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Detection of Matrix Metalloproteinase-2 by Field Effect Transistor with a Fibronectin-immobilized Gate

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Matrix metalloproteinase (MMP) is known to be involved in chronic inflammation, tumor invasion, and carcinogenesis. To detect MMP-2, we examined the use of field effect transistors (FETs). After the addition of MMP-2 to a fibronectin (FN)-immobilized gate, negative value of FET response was observed, which indicates MMP-2 decreased the amount of negative charges arising from FN molecules. This FET device successfully detected MMP-2 by utilizing degradation of FN on the gate.

Development of biosensors is an important subject in the field of health preservation, medical care diagnosis, and medicine manufacturing. Especially, field effect transistors (FETs) have been widely investigated to detect biological reactions by many researchers,[1–6] because they are capable of label-free detection, rapid measurement, and easy operation with a small volume of sample and are accessible by mass production at a low cost. In principle, FET-based biosensors detect changes in charge amounts on the gate surface as electrical signals, which are usually induced by pH changes and/or adsorption of charged biomolecules. In previous study, we have been investigating the application of FETs for the development of biosensors by utilizing self-assembled monolayers (SAMs) of organic molecules with SiO_2 gate.[7–12] Here, as a target molecule for FET-based biosensors, we focused our attention on matrix metalloproteinase (MMP). MMP is known to express on cancer cells, degrade many extracellular matrix proteins,[13,14] and are related to chronic inflammation, tumor invasion, and carcinogenesis.[15–17] To date, various methods have been investigated to detect MMP.[18–22] Considering the characteristics of FET, development of FET devices to detect MMP is expected to offer important medical guidelines for the above-mentioned diseases in early stages. There are two types of reported approaches to detect MMP by using FET. The first approach is to use MMP–antibody-modified surfaces.[21] For the immobilization of antibodies, the orientation of adsorbed antibodies and physical adsorption of target molecules have an effect on the response of FET. In addition, it should be taken into consideration the relationship between Debye length and the size of antibody, because only charges within Debye length from the gate surface can be detected as signal changes.[23,24] The second approach is to use chemically synthesized peptide probes, which possess specifically cleaving sequence sites toward MMP.[22] However, the preparation for the peptides consumes time, and marker molecules binding to the peptides are additionally required. Under these circumstances, to establish a simple detection system, we utilized fibronectin (FN) as a probe molecule to detect MMP-2. Because FN is known to be degraded by MMP-2[25,26] and be negatively charged under physiological conditions,[27] the degradation of FN immobilized on the gate is considered to be detected, which induces decrease in amounts of negative charges derived from FN molecules. In this study, we evaluated the response of FET with FN-immobilized gate to the addition of MMP-2.

We employed n-channel FET devices with SiO_2 gates that were formed using thermal oxidization (1 mm in width, 10 μm in length, and 40 nm in thickness, Toppan Printing Co., Ltd.). The modification process of 3-aminopropyltriethoxysilane (APTES, Sigma-Aldrich Inc.) and glutaraldehyde (GA, Kanto Chemical Co., Inc.) on SiO_2 surface was described in our previous paper.[11,12] A solution of FN from human plasma (Sigma-Aldrich Inc.) in 1 × phosphate buffered saline (PBS, pH 7.4) was added on the GA-modified SiO_2 surface at room temperature (reaction time: 1 h). The surface was rinsed with 1.0 mL of 1 × PBS five times and dried under N_2 gas. After the immobilization of FN, a solution of 150 ng mL^{-1} MMP-2 (Sigma-Aldrich Inc.) containing 5 mM $CaCl_2$ (Kanto Chemical Co., Inc.) in 1 × PBS was added on the FN-immobilized surface at room temperature (reaction time: 3 h). The substrate was rinsed and dried by using the same procedure as described above. To verify the immobilization of FN on the GA-modified surface and FN degradation after the addition of MMP-2, atomic force microscopy (AFM, SPM-9600, Shimadzu Co.) and X-ray photoelectron spectroscopy (XPS, JPS-9010MX, JEOL Ltd.) with Al Kα radiation were performed (Figures S1 and S2, see Supporting Information; SI).[28] The thickness and coverage factor of FN molecules immobilized on the GA-modified surface were evaluated by ellipsometry (M-240, JASCO Co.) with a He-Ne laser (λ = 632.8 nm) and fluorescence measurements (Typhoon 9410, GE Healthcare Bio-Sciences KK), respectively. The drain current–gate voltage (I_d–V_g) was evaluated at room temperature in the dark in the PBS solution with a digital source meter (2600 A model, Keithley Instruments, Inc.). V_g was scanned from −3.0 to 1.5 V with a 1.0 V drain voltage by using an Hg/Hg_2SO_4 electrode as the reference electrode. The lateral shift of the I_d–V_g curves after the addition of the solution, denoted hereafter as ΔV_g, will be discussed as an FET response.

