MoS$_2$ Field-Effect Transistor-Amyloid-$\beta_{1-42}$ Hybrid Device for Signal Amplified Detection of MMP-9

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Supporting Information

ABSTRACT: The detection of circulating protein (CP) is very important for the diagnosis and therapeutics of cancer. Conventional techniques based on a specific antibody–antigen interaction are still lacking because of a shortage of cost-effectiveness, complicated sandwich structure and tagging process, and inconsistent detection of CP due to the inherent instability of antibodies. Herein, we demonstrate a hybrid device consisting of two-dimensional (2D) nanoscale molybdenum disulfide (MoS$_2$) field-effect transistor (FET) with an amyloid-$\beta_{1-42}$ (A$_{\beta_{1-42}}$) functionalized surface, which amplifies electric signals of the FET in order to detect matrix metalloproteinase-9 (MMP-9), which is a certain type of CP that degrades A$_{\beta_{1-42}}$. With the hybrid device, we detected the concentrations of MMP-9 in the range from 1 pM to 10 nM. Moreover, using tapping-mode atomic force microscopy and Kelvin probe force microscopy, we verified that the signal amplification corresponding to the MMP-9 concentrations was caused by the reduced length and the decreased surface potential of degraded A$_{\beta_{1-42}}$ due to MMP-9. The hybrid device studied in this paper can be very useful for monitoring MMP-9 activity, as well as serving as a sensing platform for the electrical signal amplification of 2D MoS$_2$ FET-biosensors.

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been developed using the proteolytic ability of MMP-9, including surface plasmon resonance biosensors,\textsuperscript{22} zymography,\textsuperscript{23} and fluorescence resonance energy transfer-based sensors.\textsuperscript{24} These methods offer significant advantages such as a shorter processing time and fewer preparation steps as well as higher reliability compared to biosensors that rely on immune reactions. More importantly, the enzyme activity of MMP-9 can be evaluated. However, even though many advantages have been achieved in the previous studies, these assays for detecting MMP-9 activity still require complex sensing systems and do not exhibit superior sensitivity. It is also difficult to create circuit integration systems as portable chip devices. Particularly, the low carrier mobility of conventional electrochemical biosensors prevents configuring the electronic circuit for commercial sensors. In this regard, field-effect transistor (FET)-based biosensors are promising detection devices because of their rapid and easy operation, high sensitivity, nanoscale size, intrinsic amplification capability, and circuit integration potential. Furthermore, transistors based on two-dimensional (2D) nanomaterials, such as graphene and transition metal dichalcogenides, can be used for ultrasonic dimensional (2D) nanomaterials, such as graphene and MoS\textsubscript{2} FETs can be very promising candidates for the detection of MMP-9 in liquid biopsy by CP analysis. Therefore, the hybrid device studied in this paper could be a promising platform for monitoring MMP-9 activity with respect to the physiological catabolism of A\textsubscript{42} as well as for sensing electrical signal amplification in 2D MoS\textsubscript{2} FETs for liquid biopsy by CP analysis.

**EXPERIMENTAL SECTION**

**Fabrication of MoS\textsubscript{2} FET.** First, multilayer MoS\textsubscript{2} thin films were obtained from bulk MoS\textsubscript{2} (SPI Supplies, U.S.A.) through the Scotch tape method. The exfoliated MoS\textsubscript{2} films were transferred onto Al\textsubscript{2}O\textsubscript{3} (40 nm)-covered p-doped silicon (Si; 0.5 \textmu m), where the Al\textsubscript{2}O\textsubscript{3} layer was deposited by an atomic layer deposition system (Lucida D100, NCD, Korea) using trimethylaluminum and deionized (DI) water as the precursors. For electrical contact, titanium (Ti) and gold (Au) were sequentially deposited by an electron beam evaporator and patterned by a photolithography and an etching process. The devices were then annealed at 200 °C for 2 h in a vacuum chamber to remove the organic residues on the MoS\textsubscript{2} surface and enhance the electrical junction between the electrode and the MoS\textsubscript{2} channel.

