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# Electrospun polystyrene–poly(styrene-co-maleic anhydride) nanofiber as a new aptasensor platform

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ABSTRACT

Here, we report the use of an aptamer-immobilized electrospun polystyrene–poly(styrene-co-maleic anhydride) (PS–PSMA) nanofiber as a new aptasensor platform for protein detection. Two thrombin-binding aptamers (TBA29 and TBA15) were used as a model platform to facilitate efficient detection of thrombin in a sandwich manner. Thrombin concentration was measured by fluorescence microscopy and spectroscopy, in which aptamers were labeled with either fluorescein dye or quantum dots. The results indicated that thrombin was captured uniformly on the surface of the nanofiber. Using this sandwich-type biosensor, the minimum detectable concentration of thrombin was 10 pM, with a dynamic range of 0.1–50 nM, when quantum dots were used for labeling. In contrast, the limit of detection was 1 nM, with a dynamic range of 10–200 nM, when using fluorescein dye labeling. This aptamers-on-nanofiber-based biosensor showed 2500-fold higher sensitivity than a 96-microwell plate format, attributed mainly to the large surface area of the nanofibers. In addition, this novel platform also exhibited similar high sensitivity in the detection of exogenously added thrombin in diluted human serum. This aptamers-on-nanofiber system, which is competitive with other sensing platforms and clinically meaningful in terms of its detection limit, is expected to be useful for the detection of various other targets because of its ease of application and manipulation.



## 1. Introduction

Aptamers are functional biomolecules composed of ssDNA or RNA molecules that have the potential to bind to specific target molecules, such as proteins, chemicals, viruses, cells, etc., with high specificity and affinity (Jang et al., 2007; Lee et al., 2008; Niazi et al., 2008; Shamah et al., 2008). These molecules can be isolated from random libraries *in vitro* using the SELEX process (Tuerk and Gold, 1990). In addition, they can be chemically synthesized, are thermally stable, and can be easily labeled or modified without loss of binding efficiency (Willner and Zayats, 2007). These advantages over traditional methods, such as antibody-mediated detection, make aptamers attractive sensing probes compared to antibodies, and aptamers have been exploited for the detection of various targets using numerous transduction strategies (Ikebukuro et al., 2005; Peng et al., 2011; Wang and Liu, 2009; Zhao et al., 2011).

In biosensor applications, nanomaterials with three-dimensional structures have been investigated for their ability to improve sensitivity due to their large surface areas, which allow for improved immobilization of sensing probes due to their high loading capacities (Oillic et al., 2007a). For example, Qiu et al. (2010) reported that nanoporous gold electrode-based electrochemical sensors exhibited detection limits as low as 30 fM. In another study, a nanopillar structure exhibiting proper height and spacing was utilized as a DNA chip substrate, increasing the sensitivity of DNA detection by 100-fold (Kim et al., 2011b). Moreover, the immobilization of properly spaced probes on the nanostructure surface can enhance the accessibility of target molecules, leading to higher signals and reducing the quenching effect of fluorescence signals in optical biosensor systems (Lee et al., 2010).

The electrospun nanofibers produced by electrospinning processes have several advantages, including uniformity, porosity, mechanical strength, biocompatibility (depending on the material polymer), and high surface-to-volume ratios due to the large surface area (Hwang et al., 2011b; Nair et al., 2007). The large surface areas of electrospun nanofibers are inherent to the three-dimensional structure of nanomaterials (Oillic et al., 2007b). Many studies have utilized electrospun nanofibers for biomedical and industrial applications, such as supporting scaffolds for enzyme immobilization, membranes for filtration, and tissue templates (Herricks et al., 2005; Jun et al., 2010; Kim et al., 2006a; Masuda et al., 2000). In addition, because of the advantages described above, several reports have investigated the development of nanofiber-based sensing platforms (Huang

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et al., 2008; Wang et al., 2002; Yang et al., 2008). However, to the best of our knowledge, aptamers have never been applied to nanofibers to facilitate protein-sensing applications.

It is believed that electrospun nanofibers can be used as an ideal platform for highly sensitive aptamer-based biosensing applications, with all of the aforementioned advantages, including high surface area for loading, capability of functional immobilization with desired spacing, reproducibility, and long-term storage. In addition, other nanomaterials used in biosensing applications may suffer from harsh production conditions, undergo undesired aggregation, require complex instrumentation, and yield results that are challenging to reproduce (Wang et al., 2011; Zhang et al., 2011b). Therefore, the electrospun polymer nanofiber is a very promising aptasensor platform that is stable during long-term storage, reusable, and easy to produce and handle (Hwang et al., 2011a).

