Chemically exfoliated WS$_2$ nanosheets can efficiently inhibit amyloid β-peptide aggregation and use for photothermal treatment of Alzheimer’s disease

Meng Li, Andong Zhao, Kai Dong, Wen Li, Jinsong Ren and Xiaogang Qu (✉)

Nano Res., Just Accepted Manuscript • DOI 10.1007/s12274-015-0821-z
© Tsinghua University Press 2015

Just Accepted

This is a “Just Accepted” manuscript, which has been examined by the peer-review process and has been accepted for publication. A “Just Accepted” manuscript is published online shortly after its acceptance, which is prior to technical editing and formatting and author proofing. Tsinghua University Press (TUP) provides “Just Accepted” as an optional and free service which allows authors to make their results available to the research community as soon as possible after acceptance. After a manuscript has been technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Please note that technical editing may introduce minor changes to the manuscript text and/or graphics which may affect the content, and all legal disclaimers that apply to the journal pertain. In no event shall TUP be held responsible for errors or consequences arising from the use of any information contained in these “Just Accepted” manuscripts. To cite this manuscript please use its Digital Object Identifier (DOI®), which is identical for all formats of publication.
Chemically Exfoliated WS₂ nanosheets Can Efficiently Inhibit Amyloid β-Peptide Aggregation and Use for Photothermal Treatment of Alzheimer’s Disease
Meng Li, Andong Zhao, Kai Dong, Wen Li, Jinsong Ren and Xiaogang Qu*

Laboratory of Chemical Biology and State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Changchun, Jilin 130022, China.

Herein, chemically exfoliated 2D WS₂ nanosheets were employed to strongly inhibit Aβ aggregation and dissociate Aβ fibrils upon NIR irradiation by using the unique high NIR absorption property of WS₂. The low cellular toxic WS₂ nanosheets possessed the potential ability to cross blood-brain barrier (BBB) to overcome the drawback of previously reported small-molecular-weight Aβ inhibitors.
Chemically Exfoliated WS$_2$ nanosheets Can Efficiently Inhibit Amyloid β-Peptide Aggregation and Use for Photothermal Treatment of Alzheimer’s Disease

Meng Li, Andong Zhao, Kai Dong, Wen Li, Jinsong Ren and Xiaogang Qu (✉)

Laboratory of Chemical Biology and State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Changchun, Jilin 130022, China.

ABSTRACT

Polymerization of amyloid-β peptide (Aβ) into amyloid fibrils is a critical step in the pathogenesis of Alzheimer’s Disease (AD). Inhibition of Aβ aggregation and destabilization of preformed Aβ fibrils have shown promising against AD and been used in clinic trials. Herein, we demonstrate for the first time the application of WS$_2$ nanosheets to not only effectively inhibit Aβ aggregation, but also dissociate the preformed Aβ aggregates upon NIR irradiation. Further studies indicated that the biocompatible WS$_2$ nanosheets possess the potential ability to cross blood-brain barrier (BBB) to overcome the drawback of the most previously reported Aβ inhibitors. Through van der Waals and electrostatic interactions between Aβ$_{40}$ and WS$_2$, Aβ$_{40}$ monomers can selectively adsorb on the surface of the nanosheet to inhibit the aggregation process of Aβ$_{40}$. Intriguingly, by using unique high NIR absorption property of WS$_2$, amyloid aggregates can be dissolved upon NIR irradiation. These results will promote biological applications of WS$_2$ and provide new insights into design of new multifunctional nanomaterials for treatment of AD.

1 Introduction

As the most prevalent age-related neurodegenerative disease, Alzheimer’s disease (AD) has been characterized by progressive brain atrophy/neuronal death which can result in cognitive and memory impairment [1]. The accumulation of extracellular amyloid β-peptide (Aβ) plaques has been demonstrated to be a pathological hallmark of AD [2-7]. Although the mechanism of Aβ neurotoxicity is not fully understood, recent advances have demonstrated that polymerization of Aβ into amyloid fibrils is a critical step in the pathogenesis [2-7]. Therefore, diverse therapeutic strategies that inhibit Aβ aggregation and dissociate the preformed Aβ fibrils are being pursued.

For this purpose, a range of β-sheet breaker
peptides [3-10] and organic molecules [11-15] have been designed and synthesized to inhibit Aβ aggregation and toxicity. However, the weak targeting [16] and poor permeability through the blood-brain barrier (BBB) and/or toxic side effects [17,18] lead to only moderate inhibition efficiencies and weak disaggregation abilities of a majority of these traditional inhibitors. To overcome these limitations, nowadays nanomaterials as novel therapeutic agents have been designed to inhibit Aβ aggregation and disaggregate Aβ fibrils [19,20].

Graphene oxide (GO), the water-soluble derivative of graphene, is a two-dimensional (2D) single atomic layer of carbon atoms arranged in a honeycomb lattice, possessing unique properties, such as atomic layered structures, large surface area and easy functionalization [21-25]. It has been proposed that GO can adsorb Aβ monomers via π-π interactions and hydrophobic interactions to prevent Aβ aggregation [26,27]. Recently, extensive attention has been focused on the other 2D nanomaterials, including the transition metal dichalcogenides (TMDs) (e.g., WS₂, etc.) due to their 2D layer structure analogous to graphene [28-35]. Being an ultrathin direct bandgap semiconductor, WS₂ with a layered structure has been used in the area of nanoelectronics, optoelectronics, and electrocatalysis [36-38]. Further biological applications of WS₂ nanosheets remain challenging. Herein, for the first time, WS₂ nanosheets were found to strongly inhibit Aβ fibrillation and dissociate Aβ fibrils.