**Synthesis Protocol of Amyloid.** Lyophilized Aβ\textsubscript{1−42} peptide was purchased from TOCRIS, U.K. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. The peptide was dissolved in HFIP solution. A 1 mM solution of the peptide was distributed into microtubes and then dried overnight. The tubes were placed into a ScanSpeed vacuum concentrator (Gyrozen, Korea) and dried for 1 h. After drying, the peptide was dissolved in a DMSO solution with vortexing for 30 s. The concentration of the Aβ\textsubscript{1−42} solution became 5 mM. The solution was sonicated in a bath-type sonicator for at least 10 min. To synthesize Aβ\textsubscript{1−42} aggregates (i.e., fibrils), the solution was resuspended in 10 mM hydrogen chloride solution. The final concentration of the Aβ\textsubscript{1−42} solution was 100 μM. The solution was incubated in an oil bath at 37 °C for 1 day and then was dialyzed in phosphate-buffered saline (PBS; pH ∼ 7.0) for 60 h by using 50 kDa dialysis membranes (Biovision, U.S.A.).

**Adsorption of Aβ\textsubscript{1−42} and Detection of MMP-9.** Active MMP-9 (Merck Millipore, Germany) was stored at −80 °C and then diluted to various concentrations ranging from 1 pM to 10 nM in DI water. First, the as-mentioned synthesized solution consisting of 10% Aβ\textsubscript{1−42} fibrils was dropped on the MoS\textsubscript{2} channel surface for 15 min at room temperature in a humidity chamber for immobilization of Aβ\textsubscript{1−42}. The device was then rinsed with 200 μL of PBS (pH ∼ 7.4) and dried under N\textsubscript{2} gas. The proteolytic reaction of MMP-9 was carried out on the device with prediluted MMP-9 solutions for 2 h at 37 °C in a sufficiently humid environment (∼90% relative humidity). After washing the device, electrical analyses were performed using a semiconductor parameter analyzer (4200-SCS, Keithley, U.S.A.).

**Analysis Conditions of tm-AFM and KPFM.** A SiO\textsubscript{2} (500 nm)-covered p-doped Si wafer was prepared for the length and surface potential measurements of the Aβ\textsubscript{1−42} fibrils. The wafer was diced into a 10 × 10 mm\textsuperscript{2} area and then cleaned with piranha solution (mixture of 1:1 v/v H\textsubscript{2}SO\textsubscript{4} and H\textsubscript{2}O\textsubscript{2}) for 10 min prior to use (NB: always use caution when working with piranha solution). The immobilization method of Aβ\textsubscript{1−42} is the same as the above method. The topography and the concentration range of 1 pM which is under detectable threshold using MoS\textsubscript{2} FET without doping effect of Aβ\textsubscript{1−42}. The electrical current in the hybrid structure resulted from the truncation of Aβ\textsubscript{1−42} caused by MMP-9. Importantly, our previous study by quantitative detection. Therefore, the hybrid device reliably detected the MMP-9
surface potential measurements of all Aβ_{1-42} fibrils were performed using a commercial AFM (Multimode V, Veeco, U.S.A.) at room temperature. A Si cantilever (TESP-V2, Bruker, USA) was used for 10 × 10 μm² topographic imaging, and the scan rate was 0.5 Hz. For 3 × 3 μm² surface potential imaging, a conducting cantilever (SCM-PIT, Bruker, U.S.A.) was mounted in a KPFM cantilever holder (MMEFCH, Veeco, U.S.A.) that is capable of controlling the voltage of the cantilever. Surface potential measurements were performed using a lift-mode KPFM (lift height of 10 nm and scan rate of 1.0 Hz).

RESULTS AND DISCUSSION

Hybrid Structure Consisting of MoS₂ FET and Aβ_{1-42}.