Because FN molecules are utilized to function as probes for the detection of MMP-2, we first examined the state of FN molecules immobilized on the GA-modified surface. For ellipsometry measurements, the thickness of GA-modified SiO_2 surface was defined as 0 nm. We evaluated increases in thickness of FN molecules immobilized on the GA-modified surface with a refractive index of 1.46 for SiO_2,[29] APTES,[30] GA,[30] and that of 1.47 for FN,[31] respectively. Figure 1 shows increases in

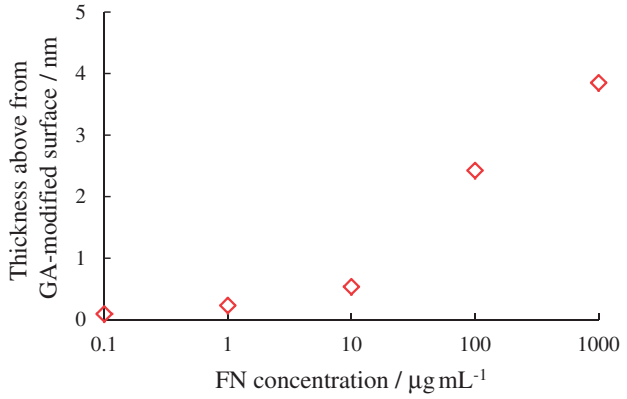

Figure 1. Relationship between FN concentration and increase in thickness above from the GA-modified surface. The thickness of GA-modified SiO_2 was defined as 0 nm.

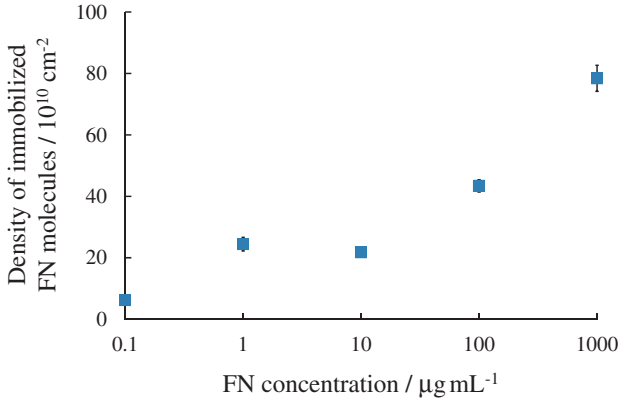

Figure 2. Relationship between FN concentration and density of immobilized FN molecules. The density was calculated from fluorescence intensity.

thickness above from the GA-modified surface upon the addition of different FN concentration. The observed increases in thickness were below 1 nm for less than 10 μg mL^{-1} and approximately 3 or 4 nm for more than 100 μg mL^{-1}. For fluorescence measurements, FN molecules conjugated SureLINK™ Fluorescein (KPL, Inc.) were added to the GA-modified surfaces. The density of immobilized FN molecules D was calculated by using following formula

