MULTICHANNEL LINEAR-ARRAY MICROBIOSENSOR USING APTAMER MODIFIED GRAPHENE OXIDE: IMPROVED SENSITIVITY BY MOLECULAR DESIGN

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ABSTRACT

We demonstrate the first example of a multichannel linear-array microbiosensor that uses aptamer-modified graphene oxide supported on a solid surface. A homogeneous graphene oxide single layer on a solid surface can be used as a satisfactory platform for this sensor. We formed a linear array of several different aptamer-based biomolecular probes, which we designed for highly sensitive protein detection, and implemented a multichannel microfluidic device across the array. The integration of several different probes into one chip is an excellent way to compare performance. A diagnosis level of thrombin detection was achieved using the best-designed probe.

KEYWORDS: Multichannel, Linear-Array, Aptamer, Graphene Oxide

INTRODUCTION

Graphene oxide (GO) has been a widely used as a material for biosensing applications owing to its unique properties. Aptasensors, which are biosensors that use aptamer-based biomolecular probes for molecular recognition, make the best use of the unique properties of GO. An aptamer is a synthetic single-stranded oligonucleotide and it forms a complex specifically with the target molecule. In many GO aptasensors, the aptamer is conjugated with a fluorescence dye and fixed on the GO surface via physisorption or chemical bond formation [1]. The dye exists close to the GO surface because a single-stranded DNA (ssDNA) such as the aptamer has strong affinity with the GO surface. The fluorescence from the dye is not observed under the above conditions because GO behaves as an efficient acceptor for fluorescence resonance energy transfer (FRET). Once the aptamer forms a complex with its target molecule, the dye leaves the GO surface because the aptamer-target complex has little affinity with GO. This can be observed as a recovery of dye fluorescence, namely as a signal indicating the detection of a target molecule. Recently, we reported an FRET aptasensor using GO fixed on a solid support [2]. In our system, we modified the GO surface with a pyrene-aptamer-dye probe. Each segment works as a linker to the sp^2 domains of the GO, a protein recognition part, and a detection probe, respectively. The molecular probe allows us to perform molecular detection on a solid surface, which is a powerful tool with which to realize a multichannel linear-array microbiosensor. A merit of the multichannel and/or microarray configuration is that it facilitates a quantitative comparison of several different samples (Fig. 1).

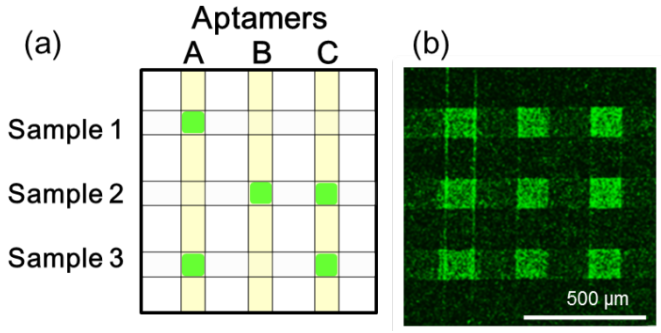

Figure 1: Concept of multichannel linear-array microbiosensor (a), molecular detection image using a 3×3 chip (b).

In this work, we demonstrated the first example of a multichannel linear-array microbiosensor using aptamer-modified GO supported on a solid surface. We formed a linear array of several different biomolecular probes by injecting the probe solutions into a microchannel device placed on a substrate (Fig. 2). After washing the solutions and removing the microchannel device, we implemented another multichannel microfluidic device across the probe pattern. Here we used newly designed biomolecular probes for highly sensitive protein detection by introducing a DNA spacer to an aptamer (Fig. 3). We compared the performance of the probes quantitatively by using a multichannel linear-array microbiosensor. The strategy of the molecular design is to enlarge *d*, which is the distance between the fluorescence dye (donor) and the GO surface (acceptor). The