The overall detection process using MoS₂ FET-Aβ_{1-42} hybrid device for label-free and highly sensitive detection of MMP-9 is presented in Figure 1A–D. Prior to the formation of the proposed hybrid structure, the MoS₂ FET was fabricated as shown in Figure 1A, where mechanically exfoliated multilayer MoS₂ was transferred onto an Al₂O₃-covered Si substrate and source/drain electrodes were arranged at both ends of the MoS₂ film. The specific large surface area of the MoS₂ channel, which plays a pivotal role in achieving superior sensing performance, was visualized by tm-AFM, as shown in Figure 1E. The inset of Figure 1E indicates a film thickness of approximately 30 nm, which is within the appropriate range to achieve high field-effect mobility (µ = 30–50 cm² V⁻¹ s⁻¹) on silicon dioxide (SiO₂)/Si substrate).³⁹,⁴⁰ As shown in Figure 1B, Aβ_{1-42} fibrils were adsorbed on the surface of the MoS₂ channel to build the hybrid structure. Aβ_{1-42} fibrils, widely considered physiological marker of Alzheimer’s disease, have great potential for the highly sensitive detection of MMP-9. First, Aβ_{1-42} can be applied to monitor the proteolytic ability of MMP-9, because the peptide is degraded by MMP-9.⁴⁰–⁴⁸

In the mass sequence of the identified fragments of Aβ_{1-42} (i.e., NH₂/DAEFRHDSGYEVHHQKVFFAEDVGSNK-GAIIGLMVGGVVIA-COOH),³⁷ the major cleavage sites degraded by MMP-9 is the Leu³⁴—Met³⁵ bond. Next, Aβ_{1-42} chemically anchored on the MoS₂ surface induced n-type doping in the MoS₂ channel because Aβ_{1-42} was rich in negative charge under our experimental conditions of pH = 7.4,⁴¹ thereby increasing the electron carrier density of the MoS₂ channel, which induced a signal amplification effect. As shown in Figure 1C, Aβ_{1-42} adsorbed on the MoS₂ channel was degraded by MMP-9 and removed by washing, reducing the n-doping effect. This reduction could be characterized by the surface potential using KPFM and the electrical sensor response of the MoS₂ FET over a wide range of MMP-9 concentrations, as shown in Figure 1D.

Functionalized Aβ_{1-42} on the Surface of MoS₂ FET for Signal Amplification.

As the first step to confirm the binding of Aβ_{1-42} on the MoS₂ films, we conducted XPS analysis of the MoS₂ surface with respect to the adsorption of Aβ_{1-42}. Figure 2A shows XPS spectra of the MoS₂ films in Mo 3d and S 2p regions after the adsorption of Aβ_{1-42}. Two strong doublet peaks were observed at 229.3 and 232.4 eV, corresponding to Mo²⁺ 3d₅/₂ and Mo⁴⁺ 3d₃/₂ signals, respectively. In addition, two peaks appeared at 162.1 and 163.3 eV, corresponding to S²⁻ 2p₃/₂ and S²⁻ 2p₁/₂ signals, respectively.⁴⁴–⁴⁶ These Mo 3d and S 2p peaks were consistent with those of pristine MoS₂ in Figure 1A, indicating that Aβ_{1-42} adsorption did not affect the Mo—S bond. Figure 2B presents the XPS spectrum of N 1s–Mo 3p deconvoluted into three peaks at 395.2, 397.6, and 399.7 eV, corresponding to Mo 3p₃/₂ N 1s (N–Mo bond), and N 1s (N–H bond), respectively.⁴⁷ However, the N 1s N–

Figure 1. Schematic illustration of the sequential process for MMP-9 detection: (A) Structure of a pristine MoS₂ FET-based biosensor, (B) surface modification of the MoS₂ FET with Aβ_{1-42}, (C) proteolytic degradation of Aβ_{1-42} on the MoS₂ FET by MMP-9, and (D) electrochemical measurements for detection of the proteolytic activity of MMP-9. (E) Tm-AFM image of the MoS₂ FET with an inset presenting its thickness of approximately 30 nm.