As an alternative to the aforementioned materials, chemically exfoliated WS₂ is a mixed phase, two-dimensional amphiphile that is easy to synthesize in large batches and is directly dispersible in water. Compared with GO, the magnitude of its ζ potential (-23 mV) provides great colloidal stability in aqueous media. The physisorption of aromatic (e.g., pyridine, purine, etc.) and conjugated compounds on the basal plane of TMDs nanosheet has been reported using either theoretical calculations or experimental studies [39-41]. Therefore, we expect that WS₂ can adsorb Aβ monomer via the van der Waals force between aromatic amino acids and the basal plane of WS₂. The sulfur existed in the nanosheet [42] can perturb the formation of hydrogen bonding in Aβ aggregation, which makes a further contribution to the inhibition effect of WS₂. Besides, the favorable electrostatic interactions between cationic cluster HHQK of Aβ [43] and negatively charged WS₂ [44] would enhance their binding. In considering these special properties, WS₂ can be a promising candidate for inhibition of Aβ fibrillation.

On the other hand, owing to the high optical absorption in the NIR region, a new direction for TMDs nanosheet is in the field of biomedicine. Recently, Chou and co-workers [45] and Liu’s group [46,47] have explored biomedical applications of MoS₂ as near-infrared photothermal agents for selective photoablation of cancer cells. As we and others reported previously, local heat generation (hyperthermia) can act as an effective means to dissolve the amyloid aggregates of Aβ [48-50]. The preformed Aβ fibrils can be dissociated by utilizing TMD nanosheets strong NIR optical absorption ability to generate local heat upon low-power NIR laser irradiation. Therefore, WS₂ nanosheet can not only effectively inhibit Aβ aggregation, but also dissociate the preformed Aβ aggregates upon NIR irradiation (Figure 1). Photothermal therapy treatment possesses several remarkable advantages, including their non-invasive property, and unique site- and time-specificity. Together with the ability of nanomaterials to cross the BBB [51,52], WS₂ can be a promising candidate for treatment of AD. To the best of our knowledge, there is no report of using WS₂-based material for treatment of AD.
Figure 1. Schematic representation of WS$_2$ nanosheets with high NIR absorbance used for AD treatment.

2 Experimental

2.1 ceWS$_2$ Synthesis

First, 100 mg of WS$_2$ powder was reacted with 3 ml of n-butyllithium (1.6 M in hexane) under a nitrogen environment. After 2 day, the mixture was filtered over Whatman #41 filter and washed 3 times with 100 ml of hexane. To achieve exfoliation, 300 ml of deionized H$_2$O was added to the semi-dry mixture and was sonicated for 1 hour. The solutions were then centrifuged and washed with deionized H$_2$O for 3 times to remove the lithium cations and the unexfoliated materials. It was then collected and dialyzed against deionized H$_2$O for 5 days.

2.2 Peptide Preparation

Aβ40 (lot no. U10012) was obtained from American Peptide and prepared as previously described. Firstly, the Aβ40 peptide power was dissolved in 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP) with a concentration of 1 mgmL$^{-1}$. The dissolved solution was kept in a sealed vial and shaken at 4 °C for 4 h for further dissolution. After that, the solution was stored at -20 °C as a stock solution. Before use, the solvent HFIP was removed by evaporation under a gentle stream of nitrogen and the peptide was dissolved in water. Aβ40 self-aggregation was accomplished by incubating the water solution in aggregation buffer (10 mM Tris in 150 mM NaCl, pH 7.3) at 37 °C for 7 days.

2.3 ThT Fluorescence Measurements

The kinetics of Aβ40 aggregation was monitored by ThT fluorescence assay. ThT is an amyloid-specific dye that exhibits enhanced fluorescence intensity upon binding to Aβ40 fibrils, instead of Aβ40 monomers. When added to samples containing β-sheet-rich aggregates, it exhibits an excitation maximum at 444 nm and enhanced emission at 482 nm. The reaction can be completed within 1 min in an aqueous environment. Fluorescence measurements were carried out using a JASCO FP6500 spectrofluorometer. The fluorescence signal was recorded between 460 and 650 nm; 10 nm slits were used for both emission and excitation measurements. In inhibition experiment, the samples of Aβ40 peptide (50 μM) with or without various concentrations of WS$_2$ were incubated at 37 °C for 7 days. At different times, aliquots of each sample were taken for fluorescence measurements. The concentration of Aβ40 used for measurements was kept at 1 μM, and the ThT concentration was 10 μM. In disaggregation experiment, preformed Aβ40 fibrils were treated with 40 μg/mL WS$_2$ nanosheets and irradiated for 5 min. Then aliquots of the Aβ40 solution were taken for fluorescence measurements. The peptide concentration used for measurements was 5 μM, and the ThT concentration was 10 μM.

2.4 Circular dichroism (CD) measurements

The samples were measured in 10 mM Tris buffer (pH 7.3) after incubation at 37 °C for 7 days. CD spectra were collected at 37 °C with a JASCO J-810 spectropolarimeter using a 1 mm path length quartz cell. The parameters were controlled as 0.1 nm intervals, 4 seconds response, and each sample was an average of three scans in a speed of 5 nmmin$^{-1}$ over the wavelength range from 200 nm to 250 nm.

