LABEL-FREE THROMBIN DETECTION IN A MICROCHANNEL USING AN APTAMER MODIFIED GRAPHENE OXIDE SURFACE

Y. Ueno[1]*, K. Furukawa[2], S. Inoue[1], K. Hayashi[1], H. Hibino[2], E. Tamechika[1]

[1]NTT Microsystem Integration Laboratories, NTT Corporation, Japan

[2]NTT Basic Research Laboratories, NTT Corporation, Japan

ABSTRACT

We have designed a label-free thrombin detection system at an aptamer modified graphene oxide (GO) surface fixed on a solid support by using the excellent fluorescence quenching property of GO. The detection system has great merit for on-chip detection because the aptamer is firmly immobilized on the GO surface. An accurate evaluation of the change in the fluorescence intensity caused by the reaction is possible by incorporating a microchannel in the system. The validity of the on-chip detection system was clearly confirmed by using thrombin detection, and the specificity for thrombin was successfully demonstrated.

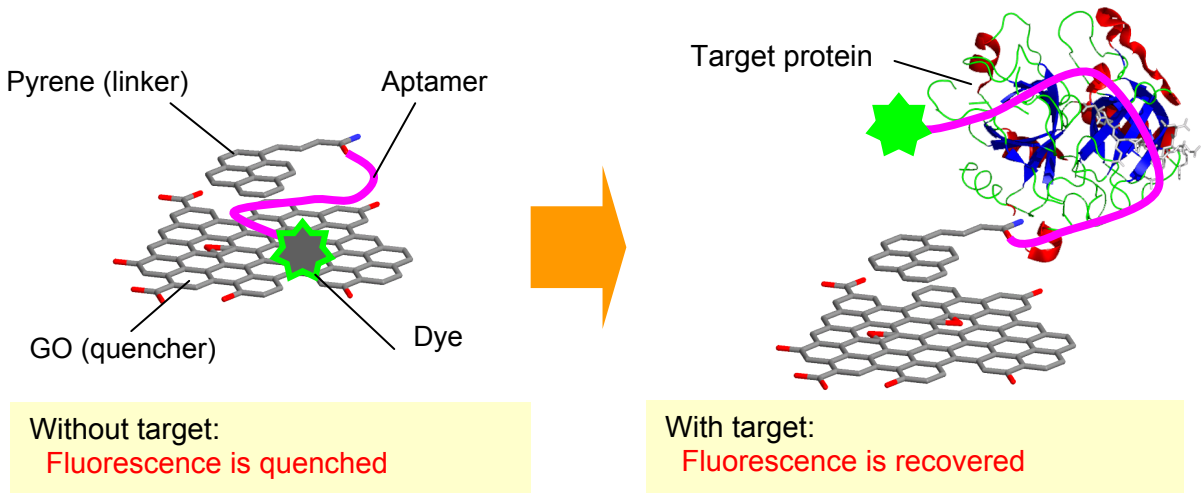
KEYWORDS: Graphene oxide, aptamer, protein, thrombin, microchannel, fluorescence

INTRODUCTION

Graphene oxide (GO), a partially oxidized graphene, is an atomically thin two-dimensional sheet thus making it an interesting material as a platform for a surface chemical reaction. It exhibits interesting optical properties such as fluorescence quenching and broadband photoluminescence due to its unique structure consisting of nanometer-size sp^2 carbon clusters isolated within an sp^3 carbon matrix. These unique properties of GO provide new opportunities for its use in biosensing applications [1-2]. Moreover, functionalization with oxygen-containing groups including hydroxyl, carboxyl, and epoxide groups gives GO good dispersibility in water thus allowing us to use a low-cost conventional wet process to fabricate optical and electronic devices.

We developed a label-free protein detection system with a new design using aptamer modified GO (Scheme 1). Aptamers are selected single-stranded oligonucleotides that can specifically bind to certain proteins or small molecules. In this system, the GO surface is fixed on a solid support and is modified by an aptamer that bonds chemically to pyrene and a dye probe at the 5'- and 3'- termini, respectively. The pyrene works as a linker to the sp^2 domains of GO to immobilize the aptamer firmly on the GO surface, and the dye works as a detection probe. At the initial stage, the fluorescence of the dye labeled aptamer, which is well adsorbed on the GO surface via π-π interactions, is quenched by GO. When the target protein is added, the fluorescence recovers as a result of the separation of the dyes from the GO surface by the formation of aptamer-protein complexes. The chemical reactions that occur on the GO surface at each reaction step are successfully monitored with AFM observations [3].