Recently, we demonstrated that alcohol dispersed polystyrene–poly(styrene-co-maleic anhydride) (PS–PSMA) nanofibers immobilized with aptamers could be used as matrices for protein purification (Kim et al., 2011a). The abundance of maleic anhydride moieties on PS–PSMA nanofibers assures sufficient coating of streptavidin (SA) for immobilization of biotin-tagged aptamers. Coated with aptamers, these PS–PSMA nanofibers were found to have sufficient capacity for capturing target molecules, such as proteins. Therefore, an aptamer-coated PS–PSMA nanofiber could be used as a sensing platform for protein detection. In this study, we developed a sandwich-type aptasensor using aptamer-immobilized PS–PSMA nanofibers. To demonstrate whether this aptamers-on-nanofiber design could be used as a sensor platform, we attempted to detect thrombin using its two binding aptamers, one as a capturing probe and the other as a signaling probe (Tennico et al., 2010; Yang et al., 2009). These signaling aptamers were labeled with fluorescein dye or quantum dots (QDs), which were expected to have enhanced sensitivity due to their unique optical properties (Kim et al., 2006b). Thrombin binding assays were performed using aptamer-immobilized nanofibers in a sandwich manner. When QDs were used, we were able to detect thrombin with higher sensitivity. Moreover, this assay could be successfully used to measure lower concentrations of protein than 96 microwell plate based detection methods. Our results demonstrated that low levels of exogenous thrombin spiked in human serum could also be detected by this method, confirming the potential use of this novel biosensor in real samples.

## 2. Experimental

### 2.1. Materials and reagents

Polystyrene (PS, MW∼900,000) was purchased from Pressure Chemicals. Poly(styrene-co-maleic anhydride) (PSMA, MW∼240,000), 99.8% pure *N,N*-dimethylformamide (DMF), human α-thrombin ( ≥ 2000 NIH units/mg protein), Streptavidin (SA), IgG (from human serum), bovine serum albumin (BSA), human serum albumin (HSA), and human serum (from human male AB plasma) were purchased from Sigma-Aldrich. All reagents were used as received.

The sequences for thrombin binding aptamers were as follows: biotin-labeled TBA29 aptamer, biotin-T10-5′-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3′; fluorescein-labeled or biotin-labeled TBA15 aptamer, 5′-GGT TGG TGT GGT TGG-3′. For the biotinylation at the 5′ end of each aptamer, poly dT (T10) was inserted as a spacer arm. All oligonucleotides were purchased in purified forms (GenoTech Co., Korea).

### 2.2. Instrumentation

SA-coated black 96 microwell plates were purchased from Thermo Fisher Scientific, Inc. (USA), and SpectraMax (Molecular Devices, USA) was used for fluorescence measurements. A confocal laser scanning microscope (CLSM) and Zeiss Axiovert 200 microscope coupled to a Zeiss LSM 510 scanning device (Carl Zeiss Co. Ltd., Germany) were used to inspect the aptamer–thrombin–aptamer complex present on nanofibers.

### 2.3. Preparation of the aptamer-immobilized PS–PSMA nanofiber

PS–PSMA nanofibers were prepared according to methods outlined in previous reports (Kim et al., 2011a; Nair et al., 2007). In brief, 120 mg PS and 60 mg PSMA were mixed in 2 mL DMF and completely dissolved at room temperature (RT). Next, the polymer solution was sonicated for 1 h at RT and loaded into a 10 mL plastic syringe equipped with a stainless steel needle. Electrospinning for nanofiber production was performed according to the following conditions: a syringe with a 23 gauge needle was fixed vertically with the flow rate set at 2.5 mL/h; a 10 cm distance was set between tip-to-collecting aluminum foil; and a 10 kV high-voltage power supply (Gamma High Voltage Research, ES series) was used. Thus, electrospun PS–PSMA nanofibers were obtained, and 0.5 mg of nanofibers were collected in individual vials. After ethanol dispersion of the nanofibers for 1 h, they were washed with excess of distilled water and 0.1 M PBS buffer (pH 7.4) and then coated with SA.