2.5 Attenuated Total Reflection Fourier-Transform Infrared (ATR-FTIR) Spectroscopy
For ATR-FTIR assay, peptides dissolved in Tris buffer (Tris in 150 mM NaCl, pH 7.3) at a concentration of 50 μM, treated with or without WS₆ nanosheets after 7 days of incubation, were lyophilized and resuspended at a concentration of 50 μM in D₂O. ATR-FTIR spectra were recorded using a Nicolet 6700 infrared spectrometer. Spectra of 128 scans were taken with a spectral resolution of 2 cm⁻¹. The ATR out-of-compartment accessory consists of a liquid jacketed Piketech ATR flow-through cell with a trapezoidal Ge-crystal (Piketech, Madison, WI, 80 × 10 × 4 mm³, angle of incidence: 45°). The ATR flow-cell was tempered to 25 °C. Spectra were processed using GRAMS software (Thermo Electron). In this study, the final concentration of Aβ40 and WS₆ nanosheets were 50 μM and 40 μg/mL.

2.6 Atomic Force Microscopy

For the atomic force microscopy (AFM) measurements, samples were diluted with deionized H₂O to yield a final concentration of 1 μM. Then the sample (20 μL) was applied onto freshly cleaved muscovite mica and allowed to dry. Data were acquired in the tapping mode on a Nanoscope V multimode atomic force microscope (Veeco Instruments, USA).

3 Results and discussion

3.1 Synthesis and Characterization of the WS₆ Nanosheets

The WS₆ nanosheet was synthesized according to the Morrison method [53-55] which broke the weak interlayer forces in bulk WS₆ through Li ion insertion and ultrasonication. As shown in X-ray photoelectron spectroscopy (XPS), the peaks at 163.4 and 162.2 eV corresponded to the S2p1/2 and S2p3/2 orbital of divalent sulfide ions (Figure 2B). Besides, the W peaks shown in Figure 2A located at 38.9, 35.5, and 33.4 eV were assigned to W5p3/2, W4f5/2, and W4f7/2, respectively. The energy positions of these peaks indicated a W valence of +4, indicating the formation of pure WS₆ phase. In addition, atomic force microscopy (AFM) (Figure 2C) demonstrated that the WS₆ separated well with an average topographic height of \(~1.7±0.2\) nm and mean longest diagonal of 200 nm (n=50 sheets). The relative small size made them possible to penetrate the BBB.

Figure 2. XPS spectra of ceWS₆ (A) W4f, (B) S2p. (C) AFM images and height profiles of the corresponding AFM topographies of typical nanosheets of WS₆.

3.2 Effect of the WS₆ Nanosheets on the Kinetics of Aβ40 Fibrillation Process

To examine the influence of WS₆ nanosheets on the fibril formation of Aβ40 (Figure 3A), incubation solutions of the Aβ40 with and without WS₆ were prepared. The kinetics of fibrillation was monitored by a commonly used thioflavin T (ThT) assay. ThT, an extrinsic fluorescent dye, is able to bind to amyloid fibrils; upon binding, its fluorescence intensity increases [56,57]. When fresh Aβ40 alone incubated at 37 °C, ThT fluorescence as a function of incubation time showed a sigmoidal shape (Figure 3B). This result was consistent with the nucleation-dependent polymerization model. However, in the presence of WS₆, ThT fluorescence did not increase, which indicated that Aβ40
amyloid formation was suppressed. We also conducted a control experiment to clarify that the fluorescence of ThT was not influenced by the addition of WS₂ (Figure S1). Interestingly, after binding with Aβ40 monomers, the ζ potential of WS₂ was changed (Figure S2). To investigate whether WS₂ nanosheets inhibit Aβ40 aggregation by adsorbing monomers on the surface, tyrosine intrinsic fluorescence and AFM assays were applied to analyze the effect. AFM images (Figure S3) clearly showed that Aβ40 monomers could be adsorbed on the surfaces of WS₂ nanosheets. Tyrosine fluorescence signals of Aβ40 peptides were acquired after titration with different concentrations of WS₂ nanosheets. Figure S4A revealed a marked quenching of the tyrosine fluorescence signal when Aβ40 was mixed with the WS₂ nanosheets, which meant that the large surface area of WS₂ sheets can adsorb and bind Aβ40 monomers to quench the tyrosine fluorescence signal. The amount of Aβ40 monomers bound to WS₂ nanosheets was determined via calculation of the difference of absorbance at 276 nm between the Aβ40 solution before and after incubation with WS₂, which was defined as 436.96 μmol/g WS₂ (Figure S4B). A significant body of data has indicated trapping the monomers and/or blocking the growing oligomer ends on the surface of nanomaterials could disturb the monomer-critical nuclei equilibrium, thereby decreasing their solution concentration and interfering with their elongation to form fibrils [19,58,59]. In order to check whether TMDs nanosheets with similar structures could inhibit Aβ40 self-assembly and demonstrate the superiority of WS₂ over GO, we examined the inhibition effects of MoS₂ and GO nanosheets. MoS₂ sheets with an average topographic height of ~1.6±0.4 nm and mean longest diagonal of 200 nm (Figure S5A) were synthesized according to the same method as WS₂. While GO obtained from graphite powder via a modified Hummers’ method [25] possessed an average topographic height of ~1.5±0.3 nm and mean longest diagonal of 200 nm (Figure S5B). Figure S5C clearly indicated that MoS₂ exhibited comparable inhibition effect on Aβ40 aggregation with WS₂. Furthermore, both of them can decrease ThT fluorescence in a dose-dependent manner (Figure S5D). Interestingly, when compared the inhibition effects of WS₂ on Aβ40 aggregation with GO, WS₂ showed a stronger inhibition effect than GO under the same experimental conditions from the results of ThT fluorescence assay (Figure S5D) and CD spectra (Figure S5E). The enhanced inhibition effect of WS₂ could be attributed to the large excess sulfur in the nanosheets [42] by forming hydrogen bonding between peptides and WS₂ nanosheets.