Our detection system has great merit as regards the fabrication of on-chip detection devices. In the work described in this paper, we fabricated an on-chip detection system with a microchannel and used it for the detection of thrombin. Thrombin detection is very important, because thrombin is a protease present in the human body that is responsible for excessive blood clotting. By employing a microchannel, it is possible to measure simultaneously the fluorescence on the GO pieces located inside the channel where the reaction occurs, and GO located outside the channel where no reaction occurs. Thus, we can improve the accuracy of our quantitative evaluation of the change in fluorescence intensity. Moreover, using a microchannel, we can reduce the sample volume to a few μL and this will be advantageous when this system is used for blood tests.



Scheme 1: Design of a protein detection system using an aptamer modified GO surface.

EXPERIMENTAL

Figure 1 shows the fabrication of an on-chip protein detection system using an aptamer modified GO surface equipped with a microchannel. An aqueous dispersion of GO was synthesized with a modified Hummers method [4] and spin coated on a hydrophilic-treated glass plate. The GO surfaces were then modified with 5 mM of 1-pyrenebutanoic acid-succinimidyl ester (PB-S, Invitrogen) dissolved in N,N-dimethylformamide (DMF, Kanto Chemical Co. Inc.). The thrombin aptamer (5'-GGTTGGTGTGGTTGG-3', TBA), which was modified at the 5'-terminus with an amine group and at the 3'-terminus with 6-carboxyfluorescein (FAM), were purchased from Sigma Genosys. 100 μM of 5'NH_2-TBA-FAM3' in 10 mM phosphate buffer was reacted with PB-S/GO to form PB-CONH-TBA-FAM. Finally, a polydimethylsiloxane (PDMS) channel, formed using a photolithographic technique, was mounted on the top of the chip. Alpha human thrombin (Wako Pure Chemical Industries, Ltd.) and human albumin (ICN Biomedicals) were dissolved in Deionized (DI) water (Millipore, >18 MΩ·cm) to prepare a certain concentration of solutions.

An Olympus BX51-FV300 confocal laser scanning microscope (LSM) was used to obtain fluorescence images. We used a 505-525 nm band-pass filter with a 488 nm laser light source for the FAM fluorescence observations. The space between the rear of the glass chip and the objective lens was filled with water throughout the observations which were undertaken using a water-immersion objective lens Plan Apo 40×WLSM (Olympus). All the measurements were performed at room temperature.

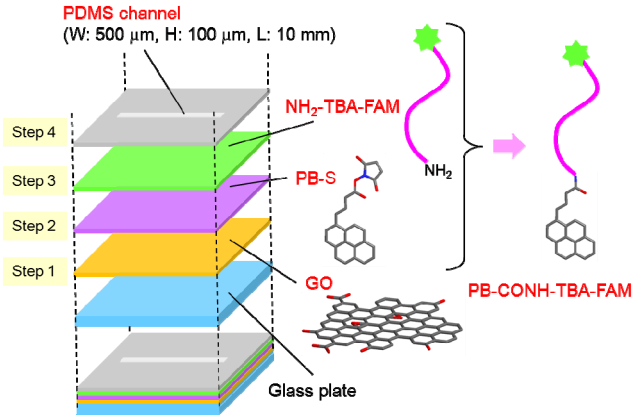


Figure 1: Fabrication of an on-chip protein detection system using aptamer modified GO surface assembled with a microchannel.