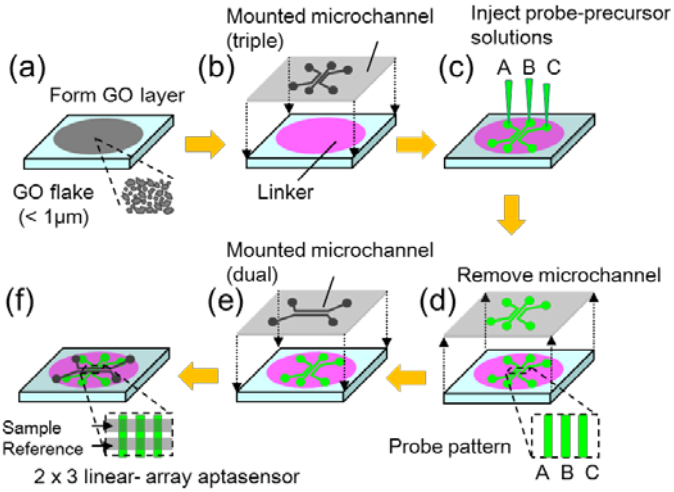

Figure 2: Preparation procedure for 2×3 multichannel linear-array GO aptasensor.

enlargement of *d* is crucial for FRET-based sensors, at the formation of a complex with the target protein (Fig. 4).

EXPERIMENTAL

Figure 3 shows the five different modification probes we designed. We introduced an ssDNA spacer with a different number of thymine segments, *N*, at 3'- and/or 5'-terminals of the thrombin aptamer (3'- and 5'-spacers). 3'- and 5'-terminals of the sequences are modified with 6-carboxyfluorescein (FAM) and a linker, respectively.

The procedure we used to prepare the on-chip GO aptasensor was a modified version of the one we reported previously [1]. We prepared a homogeneous GO single layer on a solid surface with a spin-coating technique (Fig. 2a). We reduced the GO to enlarge the sp^2 domain by exposing it to hydrazine/ammonium vapor (aqueous hydrazine solution 35wt%: aqueous ammonium solution 28wt% = 7:1) at 95˚C for 60 min. The reduced GO Fig. 2a) was covered with a drop of linker solution, namely 5 mM 1-pyrenebutanoic acid-succinimidyl ester (Invitrogen) in *N*,*N*-dimethylformamide (DMF, Kanto Chemical Co., Inc.), for 1 h. Then, a polydimethylsiloxane (PDMS) sheet with a triple-channel microfluidic device (width, depth, and gap between channels of 50, 100, and 100 μm, respectively) was placed on the substrate (Fig. 2b). The probe precursors (Sigma Genosys) were prepared as a 100 μM phosphate buffer solution (pH = 7.4, Nacalai Tesque Inc.). Each precursor was modified with an amine group at the 3'-terminal and thus the probe was formed on the GO surface by a dehydration reaction with 1-pyrenebutanoic acid-succinimidyl ester. Three different precursor solutions were injected into each microchannel (Fig. 2c). After removing the solutions from the channels, the microfluidic device was peeled off in a water bath and then the chip was rinsed with water. A linear pattern modified by the different probes was obtained on the surface (Fig. 2d). Another PDMS sheet with a dual-channel microfluidic device (width, depth, and gap between channels of 300, 100, and 80 μm, respectively) was implemented across the linear pattern (Fig. 2e) to obtain a 2×3 multichannel linear-array aptasensor (Fig. 2f). α-thrombin (ICN Biochemicals) was dissolved into DI water (Millipore, >18 MΩ·cm)

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 fluorescence observations of FAM (λ_{max}(abs)/λ_{max}(em) = 494 nm/518 nm). The fluorescence images were taken through the glass plate with an objective lens UPlan Apo 10× LSM (Olympus).

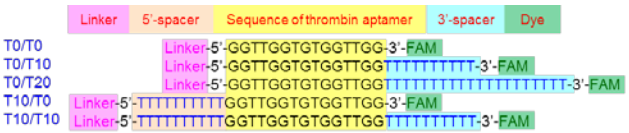

Figure 3: Design of the biomolecular probes examined in this study. They are denoted using the numbers of thymines (N) for 5' and 3' spacers as T0/T0.