Inhibition effect on Aβ40 aggregation was further studied by AFM (Figure S6A and S6B). AFM has been widely used to study the morphology of Aβ40 amyloid fibrils. The sample of Aβ40 alone formed typical unbranched Aβ40 amyloid fibrils longer than 1 μm. In the presence of the WS₂, there was almost no obvious aggregates or fibrils observed. As shown in Figure S6, Aβ40 monomers can selectively attach on the surface of WS₂ to inhibit the aggregation process.

Aβ oligomers, protofibrils and fibrils all share the common β-sheet structure which drives Aβ aggregation and toxicity. Circular dichroism studies clearly indicated that WS₂ could inhibit structural transition from native Aβ40 random coil to β-sheet conformation in solution (Figure 3C). As a control experiment, the presence of WS₂ nanosheets in the Aβ40 monomer solution could not change the Aβ40 random coil structures (Figure S6C). For further to study the structures of Aβ40 on the surface of WS₂ nanosheets, we applied the surface-sensitive attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy technique [60-62] to be able to monitor the amyloid formation in the presence of WS₂. As shown in Figure 3D, after Aβ40 alone incubated for 7 days, formation of...
β-sheet-rich amyloid fibrils was characterized by their specific amide I subband which appeared at approximately 1623 cm⁻¹. When WS₂ nanosheets were included in the buffer, no such aggregation subband was observed. Rather, broad IR bands at about 1640-1645 cm⁻¹ appeared, which were characteristic of a large contribution of disordered conformations arising from the presence of monomeric and small oligomeric Aβ40 particles in solution.

Figure 3. (A) Inhibition of Aβ40 aggregation by WS₂ nanosheets. (A) Schematic representation of WS₂ nanosheets used for inhibiting Aβ40 aggregation. (B) Fibrillation kinetics of Aβ40 as monitored by the development of thioflavin T binding in the absence or presence of WS₂ nanosheets. The concentration of Aβ40 was 50 μM. The data points shown are the mean values ± SEM from three independent experiments. (C) CD spectra of Aβ40 in the absence or presence of WS₂ nanosheets. The samples were measured in 10 mM Tris buffer (pH 7.3) after incubation at 37°C for 7 days. (D) ATR-FTIR spectra of Aβ40 in the absence or presence of WS₂ nanosheets. In this study, the final concentrations of Aβ40 and WS₂ nanosheets were 50 μM and 40 μg/mL, respectively.

3.3 The Hyperthermic Effects of WS₂ on Dissociating the Aβ Fibrils

Having established the inhibition effect of the WS₂ nanosheets, we next investigated the ability of the novel nanosheet to dissociate the Aβ40 aggregates upon NIR irradiation. Similar to GO, WS₂ exhibited a high optical absorption (Figure S7). To verify the potential of using WS₂ in photothermal therapy, the WS₂ solution was exposed to an 808 nm NIR laser with water as the control. In marked contrast to the water sample, the WS₂ solution showed a rapid increase of temperature when exposed to the laser within a short time. Furthermore, the solution of WS₂ exhibited a concentration-dependent (from 10 to 40 μg/mL⁻¹) and radiant energy-dependent (from 0.5 to 2 W cm⁻²) photothermal heating effect (Figure S8).

Motivated by the strong NIR optical absorption property of WS₂, we then carried out a photothermal study on Aβ40 aggregation (Figure 4A). Aβ40 fibrils in aggregation buffer (10 mM Tris, 150 mM NaCl, pH 7.3) were incubated with WS₂ for 20 min at 37°C to maximize the targeting effect of WS₂ and Aβ40 fibrils. The presence of Aβ40 fibrils enhanced the fluorescence of ThT. However, after we exposed the solution to a laser with a power density of 1 Wcm⁻² to irradiate the WS₂-Aβ40 aggregates, the fluorescence signal of ThT decreased gradually following the increase of irradiation time, indicating that the amount of Aβ40 aggregates was diminished by the local heat generated by WS₂ upon NIR irradiation (Figure S9).

In marked contrast, for the irradiation of Aβ40 fibrils alone or the WS₂ with Aβ40 aggregates without laser irradiation, the fluorescent signals remained almost unchanged (Figure 4B), demonstrating that neither the NIR laser irradiation alone nor WS₂ by itself can affect the Aβ40 structure in a short time under our experiment condition. To demonstrate the ability of WS₂ for dissociating Aβ40 aggregates upon NIR irradiation more clearly, CD spectroscopy was employed to measure the secondary structure change (Figure 4C). According to the computer fit results, after photothermal treatment, the content of random-coil had an increase of 22.1% (from 21.2% to 43.3%), while the β-sheet decreased from 54.8% to 22.4%. The decreased content of β-sheet conformation supported that Aβ fibrils were dissociated after photothermal treatment.
The same results were also observed by using AFM. When incubated with WS\textsubscript{2}, the morphology of the Aβ40 fibrils remained almost unchanged illustrating that WS\textsubscript{2} could hardly alter the assembly of Aβ40 (Figure 4D). However, after irradiation with NIR laser, numerous small, relatively amorphous aggregates were observed in the WS\textsubscript{2} treated Aβ40 samples, demonstrating the excellent efficacy of WS\textsubscript{2} to disaggregate the preformed Aβ40 aggregates upon NIR laser irradiation (Figure 4E). In contrast, without WS\textsubscript{2}, exposure to the laser alone could not change the morphology of the amyloid fibrils (Figure S10). These results further supported the above results and indicated that our design can effectively dissociate Aβ40 fibrils.