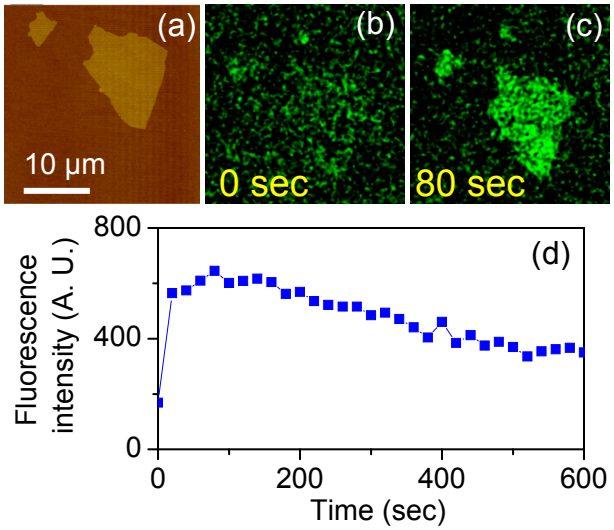


Figure 2: AFM image of GO before modification on a Si(100) surface with a 285 nm SiO_2 layer (a); Fluorescence images of the same modified GO piece. After adding thrombin at 0 sec (b) and 80 sec (c); change in the average fluorescence intensity on the GO piece (d).

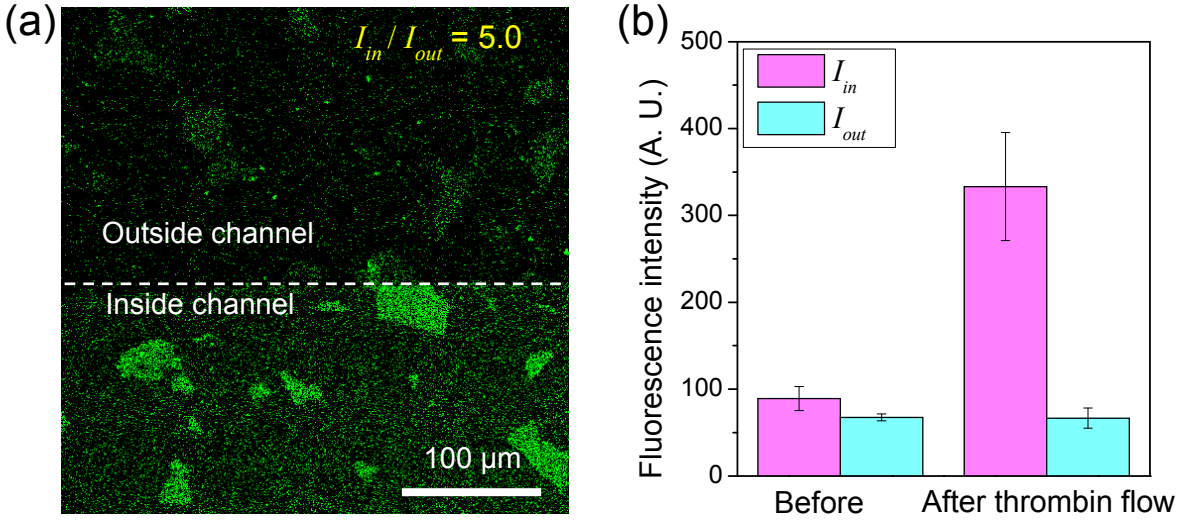


Figure 3: Fluorescence image of modified GO pieces around the boundary of the channel after flowing 100 unit/mL of thrombin solution (a); the signal intensity inside (I_{in}) and outside of the channel (I_{out}) before and after adding thrombin solution (b).

RESULTS AND DISCUSSION

Figure 2a shows the topography of GO on a Si(100) surface with a 285 nm SiO_2 layer before aptamer modification obtained with an atomic force microscope (AFM). Two GO pieces were observed as bright patterns in the image. The GO shape was irregular like a torn paper, and the GO size also varied. The GO was uniformly flat and the average height was 1.1 nm. The change in the fluorescence intensity from the same GO pieces before and after adding 50 units/mL of thrombin solution was monitored with LSM (Fig. 2b and c). The fluorescence intensity had increased approximately threefold 80 seconds after the reaction started (Fig. 2d). The results show the successful label-free detection of thrombin on the aptamer modified GO surface. The time needed to reach maximum intensity can be assigned to the time needed to form the aptamer-thrombin complexes. As a result of the structural change in TBA, the dyes are separated from the GO surface, which leads to the fluorescence recovery. The subsequent gradual decrease in intensities indicates the fluorescence degradation of the dyes caused by repeated observations.