$$I = kC \quad (1)$$

$$D = \frac{CVN_A}{MS} \quad (2)$$

where I is observed fluorescence intensity, k is coefficient of standard curve, C is FN concentration, V is volume of added solution, N_A is Avogadro constant, M is molar weight of FN molecules, and S is surface area where the solution was added. Figure 2 shows the density of immobilized FN molecules after the addition of different FN concentration. With the increase in FN concentration, the density of immobilized FN molecules increased drastically. Here, the height of FN molecules immobilized on the GA-modified surface was reported to be approximately 3.0 nm for the addition of 3.0 μg mL^{-1} FN.[32] Considering the size of FN molecules (3 nm in diameter and 30 nm in length),[33] although it should be taken into account the differences of experimental conditions, such as substrate treatment and modification process, FN molecules are densely immobilized for 1000 μg mL^{-1}. If FN molecules were immobilized on the GA-modified substrate without spaces in a monolayer fashion, the density of immobilized FN molecules is estimated to be approximately 1.11×10^{12} cm^{-2}. Considering the value, for the addition of 1000 μg mL^{-1} FN, coverage factor was estimated to be approximately 75%. In addition, FN possesses binding sites with FN monomers and 76 lysine residues within a FN molecule,[32] which have the ability to bind to aldehyde groups within a GA molecule modified on the gate. Thus, after the addition of FN to the GA-modified surfaces, FN molecules are considered to bind not only onto GA-modified surface but also among FN monomers to form partly laminated state.

For FET measurements (Figure S3, see SI),[28] after the addition of FN to the GA-modified gate, negative charges arising from FN molecules should be detected. Taking the size of FN molecules into consideration, a relatively large Debye length was required. First, 0.01 × PBS (Debye length; 7.5 nm) was used as an electrolyte to detect charge amount changes on the gate certainly. After 1 h from the addition of 1000 μg mL^{-1} FN to the GA-modified gate, a positive value of FET response of approximately 50 mV was observed (Figure 3a), which is considered to be due to negative charges of FN. After 3 h from the addition of MMP-2 to the FN-immobilized gate, negative value of FET response of approximately 40 mV was observed (Figure 3a). We evaluated the effect of MMP-2 concentration on FET responses (Figure S4, see SI).[28] Although in PBS solution, sufficient large FET response was observed for 150 ng mL^{-1}, which can be applied to the detection of several diseases in biological samples.[34–36] Taking into account that negative values were not observed in the case without MMP-2, it is indicated that MMP-2 decreased the amount of negative charges arising from FN molecules because of FN degradation on the gate. Here, FN degradation after the addition of MMP-2 was confirmed by AFM and XPS (Figures S1 and S2, see SI).[28] Therefore, for using 0.01 × PBS, both increase in negative charges derived from FN molecules and decrease in the negative charges induced by MMP-2 were successfully detected. We also examined the relationship between FN concentration and FET responses to the addition of MMP-2 (data not shown). Because sufficient FET responses were not observed for less than 100 μg mL^{-1} FN, we used 1000 μg mL^{-1} FN for FET measurements. To confirm the detectable range and consider the detection mechanism, 0.1 × PBS (Debye length; 2.5 nm) was used as an electrolyte for FET measurements instead of 0.01 × PBS. After 1 h from the addition of 1000 μg mL^{-1} FN to the GA-modified gate, similarly to Figure 3a, a positive value of FET response of approximately 50 mV was observed (Figure 3b). However, unlike Figure 3a, after 3 h from the addition of MMP-2 to the FN-immobilized gate, positive FET response of approximately 20 mV was observed (Figure 3b), which is not due to the degradation of FN immobilized on the GA-modified surface. Although further investigations are required, FET responses are considered to be affected by instable changes with time in the orientation of immobilized FN molecules. For the range above 2.5 nm from the GA-modified