Figure 2. XPS spectra of (A) Mo 3d, S 2p and (B) N 1s from the MoS₂ surface modified with Aβ_{1-42}. (C) Raman spectra of the MoS₂ surface before and after the adsorption of Aβ_{1-42}.

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Mo and N–H bond peaks were absent in the pristine MoS$_2$ spectra (Figure S1B). These results indicated that nitrogen atoms from the amide groups of A$eta_{1\text{--}42}$ were chemically adsorbed on the MoS$_2$ surface and formed Mo–N bonds. Thus, XPS analysis clearly indicates that the A$eta_{1\text{--}42}$ fibrils were successfully adsorbed on the MoS$_2$ channel surface.

In addition, A$eta_{1\text{--}42}$ has a negative potential at pH $\sim$ 7.4 because its isoelectric point is $5.2^{16,41}$ which has also been characterized in our previous study. Therefore, the adsorbed A$eta_{1\text{--}42}$ made the MoS$_2$ surface negative and pushed electrons away from the interface. It increased the free electron carrier density and induced the n-doping effect on MoS$_2$. To confirm that MoS$_2$ could be doped by A$eta_{1\text{--}42}$ through surface adsorption, we performed Raman spectroscopy on the MoS$_2$ film before and after attaching A$eta_{1\text{--}42}$, as shown in Figure 2C. The pristine MoS$_2$ sample showed an in-plane vibration peak (E$_{2g}$) and out-of-plane vibration peak (A$_{1g}$) at 384.8 and 409.4 cm$^{-1}$, respectively (black dashed line). When A$eta_{1\text{--}42}$ was adsorbed on the MoS$_2$ film, the two Raman peaks were red-shifted by 1.3 cm$^{-1}$ in E$_{2g}$ and 1.4 cm$^{-1}$ in A$_{1g}$. It has been reported that n-doping in MoS$_2$ leads to a red-shift of the two peaks because of the increased electron density, which is attributed to a remarkable change in the electron–phonon coupling. Additionally, a reduction in the A$_{1g}$ peak intensity was induced by the softening of the A$_{1g}$ phonon due to n-doping. However, the intensity of E$_{2g}$ did not change because it was insensitive to electron doping.

To further confirm the n-doping effect of A$eta_{1\text{--}42}$ on MoS$_2$, we conducted electrical measurements of the MoS$_2$ FET with relation to the adsorption of different concentrations of A$eta_{1\text{--}42}$. Tm-AFM was also used to visualize the adsorption of A$eta_{1\text{--}42}$ on the surface of MoS$_2$. In particular, to exhibit it, the solution with A$eta_{1\text{--}42}$ fibrils was separately diluted to 10% and 1%. These different concentrations of A$eta_{1\text{--}42}$ were adsorbed on the surface of the MoS$_2$ FET for 15 min inside a humidity chamber and rinsed with PBS (pH $\sim$ 7.4) to eliminate nonimmobilized proteins. As shown in Figure 3A–D, A$eta_{1\text{--}42}$ fibrils were randomly distributed on the MoS$_2$ FET surface, and the adsorption increased at higher concentration of A$eta_{1\text{--}42}$.

The good adhesion of the fibrils on the MoS$_2$ surface was expected due to the chemical bonding between Mo and N atoms of the amide groups of A$eta_{1\text{--}42}$ (Figure 2B), as well as physical bonding caused by hydrophobicity of MoS$_2$ (contact angle $\sim$ 75.77$^\circ$). When negatively charged A$eta_{1\text{--}42}$ fibrils were adsorbed on the MoS$_2$ surface, the n-doping effect lowered the energy band of the MoS$_2$ channel, which was accompanied by a decrease in the effective barrier height of the MoS$_2$ and Ti electrode junction, as shown in Figure 3E. The electrical properties of the MoS$_2$ FET corresponding to the adsorption of different concentrations of A$eta_{1\text{--}42}$ are depicted in Figure 3F. The drain current ($I_{ds}$) was measured as a function of gate voltage ($V_{G}$) from $-4$ to 2 V with a drain voltage ($V_{D}$) of 1 V. The results revealed a significant increase in $I_{ds}$ values with increasing concentrations of A$eta_{1\text{--}42}$. The same results were observed in three different MoS$_2$ FETs, as shown in Figure S2. Figure 3G shows the calculated threshold voltage ($V_{th}$) values obtained from Figure 3F. Obviously, the $V_{th}$ values were gradually negatively shifted from $-1.3$ to $-2.47$ V, which was attributed to the lowering of the effective barrier height by n-doping. This result is in good agreement with the Raman analysis result.