It is well known that Aβ accumulated in cerebrospinal fluid (CSF) is a diagnostic and therapeutic target for AD [50]. Therefore, it is important to perform the experiment in CSF in order to determine whether WS\textsubscript{2} can work well in a biological matrix which contains various other proteins. As shown in Figure 5, WS\textsubscript{2} could not only effectively inhibit Aβ40 aggregation (Figure 5A) but also dissociate the preformed Aβ40 aggregates upon NIR irradiation (Figure 5B) even in CSF.

To show whether other proteins can also be adsorbed by WS\textsubscript{2}, we took bovine serum albumin (BSA) as the example of proteins. As shown in Fig. S11, BSA could associate with WS\textsubscript{2} with an efficiency of 0.63 g/g WS\textsubscript{2} to form a protein corona on the surface of WS\textsubscript{2}. Although the interactions between WS\textsubscript{2} and Aβ were not specific, the protein coated WS\textsubscript{2} (WS\textsubscript{2}-BSA) nanosheets still possessed the ability to inhibit Aβ aggregation via binding and adsorbing Aβ monomers, which were clearly demonstrated by the fluorescence titration, ThT assay and AFM study (Figure S12). It has been widely reported that formation of the protein corona can largely define the biological identity of the nanomaterials [26]. However, our study clearly demonstrated that the ability of WS\textsubscript{2} to inhibit Aβ aggregation was not affected by adsorbing other proteins on the surface.

**Figure 4.** The influence of the photothermal effect of WS\textsubscript{2} nanosheets on Aβ40 disaggregation. (A) Schematic representation of WS\textsubscript{2} nanosheets used for dissociating Aβ40 aggregation. (B) The influence of the photothermal effect of WS\textsubscript{2} nanosheets on Aβ40 aggregation monitored by fluorescence spectroscopy. The signals were collected after irradiation for 5 min. The concentrations of Aβ40 and WS\textsubscript{2} nanosheets were 50 μM and 40 μg/mL, respectively. (C) CD spectra of Aβ40 fibrils in the presence of WS\textsubscript{2} nanosheets treated with or without NIR irradiation. The final concentrations of Aβ40 and WS\textsubscript{2} nanosheets were 50 μM and 40 μg/mL, respectively. (D) The morphology of Aβ40 fibrils in the presence of WS\textsubscript{2} nanosheets. (E) The morphology of Aβ40 fibrils in the presence of WS\textsubscript{2} nanosheets under laser irradiation.

**Figure 5.** The influence of WS\textsubscript{2} on Aβ aggregation monitored by ThT assay in mice CSF. (A) The dose-dependent inhibition effects on Aβ40 aggregation of WS\textsubscript{2}. The concentration of Aβ40 was 50 μM. (B) The influence of the photothermal effect of WS\textsubscript{2} nanosheets on Aβ40 fibrils monitored by fluorescence spectroscopy. The concentrations of Aβ40 fibrils and ThT were 5 μM and 10 μM, respectively.
3.4 The WS₂ Nanosheets Rescue Aβ40-Induced Cytotoxicity

Since WS₂ nanosheets can inhibit Aβ40 aggregation, disaggregation of the amyloid aggregates upon laser irradiation, the question arose as to whether the nanosheets can be used to block Aβ40-mediated cellular toxicity. To demonstrate this question, we used PC12 cells to perform 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliun m bromide (MTT) assay to probe cellular metabolism. Figure 6 showed that Aβ40 fibrils (5 μM) led to a decrease of 44% in cellular reduction of MTT (fAβ40 sample). However, treatment of the cells with Aβ40 which was pre-incubated with 4 μg/mL WS₂ for 7 days increased the survival of the cells to about 80% within the experimental errors (Aβ40-WS₂ samples). On the other hand, even after the Aβ40 fibrils preformed, treatment of the cells with Aβ40 fibrils in the presence of WS₂ under NIR laser irradiation for 5 minutes could effectively decrease the cytotoxicity of Aβ40 (fAβ40-WS₂-NIR sample). In order to decrease the side toxicity effect of WS₂ on PC12 cells upon irradiation, we used the lower power density of laser (0.5 W/cm²) to carry out this experiment. Furthermore, WS₂ can prevent Aβ40-induced cell death in a dose dependent manner (Figure 6). Aβ40 fibrils treated with WS₂ in the absence of NIR laser irradiation (fAβ40-WS₂ samples) or Aβ40 untreated with WS₂ under NIR laser irradiation (fAβ40 sample), however, did not increase the cell viability. As depicted in Figure S13, WS₂ nanosheets themselves with or without NIR irradiation did not affect the survival of PC12 cells at low dose of less than 20 μg/mL and WS₂ incubated with cells did not affect the output of the MTT assay under the same conditions as Aβ40. Furthermore, the effect of WS₂ on MTT was ruled out by the unchanged signal in presence of WS₂ (Figure S14).