The dispersed nanofibers (0.5 mg/vial) were incubated with 50 μg SA in PBS solution for 1 h at RT and overnight at 4 °C for covalent attachment of SA onto the nanofibers. After coating, the concentration of the remaining SA was measured by Micro BCA test (Pierce, US) to calculate the amount of bound SA molecules, and nanofibers were then washed with PBST (0.1 M PBS, pH 7.4, and 0.02% Tween 20) several times. Next, nanofibers were treated with 100 mM ethanolamine for 1 h at RT to block unoccupied regions of the nanofibers. Finally, SA-coated nanofibers were washed with TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.4) and immersed in B&W buffer (5 mM Tris–HCl, 0.5 mM EDTA, 1 M NaCl, pH 7.5) to immobilize the aptamers. Biotin-labeled TBA29 (1 nmol) was mixed with nanofibers in B&W buffer, and binding was achieved under mild rotation at RT for 1 h. After TBA29 immobilization on the nanofiber, the remaining TBA29 was precipitated with ethanol, and the concentration was measured by NanoDrop (ND-1000 Spectrophotometer, Nanodrop Technologies, Inc.). TBA29-immobilized nanofibers were rinsed with TE buffer 8 times and stored in storage buffer (100 mM NaCl, 10 mM Tris–HCl, 1 mM EDTA, 10 mM $NaN_3$, pH 8.0) until use.

### 2.4. Thrombin binding assays with fluorescein-labeled TBA15 aptamers

Sandwich assays for thrombin detection were performed using TBA29 aptamers immobilized on nanofibers as capturing probes and fluorescein-labeled TBA15 (F-TBA15) aptamers as signaling probes. Various concentrations of thrombin (0–200 nM) were applied to the nanofibers based aptasensor system. For this, nanofibers with immobilized TBA29 were immersed in 500 μL of thrombin solution in TB buffer (140 mM NaCl, 20 mM Tris–HCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.4) and mixtures were incubated for 30 min with rotation. After target binding, nanofibers that had captured thrombin were washed thoroughly with TBT buffer (TB containing 0.02% Tween 20) 5 times. Then, nanofiber-target complexes were incubated with 500 μL of TB buffer containing F-TBA15 aptamers (100 pmol). In this step and subsequent steps, black microtubes were used to protect the samples from light. After a 30 min incubation period, nanofiber complexes were washed thoroughly to remove any nonspecific binding, and the solutions were completely removed. Finally, 200 μL of 2 M $NaClO_4$ was added to elute the fluorescent moieties

from the nanofibers for 30 min, and solutions were transferred to black 96 microwell plates for fluorescence measurements. In the case of fluorescein dye, excitation and emission wavelengths were 485 nm and 516–570 nm, respectively.

### 2.5. Thrombin binding assays with QD-labeled TBA15 aptamers

To enhance the fluorescent signal, QDs were bound to signaling TBA15 aptamers. SA coated QDs (SA-QDs; 15–20 nm, Invitrogen, USA) were functionalized with biotin-labeled TBA15 aptamers following methods outlined in the literature (Kim et al., 2006b). Briefly, 0.1 μM SA-QDs were incubated with 0.5 μM biotin-labeled TBA15 aptamers (1:5 ratio) in B&W buffer. Conjugation was completed for 1 h at RT with rotation, and QDs conjugated TBA15 aptamers (Q-TBA15) were stored at 4 °C until use. To confirm the Q-TBA15 conjugation, the solution was centrifuged with a filter; no filtered DNA was found in the centrifuged sample. Thrombin binding assays were conducted as described above, except that 25 pmol of Q-TBA15 was used in place of F-TBA15, and fluorescence intensity was measured at excitation and emission wavelengths of 355 nm and 480–570 nm, respectively. Thrombin samples in buffer solution and 10-fold diluted human serum were examined for binding assays. For specificity tests, 200 nM each of HSA, BSA, and IgG were applied to nanofiber based aptasensors using both F-TBA15 and Q-TBA15 aptamers.

### 2.6. Thrombin binding assays on 96-well plates

We compared the thrombin binding abilities of nanofibers based aptasensors to conventional SA-coated 96 microwell plates using the same biotin-labeled TBA29 aptamers as capturing probes and F-TBA15 or Q-TBA15 aptamers as signaling probes. Aptamer immobilization was performed according to the manufacturer's protocol. Before the addition of aptamers, the wells were washed 3 times with 200 μL of B&W buffer. Then, 100 μL of 0.4 μM biotin-labeled TBA29 aptamers in B&W buffer was loaded into the wells, and plates were incubated for 2 h at RT with mild shaking. After aptamer immobilization, the wells were washed 3 times with 200 μL of the same buffer, and 100 μL of target protein solutions (spanning the same concentration range) in TB buffer were immediately added to the wells. The binding reactions were allowed to proceed for 30 min with mild shaking, after which the wells were washed 3 times with 200 μL TBT. For binding of signaling aptamers, 100 μL of F-TBA15 (100 pmol) or Q-TBA15 aptamers (25 pmol) were added to the wells. After incubation for 30 min and washing with TBT, the fluorescence signals were immediately measured by fluorescence spectroscopy. All experiments above were run in triplicates to confirm the reproducibility of the sandwich assays, and the coefficient of variation (%CV) was calculated.