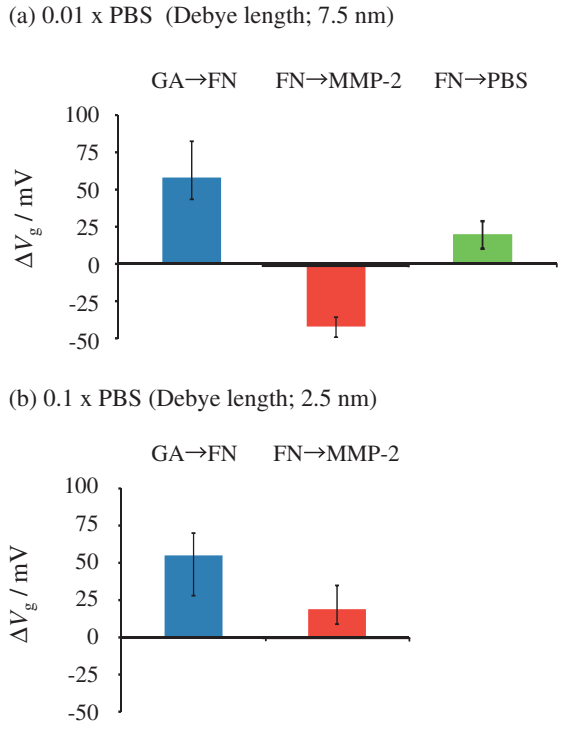

Figure 3. Comparison of the magnitudes of ΔV_g after 1 h from the addition of FN to the GA-modified gate (blue bar), after 3 h from the addition of MMP-2 + $CaCl_2$ (red bar) and after 3 h from the addition of PBS + $CaCl_2$ (green bar) to the FN-immobilized gate. (a) 0.01 × and (b) 0.1 × PBS were used as electrolyte. The concentrations of FN, MMP-2, and $CaCl_2$ were constant at 1000 μg mL^{-1}, 150 ng mL^{-1}, and 5.0 mM, respectively.

surface, FN molecules and/or FN fragments are considered to be detached from the gate. On the other hand, for the range within 2.5 nm from the GA-modified surface, FN molecules and/or FN fragments are thought to remain on the gate even after FN degradation.

In this study, at high coverage of FN molecules immobilized on the gate of FET, MMP-2 was detected based on FN degradation on the gate. By changing types of probe proteins and MMP, this sensing system is expected to be applied to the detection of MMP-expressed cancer cells and various types of MMPs.