**Electrical Detection of MMP-9 with Their Proteolytic Activity.** The electrical characteristics originating from A$eta_{1\text{--}42}$ adsorbed on the MoS$_2$ FET were expected to be changed by the truncation of A$eta_{1\text{--}42}$ in the presence of MMP-9. Prior to investigation of the electrical variations, we monitored the degradation of A$eta_{1\text{--}42}$ using tm-AFM. Figure 4A–D depict representative 3D topological images of A$eta_{1\text{--}42}$ fibrils adsorbed on Si substrates after degradation by different concentrations of MMP-9 in the range from 1 pM to 1 nM. From these images, we found that the density of A$eta_{1\text{--}42}$ fibrils decreased as the concentration of MMP-9 increased. For more precise analysis, the length of the A$eta_{1\text{--}42}$ fibrils was measured by using commercial image processing software (i-Solution DT, IMT i-solution, Canada), and the corresponding analysis results are shown in Figure 4E–H. After functionalization of A$eta_{1\text{--}42}$, the average contour length of A$eta_{1\text{--}42}$ was measured as 0.844 $\mu$m. The proteolytic activity of MMP-9 was verified by a remarkable decrease in the average contour length of A$eta_{1\text{--}42}$ from 0.338, 0.292, and 0.181 $\mu$m with respect to increasing MMP-9 concentrations. The observed changes in the density and contour length of A$eta_{1\text{--}42}$ on the surface can be used to predict the decrease in the n-doping effect of A$eta_{1\text{--}42}$ fibrils, which can change the electrical characteristics of the MoS$_2$ FET-A$eta_{1\text{--}42}$ hybrid device.

To further confirm the electrical correlation, we measured the surface potential of A$eta_{1\text{--}42}$ fibrils before and after degradation by using high-resolution KPFM. For precise measurement with the KPFM, we optimized the lift-mode conditions (e.g., the lift scan height and scan speed of the conductive cantilever tip on the KPFM) according to our previous work. Figure 5A and B are high-resolution KPFM images of A$eta_{1\text{--}42}$ fibrils before and after MMP-9 treatment, and the corresponding tm-AFM images are shown in Figure S3A and B. Figure 5C and D show the statistical distribution profiles of surface potential obtained from Figure 5A and B.
which exhibit a significant change in average surface potential before and after MMP-9 treatment from $-152.58 \pm 37.21$ to $-60.08 \pm 37.89$ mV. This result was attributed to decomposition of $\alpha\beta_{1-42}$ fibrils, and this surface potential difference also supported the decrease in the n-doping effect of $\alpha\beta_{1-42}$ fibrils on the surface of the MoS$_2$ FET.

Figure 4. (A–D) Tm-AFM images of 10% $\alpha\beta_{1-42}$ adsorbed on SiO$_2$ with 1 pM, 100 pM, and 1 nM MMP-9 treatment. (E–H) Histograms of $\alpha\beta_{1-42}$ fibril length distribution based on each MMP-9 concentration and average contour length.

Figure 5. KPFM images of 10% $\alpha\beta_{1-42}$ adsorbed on SiO$_2$ (A) before and (B) after treatment of 1 nM MMP-9. Histograms of surface potential distribution of $\alpha\beta_{1-42}$ (C) before and (D) after decomposition by 1 nM MMP-9 and the average surface potential.