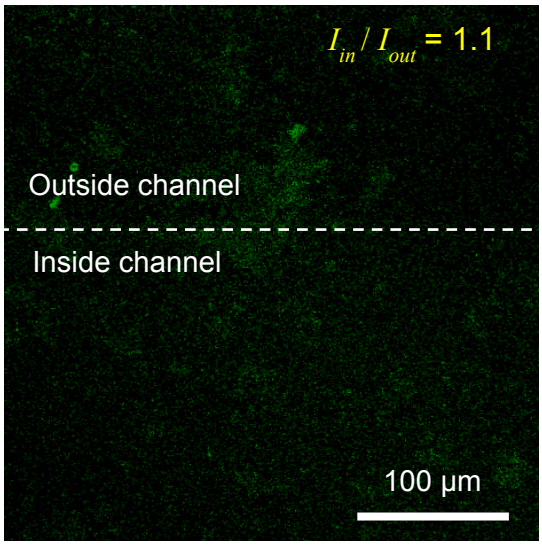


Figure 4: Fluorescence images with the addition of 50 mg/mL of albumin solution to the system's microchannel.

Next, we installed the detection system in a microchannel configuration. Figure 3a shows the fluorescence image of the aptamer modified GO pieces around the boundary of the PDMS channel a few minutes after adding < 5 μL of thrombin solution (100 units/mL). The fluorescence intensity of the GO pieces inside the channel was much stronger than that outside the channel. We calculated the signal intensity inside (I_{in}) and outside the channel (I_{out}) by subtracting the average fluorescence intensity at points where there were no GO pieces, from that at the GO pieces. We used the ratio I_{in} / I_{out} to evaluate the sensitivity of the thrombin detection. Note that the effect of the fluorescence degradation is eliminated here. The ratio I_{in} / I_{out} after adding thrombin to the channel was 5.0, whereas the I_{in} / I_{out} was about 1.3 before the reaction occurred (Fig. 3b). The results indicate that the validity of the system was clearly confirmed by using thrombin detection. We found that the fluorescence intensity inside the channel was always slightly larger than that outside the channel, either on the GO pieces or the places without GO, even before reaction occurred. This was due to the difference between the scattering inside and outside of the channel. A dual microchannel system is now being fabricated to completely remove such scattering effects and other noises.

Lastly, to demonstrate the specificity for thrombin detection, we also measured the fluorescence images when 50 mg/mL of albumin solution was added to the system (Fig. 4). The albumin caused no change in the fluorescence intensity either inside or outside of the channel, and the I_{in} / I_{out} ratio was 1.1. The results clearly show that our detection system can achieve the highly selective detection of thrombin.

CONCLUSION

In summary, we have proposed a new protein detection system using an aptamer modified graphene oxide surface and fabricated an on-chip detection system with a microchannel. The validity of the proposed system was clearly verified by using thrombin detection. The specificity for thrombin was also successfully demonstrated. It is noteworthy that the proposed system has great potential for application to many other biological molecules by employing different aptamers. Moreover, the system can be easily developed into a multichannel detection system that can realize multiple detection simultaneously. Our system is thus promising for biosensor and diagnostics applications.

REFERENCES

[1] J. H. Jung, D. S. Cheon, F. Liu, K. B. Lee, and T. S. Seo, "A Graphene Oxide Based Immuno-biosensor for Pathogen Detection", Angew. Chem. Int. Ed., 49, 5708-5711 (2010).

[2] H. Chang, L. Tang, Y. Wang, J. Jiang, and J. Li "Graphene Fluorescence Resonance Energy Transfer Aptasensor for the Thrombin Detection", Anal. Chem., 82, 2341-2346 (2010).

[3] K. Furukawa, Y. Ueno, E. Tamechika, and H. Hibino, "Graphene Oxide as Optical Platform for Protein Recognition" ISOME 2012, O6-2 (2012).

[4] W. Hummers and R. Offeman, "Preparation of Graphitic Oxide" J. Am. Chem. Soc., 80, 1339 (1958).

CONTACT

*Yuko Ueno +81-46-240-3549 or ueno.yuko@lab.ntt.co.jp