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References and Notes

1 P. Bergveld, *Sens. Actuators, B* **2003**, *88*, 1.
2 M. J. Schöning, A. Poghossian, *Electroanalysis* **2006**, *18*, 1893.
3 S. V. Dzyadevich, Y. I. Korpan, V. N. Arkhipova, M. Y. Alesina, C. Martelet, A. V. El'Skaya, A. P. Soldatkin, *Biosens. Bioelectron.* **1999**, *14*, 283.
4 D.-S. Kim, Y.-T. Jeong, H.-J. Park, J.-K. Shin, P. Choi, J.-H. Lee, G. Lim, *Biosens. Bioelectron.* **2004**, *20*, 69.
5 A. Matsumoto, H. Cabral, N. Sato, K. Kataoka, Y. Miyahara, *Angew. Chem., Int. Ed.* **2010**, *49*, 5494.
6 K. Maehashi, T. Katsura, K. Kerman, Y. Takamura, K. Matsumoto, E. Tamiya, *Anal. Chem.* **2007**, *79*, 782.
7 D. Niwa, K. Omichi, N. Motohashi, T. Homma, T. Osaka, *Sens. Actuators, B* **2005**, *108*, 721.
8 J. Wang, K. Ito, T. Nakanishi, S. Kuroiwa, T. Osaka, *Chem. Lett.* **2009**, *38*, 376.
9 T. Nakamura, Y. Sakurai, S. Hideshima, S. Kuroiwa, T. Osaka, *Chem. Lett.* **2010**, *39*, 1245.
10 S. Hideshima, H. Einati, T. Nakamura, S. Kuroiwa, Y. Shacham-Diamand, T. Osaka, *J. Electrochem. Soc.* **2010**, *157*, J410.
11 S. Hideshima, R. Sato, S. Kuroiwa, T. Osaka, *Biosens. Bioelectron.* **2011**, *26*, 2419.
12 S. Hideshima, R. Sato, S. Inoue, S. Kuroiwa, T. Osaka, *Sens. Actuators, B* **2012**, *161*, 146.
13 H. Nagase, J. F. Woessner, Jr., *J. Biol. Chem.* **1999**, *274*, 21491.
14 L. J. McCawley, L. M. Matrisian, *Curr. Opin. Cell Biol.* **2001**, *13*, 534.
15 W. C. Parks, C. L. Wilson, Y. S. López-Boado, *Nat. Rev. Immunol.* **2004**, *4*, 617.
16 I. Stamenkovic, *Semin. Cancer Biol.* **2000**, *10*, 415.
17 M. J. Duffy, T. M. Maguire, A. Hill, E. McDermott, N. O'Higgins, *Breast Cancer Res.* **2000**, *2*, 252.
18 S. Zucker, P. Mancuso, B. DiMassimo, R. M. Lysik, C. Conner, C.-L. Wu, *Clin. Exp. Metastasis* **1994**, *12*, 13.
19 R. Kikuchi, T. Noguchi, S. Takeno, N. Kubo, Y. Uchida, *Br. J. Cancer* **2000**, *83*, 215.
20 G. Liu, J. Wang, D. S. Wunschel, Y. Lin, *J. Am. Chem. Soc.* **2006**, *128*, 12382.
21 H.-S. Lee, J.-S. Oh, Y.-W. Chang, Y.-J. Park, J.-S. Shin, K.-H. Yoo, *Curr. Appl. Phys.* **2009**, *9*, e270.
22 S. Lee, K. Jeon, W. Lee, A. Choi, H.-I. Jung, C.-J. Kim, M.-H. Jo, *J. Korean Phys. Soc.* **2009**, *55*, 232.
23 J. P. Kim, B. Y. Lee, S. Hong, S. J. Sim, *Anal. Biochem.* **2008**, *381*, 193.
24 E. Stern, R. Wagner, F. J. Sigworth, R. Breaker, T. M. Fahmy, M. A. Reed, *Nano Lett.* **2007**, *7*, 3405.
25 M. D. Sternlicht, Z. Werb, *Annu. Rev. Cell Dev. Biol.* **2001**, *17*, 463.
26 A. Omura, T. Matsuzaki, K. Mio, T. Ogura, M. Yamamoto, A. Fujita, K. Okawa, H. Kitayama, C. Takahashi, C. Sato, M. Noda, *J. Biol. Chem.* **2009**, *284*, 3461.
27 D. E. MacDonald, B. Markovic, A. L. Boskey, P. Somasundaran, *Colloids Surf., B* **1998**, *11*, 131.
28 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
29 E. A. Taft, *J. Electrochem. Soc.* **1978**, *125*, 968.
30 H. Ouyang, C. C. Striemer, P. M. Fauchet, *Appl. Phys. Lett.* **2006**, *88*, 163108.
31 L. Guemouri, J. Ogier, J. J. Ramsden, *J. Chem. Phys.* **1998**, *109*, 3265.
32 K. Vallières, P. Chevallier, C. Sarra-Bournet, S. Turgeon, G. Laroche, *Langmuir* **2007**, *23*, 9745.
33 Y. Mao, J. E. Schwarzbauer, *Matrix Biol.* **2005**, *24*, 389.
34 L. Incorvaia, G. Badalamenti, G. Rini, C. Arcara, S. Fricano, C. Sferrazza, D. D. Trapani, N. Gebbia, G. Leto, *Anticancer Res.* **2007**, *27*, 1519.
35 H. Narumiya, Y. Zhang, C. Fernandez-Patron, L. J. Guilbert, S. T. Davidge, *Hypertens. Pregnancy* **2001**, *20*, 185.
36 A. N. Paisley, C. J. O'Callaghan, K. C. Lewandowski, C. Parkinson, M. E. Roberts, W. M. Drake, J. P. Monson, P. J. Trainer, H. S. Randeva, *J. Clin. Endocrinol. Metab.* **2006**, *91*, 4635.