Figure 6. Effect of WS₂ on the cell toxicity of Aβ40. Cell viability was determined using the MTT method and data points shown are the mean values ± standard error of the mean (SEM) from four independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001. Control: Aβ40 untreated cells, [Aβ40] = 5 μM.

Figure 7. (A) Schematic illustration for WS₂ decreasing Aβ40 aggregates-induced cytotoxicity. (B) Measurements of 5(6)-CF leakage from liposome induced by Aβ40 aggregates (10 μM) at 4 h, 12 h and 24 h in the absence or presence of incubation with 8 μg/mL WS₂ nanosheets.
In order to clarify the mechanism of WS\(_2\) reduced the Aβ40-induced cytotoxicity, fluorescent leakage assay was performed. Previous studies suggested that nerve cell of AD patients were somehow killed through Aβ-induced damage of the cell membrane [63,64]. Herein, we took the liposome to mimic the cell membrane [65]. As shown in Figure 7, with the addition of Aβ40 aggregates, the leaked 5(6)-carboxyfluorescein (5(6)-CF) [66] from the liposome was increased with increasing the incubation time. In contrast, the leakage was decreased significantly when Aβ40 was co-incubated with WS\(_2\) in advance (Figure 6). Furthermore, introduction of the Aβ40 aggregates which was treated with NIR irradiation in the presence of WS\(_2\) to the liposome also caused a decrease of 5(6)-CF leakage (Figure S15A). We conducted a control experiment to clarify that leaked 5(6)-CF would not be quenched by the added WS\(_2\) nanosheets (Figure S15B). These results demonstrated that WS\(_2\) could effectively prevent Aβ40-induced damage of the cell membrane.

3.5 The Ability of WS\(_2\): Nanosheets to Cross the BBB

As suitable candidates for AD treatment, the multifunctional nanosheets should cross BBB. To determine whether WS\(_2\) can passively accumulate in the brain of living animals, we used ICP-MS to measure the amount of WS\(_2\) in the cerebrospinal fluid after intraperitoneal injection for 6 hours. A significant level of tungsten was found in the cerebrospinal fluid (CSF) of the mouse that treated with WS\(_2\) compared to the control group. The efficiency of WS\(_2\) accumulation in the cerebrospinal fluid was about 0.53 ± 0.05 % (Table 1), indicating WS\(_2\) possessed the potential ability to cross BBB. Similar with other nanomaterials, the accumulation of the nanosheets in the CSF may occur via endocytosis of the nanosheets by the brain capillary endothelial cells followed by nanosheets transcytosis through these cells [67,68]. Critically, the higher X-ray attenuation and shortening the longitudinal relaxation time by tungsten [69-72] can endow WS\(_2\) nanosheets with excellent X-ray computed tomography imaging capabilities. These results further supported that WS\(_2\) could act as promising therapeutic agents and contrast agents for AD treatment and brain tumor diagnosis.

### Table 1 The amount of WS\(_2\) accumulation in the cerebrospinal fluid of the mouse treated with or without the nanosheets.

<table>
<thead>
<tr>
<th>Samples treated with WS(_2)</th>
<th>Control samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICP results (ng/mL(^{-1}))</td>
<td>Amounts (%)</td>
</tr>
<tr>
<td>1 26.16</td>
<td>0.47</td>
</tr>
<tr>
<td>2 31.31</td>
<td>0.57</td>
</tr>
<tr>
<td>3 29.65</td>
<td>0.54</td>
</tr>
</tbody>
</table>

4. Conclusions

In conclusion, WS\(_2\) nanosheet can effectively inhibit Aβ40 aggregation. Through van der Waals and electrostatic interactions between Aβ40 and WS\(_2\), Aβ40 monomers can selectively adsorb on the surface of the nanosheet. More importantly, with the unique high NIR absorption property of WS\(_2\), WS\(_2\) can also dissociate the preformed Aβ40 fibrils upon NIR irradiation. Compared with traditional small-molecular-weight Aβ inhibitors, WS\(_2\) nanosheets can cross the BBB which overcomes the drawbacks of unstable small chemicals or peptides. Furthermore, the hyperthermic effects of WS\(_2\) have been considered important and useful in clinical treatment with reduced side effects. Our results would accelerate biomedical applications of WS\(_2\) and provide new insights into design of new multifunctional nanomaterials for treatment of AD.

Acknowledgements

This work was supported by 973 Project
References


[19] Cabaleiro-Lago, C.; Quinlan-Plück, F.; Lynch, I.; Lindman...


Electronic Supplementary Material

Chemically Exfoliated WS$_2$ nanosheets Can Efficiently Inhibit Amyloid β-Peptide Aggregation and Use for Photothermal Treatment of Alzheimer’s Disease

Meng Li, Andong Zhao, Kai Dong, Wen Li, Jinsong Ren and Xiaogang Qu$^1$(✉)

Supporting information to DOI 10.1007/s12274-****-**** (automatically inserted by the publisher)

**Materials and Chemicals.**

WS$_2$ powder, n-butyllithium and hexane were purchased from Aladdin. All other chemicals were purchased from Sigma-Aldrich and used as supplied. Bovine Serum Albumin (BSA) was obtained from Sangon Biotechnology (Shanghai, China). Nanopure water (18.2 MΩ; Millipore Co., USA) was used for the preparation of all solutions throughout the experiments.