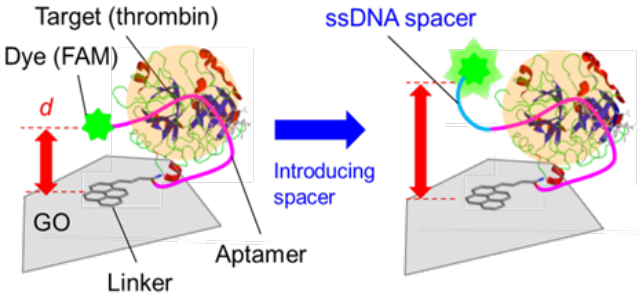

Figure 4: Schematic diagram showing how to increase fluorescence intensity by introducing an ssDNA spacer to an aptamer. Left: Protein detection system of the GO aptasensor. Right: Increase in d becomes potent by introducing a spacer between the aptamer sequence and FAM.

RESULTS AND DISCUSSION

We first describe the preparation of a sensing platform, that is, a solid surface densely covered with a GO single layer with high coverage on a mm scale. Since the optical property of GO may differ with the number of the layers, we try to limit the amount of overlapping. An aqueous dispersion of as-synthesized GO contained pieces of various sizes ranging from a few μm to larger than 10 μm. We also prepared a GO aqueous dispersion containing smaller (sub-μm) GO pieces by breaking the large GO pieces into small pieces using ultrasound. The GO dispersion was spin coated on a SiO_2/Si chip and the size of the GO pieces in the dispersion was analyzed with an AFM (Fig. 5(a), (b)). We determined that the most appropriate size for the GO pieces, which allowed us to form a homogeneous single GO layer with the least amount of overlapping, was a few hundred nm (Fig. 5b). The coverage exceeded 70% when the absorbance of the GO dispersion was about 0.1 at 400 nm. We then prepared a dual channel GO aptasensor under the optimum conditions and examined its performance. Figure 5 (c) and (d) compare the fluorescence images of GO aptasensors that were prepared with T0/T0 by using large and small GO pieces, respectively. The images were obtained after injecting α-thrombin solution (100 μg/mL) into the top channel. The bottom channel was filled with water as a reference. We clearly observed fluorescence recovery on the GO surface in the sample channel for both chips and there was little fluorescence recovery in the reference channel. With the chip prepared using large GO pieces, the coverage of the sample and the reference channels was not the same. In contrast, with the chip prepared using small GO pieces, the fluorescence in the channel was almost homogeneous at least up to the resolution of the obtained fluorescence image. Thus, we can consider the GO coverage of the glass plate to be the same over the entire surface. Therefore, the prepared homogeneous GO single layer on a solid surface, with high coverage and with the least amount of overlapping, can be used as a sufficient platform on which to build a multichannel linear-array aptasensor.

Next, we compared the performance of the designed probes by using a 2×3 multichannel linear-array aptasensor. Figure 6a shows a fluorescence image of the 2×3 aptasensor obtained after injecting α-thrombin solution and water into the top and

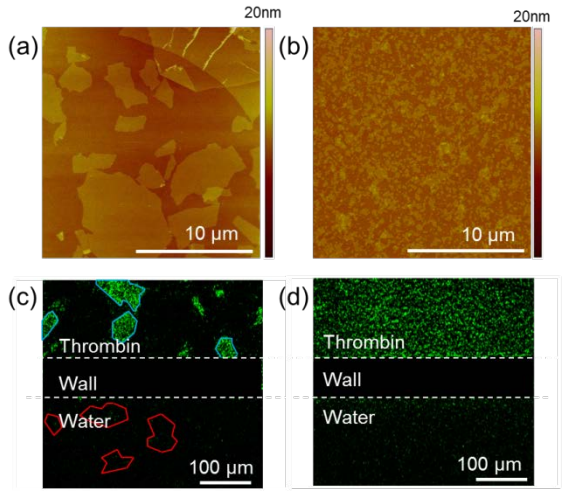

Figure 5: AFM topographical images of GO on a SiO_2/Si chip; (a) as synthesized and (b) after ultrasound treatment (b). Fluorescence images of the 2×1 GO aptasensors prepared by using the GO-dispersion containing the GO pieces (c) as synthesized and (d) after ultrasound treatment, taken in the vicinity of the wall between the two microchannels

