System as indicative materials (Worthington Biochemical Corp). Neurospheres composed of NSCs formed in 1 week of initial planting in the Neurobasal medium (Invitrogen) containing 2% (v/v) NS21 supplement, 0.5 mM L-glutamine, 100 units/mL penicillin and 100 mg/mL streptomycin in an incubator with 95% humidity, 37 °C and 5% (v/v) CO2. The NSCs were passaged weekly by enzymatic dissociation of the neurospheres. Both SH-SYSY and NSC viable cells were counted following staining with 0.2% (w/v) trypan blue (Gibco/Invitrogen) and plated at a density of 10,000 cells per well (100,000 cells/mL) in a 96-well black-walled imaging plate (BD Falcon). To investigate the effect of surface-directed amyloids on NSCs, specially cut mica pieces with and without preattached amyloid structures were positioned on the bottom of the well, followed by the same procedure of cell culturing (Figure S5).

WST-1 Cell Viability Assay. In viable cells, WST-1 undergoes reduction by mitochondrial dehydrogenases (succinatetetrazoliumreductase system) to soluble formazan, which serves as an indicator of the quantity of metabolically active cells. 10 μL of WST-1 reagent was added and coincubated with the cell solutions at 37 °C for 4 h to evaluate cell viability. The absorbance was measured using an ELISA plate reader (LabSystems Multiskan RC) at 450 nm. The cell viability was expressed as a percentage of absorbance in wells containing cells treated with amyloids compared to the control cells.

Live-Cell Fluorescence Microscopy. For image acquisition, a Nikon Ti2E microscope with an automated translational stage and a digital CMOS camera (ORCA-Flash 4.0, Hamamatsu, Japan) was used. The stage and image acquisition was controlled via NIS Elements software (Nikon, Japan). During imaging, the external conditions were set to standard culture conditions (5% CO2 and 37 °C external temperature) and maintained at this level.

ASSOCIATED CONTENT
Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03671.

Assessment of Aβ(1–40) and HEWL amyloid formation with and without continuous shaking using ThT binding assay (Figure S1); fluorescence images showing mica surface-directed Aβ(1–40) amyloid formation (Figure S2); atomic force microscopy (AFM) height images showing the Aβ(1–40) amyloid formation process without shaking (Figure S3); schematic drawing shows the possible underlying mechanism of surface-directed Aβ amyloid fibril formation (Figure S4); AFM images of HEWL and Aβ(1–40) in a gel-form after shaking for 2 weeks (Figure S5); experimental procedure of cell culturing on the mica surface (Figure S6) (PDF) Comparison between NSCs deposited on the mica surface coated with freshly dissolved Aβ(1–40) and the mica surface with preformed Aβ(1–40) amyloids (Video S1) (AVI)

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H.C. and D.S. contributed equally to this work.

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H.C. and D.S. made equal contributions. H.F., H.C., and Y.T. performed the experiments. Y.T. performed calculations and
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