**Characterization of Interaction of WS$_2$ with Aβ40 and BSA Proteins**

A 200 μL amount of BSA solution (1 mgmL$^{-1}$) was mixed with 80μg WS$_2$, and then the mixture was incubated for 6 h at 37 °C. For Aβ40, the WS$_2$ solution (80 μg/mL, 100μL) was mixed with Aβ40 monomer solution (200 μM, 100μL) at 37 °C for 6 h. Finally, the nanosheets were centrifuged and washed with a Tris buffer. All of the supernatant was collected for UV/vis absorbance measurement. Through calculation of the difference of absorbance at 276 nm between the Aβ40 or BSA solution before and after incubation, the Aβ40 or BSA bound efficiency was determined as 1.89 g/g WS$_2$ or 0.63 g/g WS$_2$, respectively.
Permeability measurements

Liposome was prepared using egg phosphatidylcholine (egg-PC) and cholesterol with molar fractions of 55:45. The large unilamellar vesicles (LUVs) were obtained from the multilamellar vesicles through extruded ten times through two stacked polycarbonate membrane filters (pore size: 400 nm and 100 nm) using a Mini-Extruder (Avanti). 5(6)-carboxyfluorescein (5(6)-CF) was encapsulated into liposome as a solute marker, and the 5(6)-CF leakage from the liposome was measured by using JASCO FP-6500 spectrofluorometer. For inhibition experiment, Aβ40 peptide (50 μM) was incubated with or without WS2 (40 μgmL⁻¹) at 37 °C for 7 days. Then the aged Aβ40 with the final concentration of 10 μM were added directly to incubate with liposome. While for the disaggregation experiment, the sample of aged Aβ40 with the final concentration of 10 μM was mixed with WS2 (8 μgmL⁻¹), which followed by irradiated using an 808 nm NIR laser at a power density of 0.5 Wcm⁻² for 5 min. Immediately, after the irradiation, the liposome were treated with the irradiated samples.

Cytotoxicity assay

For the inhibition experiment, Aβ40 peptide (50 μM) was incubated with or without different concentrations of WS2 (20 μgmL⁻¹ or 40 μgmL⁻¹) at 37 °C for 7 days. PC12 cells (rat pheochromocytoma, American Type Culture Collection) were cultured in IMDM (Iscove-modified Dulbecco medium, Gibco BRL) medium supplemented with 5% FBS, 10% horse serum in a 5% CO₂ humidified environment at 37 °C. For the MTT (Sigma-Aldrich) assay, cells were plated at a density of 10 000 cells per well on 96-well plates for 24 h, and then Aβ40 peptides (50 μM) that had been aged with or without various concentrations of WS2 (20 μgmL⁻¹ or 40 μgmL⁻¹) were
dispensed into the PC12 cells. The final concentration of Aβ40 treated with cells was 5 μM. For the disaggregation experiment, the IMDM medium was replaced by a dispersion of WS₂ (2 μgmL⁻¹ or 4 μgmL⁻¹) in IMDM medium. Aged Aβ40 (Final concentration was 5 μM.) was added to the cells immediately. Afterwards, the plates were irradiated using an 808 nm NIR laser at a power density of 0.5 Wcm⁻² for 5 min. As a control experiment, the PC12 cells containing WS₂-Aβ40 was kept in the dark during the same time. After 48 h of incubation, the cells were treated with 10 μL of MTT (5 mgmL⁻¹ in PBS (phosphate buffered saline, 10 mM, pH 7.4)) for 4 h at 37 °C and were then lysed in DMSO for 10 min at room temperature in the dark. Absorbance values of formazan were determined at 570 nm with 630 nm as the background absorbance using a Bio-Rad model-680 microplate reader. For WS₂ themselves, cells were plated at a density of 10000 cells per well on 96-well plates for 24 h, and then different concentrations of WS₂ nanosheets were dispensed into the PC12 cells. Afterwards, the plates which were used for determining the cell viabilities of WS₂ under NIR irradiation were irradiated using an 808 nm NIR laser at a power density of 0.5 Wcm⁻² for 5 min. After that, the cells were continuously incubated with 48h. To rule out the effect of WS₂ on MTT, after incubation cells for 48h, the medium was replaced by the mixture of WS₂ and MTT buffer and incubated for 4 h. Then the medium was extracted and replaced with 100 μL DMSO to dissolve the formazan salt. Absorbance values of formazan were determined at 570 nm with 630 nm as the background absorbance using a Bio-Rad model-680 microplate reader.

Handling of Mice

S4880202 normal mice were chosen as test animals, in a weight range of 20-25 g (8-12 weeks old) and random in sex. All animal studies were conducted in accordance with the principles and
procedures outlined in “Regulations for the Administration of Affairs Concerning Laboratory Animals”, approved by the National Council of China on October 31, 1988, and “The National Regulation of China for Care and Use of Laboratory Animals”, promulgated by the National Science and Technology Commission of China, on November 14, 1988 as Decree No. 2. Protocols were approved by the Committee of Jilin University Institutional Animal Care and Use. After intraperitoneal injection of 600 μL WS₂ (100 μg/mL⁻¹), the cerebrospinal fluid (CSF) were collected immediately and diluted in 0.5 mL water. The mouse that treated without WS₂ was used as the control group. The tungsten content of the samples was measured by ICP-MS (Varian 720-ES). The data points shown are the mean values ± SEM from three independent experiments (Three mice were used for groups treated with WS₂ and three mice for control group.).