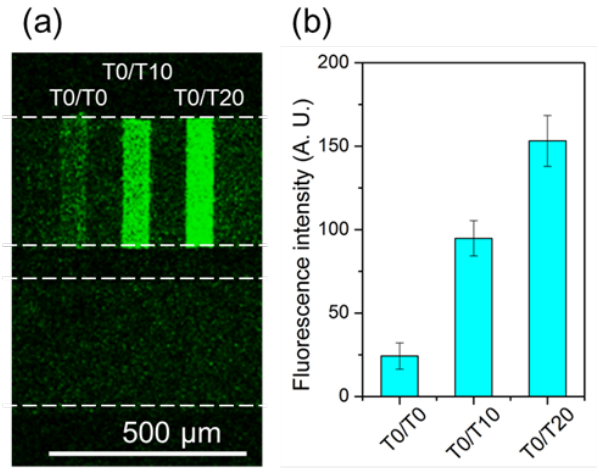

Figure 6: (a) Fluorescence image of the 2×3 multichannel linear-array aptasensor patterned by T0/T0, T0/T10, and T0/T20, from left to right. Thrombin solution (100 μg/mL) and water were injected into the top and bottom microchannels, respectively. (b) Average fluorescence intensity of the patterned area in the top microchannel.

bottom microchannels, respectively. The three green lines in the top channel correspond to the position where T0/T0, T0/T10, and T0/T20 were fixed, from left to right, respectively. Although the same probes were also fixed in the bottom channel, almost no fluorescence was observed in the corresponding areas. The result clearly shows that the designed probes function properly as a thrombin detector, but do not react with the reference water. The fluorescence intensity was large in order T0/T20, T0/T10, and T0/T0. Thus, the 3' spacer has a great effect on increasing the sensitivity of the aptasensor. Figure 6b compares the average fluorescence intensity of the patterned area in the top microchannel. The ratio of the fluorescence intensity of T0/T0, T0/T10, and T0/T20 was 1:4:6. We thus achieved our aim of increasing the sensitivity by using our molecular design. The result indicated a significant *N* dependence in the fluorescence intensity. On the other hand, the 5' spacer has little effect on changing the fluorescence intensity. This suggests that *d* with the 5' spacer was almost the same as that without a spacer. Since GO shows a great affinity for ssDNA, the 5' spacer may simply adsorb on the GO surface and not help to increase *d*. Model calculations supported the view that distance control is a major contributor to the enhancement and the 6-fold increase in the sensitivity with the T20 spacer. It is noteworthy that the integration of several different probes into one chip is an excellent way to avoid the problem of reaching a misleading conclusion in a quantitative discussion, especially when comparing fluorescence intensities. Finally we experimentally evaluated the detection limit for thrombin by using T0/T20, the best design in our present study. We successfully detected ~1nM (37 ng/mL) of thrombin, which is in the *in vivo* concentration range during blood clotting.

CONCLUSION

A multichannel linear-array microbiosensor using aptamer-modified graphene oxide supported on a solid surface was successfully realized. The performance of the designed biomolecular probes for highly sensitive thrombin detection was examined using a 2×3 multichannel linear-array aptasensor. We concluded that introducing an ssDNA spacer at the correct position is an effective strategy for increasing the sensitivity of the sensor. A diagnosis level of thrombin detection was achieved by using the best-designed probe. The simple fabrication procedure can be employed to form many other multichannel linear-array microbiosensors. Our findings will provide a useful platform for developing multi-target multichannel aptasensors and will help the field of on-chip microsensors to progress.

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