Figure 6. Variations in (A) transfer curves and (B) output curves of MoS$_2$ FET with different MMP-9 concentrations after adsorption of $\alpha\beta_{1-42}$. (C) Comparison of sensing response of MoS$_2$ FET with and without $\alpha\beta_{1-42}$ in MMP-9 concentrations range of 1 pM to 10 nM (limit of detection for the device is 1 pM, see also Figure S6).
The electrical detection of proteolytic MMP-9 was performed by measuring $I_{ds} - V_{th}$ of the MoS$_2$ FET modified with $A\beta_{1−42}$, as shown in Figure 6A. The adsorption of $A\beta_{1−42}$ on the MoS$_2$ surface resulted in a negative shift in $V_{th}$ values due to the n-doping effect, which was consistent with the results in Figure 3F. The proteolytic degradation of MMP-9 led to a decrease in the n-doping effect of $A\beta_{1−42}$ accompanied by a stepwise positive shift in $V_{th}$ with increasing MMP-9 concentrations. The corresponding output characteristics are plotted in Figure 6B. $I_{ds}$ was measured by sweeping $V_{ds}$ from 0 to 5 V near the $V_{th}$ range ($V_{gs} = −3$ to $−1$ V), exhibiting that $I_{ds}$ decreased with increasing MMP-9 concentrations due to the positive shift in $V_{th}$ values. Based on these results, the signal amplification effect from chemical doping of $A\beta_{1−42}$ on the MoS$_2$ channel could be verified from the comparison in the responses of the MoS$_2$ FET biosensors with and without $A\beta_{1−42}$ as shown in Figure 6C. Noteworthy is that the MoS$_2$ FETs with $A\beta_{1−42}$ (Figure S4) can detect even 1 pM of MMP-9 with $V_{th}$ variation ($\Delta V_{th}$) value of 0.138 V, while the MoS$_2$ FET without $A\beta_{1−42}$ (Figure S5) showed a value of 0.02 V at 1 pM of MMP-9. More importantly, the proposed hybrid devices exhibited the large detectable range from 1 pM to 10 nM with great sensing linearity and low standard error, representing that high precision and reproducibility.

In addition, device performance factors, such as field-effect mobility ($\mu_{FE}$), On/Off ratio, and subthreshold swing (SS) under continuous solution-based process, were measured to examine whether O$_2$ and H$_2$O in external environment can deteriorate the MoS$_2$ devices (Tables S1 and S2). No remarkable change in whole process shown in Tables S1 and S2 indicated that the MoS$_2$ FET-based hybrid device have a fully high stability and durability for a biosensor. Lastly, we measured the MMP-9 level of the cell media of Michigan Cancer Foundation-7 (MCF-7) cells using a MoS$_2$ FET-Amyloid-$\beta_{1−42}$ hybrid device, where MCF-7 cells are a type of breast cancer cell line known to secrete MMP-9. The electrical response of the hybrid device is shown in Figure S7. A gradually increased response demonstrated the MoS$_2$-Amyloid-$\beta_{1−42}$ hybrid sensor can be used for the detection of the MMP-9 generated from MCF-7 at increased incubation time, implying that MoS$_2$ FET-Amyloid-$\beta_{1−42}$ hybrid device has potential for clinical application.

### CONCLUSION

In conclusion, we demonstrated a highly sensitive MMP-9 biosensor based on $A\beta_{1−42}$-immobilized multilayer MoS$_2$ FET. The proteolytic activity of MMP-9 at the interface between MoS$_2$ and $A\beta_{1−42}$ can be measured by a direct variation in the $V_{th}$ electrical property. Compared with the MoS$_2$ FET without $A\beta_{1−42}$, the hybrid structure of MoS$_2$ with its surface functionalized with $A\beta_{1−42}$ achieved a significant enhancement in the detection of MMP-9 with a large detection range from 1 pM to 10 nM because of the signal amplifying effect. Tm-AMF and KPFM analyses confirmed the proteolytic activity of MMP-9 and the electrical variation in the surface on which $A\beta_{1−42}$ was adsorbed with respect to the MMP-9 concentration. The results presented in this work will provide an excellent practical method to detect CP in the field of 2D bioelectronics.