Cerebrospinal fluid (CSF) of mice collection

CSF was collected under anesthesia using a glass pulled micropipette after exposure of the cisterna magna, taking care not to contaminate the CSF with blood. Two to 10 μL was routinely collected. The CSF was immediately diluted 1:10 in 1% 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propane sulfonate (CHAPS) in PBS with protease inhibitors (Roche Diagnostics, Mannheim, Germany) before freezing in liquid nitrogen and storage at -80 °C.

Address correspondence to xqu@ciac.ac.cn
Figure S1 The influence of WS$_2$ nanosheets on the fluorescence of ThT with or without NIR irradiation. The concentration of WS$_2$ was 100 μg/mL.
Figure S2 Zeta potential of the WS₂ nanosheets in the absence (A) or presence (B) of Aβ40. The concentrations of Aβ40 and WS₂ nanosheets were 10 μM and 20 μg/mL, respectively.
Figure S3 AFM images and height profiles of the corresponding AFM topographies of typical nanosheets of WS$_2$ incubated with different concentration of A$\beta$40. (A) WS$_2$ nanosheet with A$\beta$40 at the concentration of 0.25 mmol/g; (B) WS$_2$ nanosheet with A$\beta$40 at the concentration of 2.5 mmol/g. The change of height indicated that the A$\beta$40 monomers could selectively adsorb on the surface of WS$_2$ nanosheets.
Figure S4 (A) Fluorescence titration of Aβ40 (3 μM) with various concentrations of WS₂ nanosheets in 10 mM Tris buffer (pH 7.4). The excitation wavelength was 278 nm and the emission intensity at 306 nm was used for analysis. (B) The UV/Vis spectrum of the Aβ40 monomers before incubation (black) and after incubation (red) with WS₂ nanosheets.
Figure S5 (A) The morphology of MoS$_2$. (B) The morphology of GO. (C) The kinetics of Aβ40 with these three different nanomaterials. The concentration of Aβ was 50 μM and the concentrations of WS$_2$, MoS$_2$ and graphene nanosheets were all kept at 40 μg/mL. (D) The dose-dependent inhibition effects on Aβ40 aggregation of WS$_2$, MoS$_2$ and GO. The concentration of Aβ40 was 50 μM. (E) CD spectra of Aβ40 in the absence or presence of WS$_2$ nanosheets and GO. The concentration of Aβ was 50 μM and the concentrations of WS$_2$ and graphene nanosheets were kept at 40 μg/mL. The samples were measured in 10 mM Tris buffer (pH 7.3) after incubation at 37°C for 7 days.
Figure S6 AFM images of Aβ40 in the absence (A) or presence (B) of WS$_2$ nanosheets. The concentration of Aβ40 and WS$_2$ nanosheets were 50 μM and 40 μg/mL. (C) CD spectra of Aβ40 at the beginning with or without WS$_2$. 
Figure S7 Absorbance profile of different concentrations of ceWS$_2$. 
Figure S8 Temperature change curves of the WS$_2$ nanosheets solution and the water exposed to the 808 nm laser at a power density of 0.5 W/cm$^2$ (A), 1 W/cm$^2$ (B) and 2 W/cm$^2$ (C).
Figure S9 Plot of the fluorescence intensity against irradiation time. The excitation wavelength was 444 nm, and the emission intensity at 480 nm was used for analysis.
Figure S10 The morphology of Aβ40 aggregates was analyzed by AFM images. (A) Aβ40 fibrils. (B) Aβ40 fibrils in absence of WS₂ nanosheets under the laser irradiation. The concentration of Aβ40 was 50 μM.
Figure S11 (A) The UV/Vis spectrum of BSA before incubation (black) and after incubation (red) with WS$_2$ nanosheets. (B) AFM images (left) and height profiles (right) of the corresponding AFM topographies of typical nanosheets of WS$_2$ incubated with BSA. The amount of BSA adsorbed on the surface of WS$_2$ nanosheets was 0.63 g/g WS$_2$. 
Figure S12 (A) Fluorescence titration of Aβ40 (3 μM) with various concentrations of WS2-BSA nanosheets in 10 mM Tris buffer (pH 7.4). The excitation wavelength was 278 nm and the emission intensity at 306 nm was used for analysis. (B) The influence of WS2-BSA nanosheets on Aβ aggregation monitored by ThT assay in Tris buffer. The concentration of Aβ40 was 50 μM. AFM images of Aβ40 in the absence (C) or presence (D) of WS2-BSA nanosheets. The concentration of Aβ40 and WS2-BSA nanosheets were 50 μM and 40 μg/mL.
Figure S13 Effect of WS$_2$ nanosheets on PC12 cell viability determined by MTT method.
Figure S14 The effect of WS$_2$ on MTT. After incubation cells for 48 h, the medium was replaced by the mixture of WS$_2$ and MTT buffer and incubated for 4 h. Then the medium was extracted and replaced with 100 μL DMSO to dissolve the formazan salt. Absorbance values of formazan were determined at 570 nm with 630 nm as the background absorbance using a Bio-Rad model-680 microplate reader.

Figure S15 (A) Measurements of 5(6)-CF leakage from liposome induced by Aβ40 aggregates (10 μM) in the presence of WS$_2$ (8 μg/mL) with or without NIR irradiation at 4 h, 12 h and 24 h
incubation time. (B) The influence of WS$_2$ nanosheet on the fluorescence of 5(6)-CF. The concentration of 5(6)-CF was 5 μM. The concentrations of Aβ40 aggregates and WS$_2$ were 10 μM and 8 μg/mL, respectively.