## 3. Results and discussion

### 3.1. Development of aptasensors consisting of immobilized aptamers on PS–PSMA nanofibers

A schematic diagram of the sandwich assay with TBA aptamers immobilized on PS–PSMA nanofibers is shown in Fig. 1. Initially, each PS–PSMA nanofiber was dispersed in an ethanol solvent (50%) to enlarge and swell the polymeric chains and was subsequently treated with aqueous buffer to expose the maleic anhydride groups of the PSMA block (Nair et al., 2007). The maleic anhydride groups reacted covalently with 32.27 μg (482 pmol) of SA per 0.5 mg PS–PSMA nanofiber. SA-immobilized nanofibers were treated with biotinylated thrombin binding ssDNA aptamers (TBA29) and then placed in solution for thrombin capture. The binding capacity of TBA29 aptamer on the nanofibers was 713 pmol (CV, 7%) per 0.5 mg of the nanofibers. According to a previous study, 1 mg of PS–PSMA nanofibers can accommodate 2 nmol of SA (Kim et al., 2011a). These nanofibers exhibited a several-fold higher capacity than commercially available SA-coated 96 microwell plates, which only allow binding of up to 125 pmol of free biotin molecules per well or SA-coated magnetic beads that can bind up to 500 pmol of biotin per 1 mg of beads. Thus, the increased binding capacity of nanofibers compared to typical spherical magnetic beads (2.8 μm in diameter) or 96 microwell plates makes nanofibers potential viable alternatives to beads or plates, especially since nanofibers can be massively produced, are stable for long-term storage, and can be recycled (Hwang et al., 2011b).

To reduce nonspecific protein binding, the nanofibers were washed more than 3 times with buffer solution at the end of each experimental step. After thrombin and TBA29 aptamers were bound to the nanofibers, fluorophore-labeled TBA15 aptamers were added to the aptamer–nanofiber complexes, forming an aptamer–nanofiber–aptamer sandwich. Due to the presence of separate binding sites, both thrombin binding aptamers (TBA29 and TBA15) bound to thrombin without any significant effects on their individual binding activities (Dong et al., 2008; Edwards and Baeumner, 2010; Huang and Zhu, 2009; Ikebukuro et al., 2005).

### 3.2. Dose-dependent detection of thrombin by the aptamers-on-nanofiber based sandwich assay

Dose-dependent sandwich assays with varying thrombin concentrations, ranging from 0 to 200 nM, were performed to demonstrate the binding of thrombin to the aptamer/nanofiber complexes. Fig. 2a shows dose–response fluorescence emission spectra with increasing thrombin concentrations resulting from TBA29 aptamer–thrombin–TBA15 aptamer sandwiches on nanofibers. In this assay, fluorescein-labeled TBA15 (F-TBA15) was the signaling probe. In accordance with typical aptasensor detection systems, the results in Fig. 2a demonstrate that fluorescence

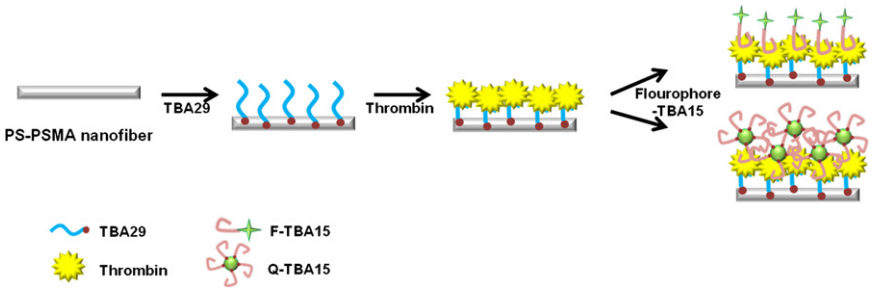


**Fig. 1.** Schematic diagram of aptamers-on-nanofiber based biosensors for thrombin detection: (a) aptamers (TBA29) were immobilized on alcohol dispersed electrospun polystyrene–poly(styrene-co-maleic anhydride) (PS–PSMA) nanofibers; (b) thrombin binds to TBA29 aptamers immobilized on nanofibers; (c) sandwich type thrombin detection using fluorescein-labeled or (d) quantum dot (QD)-labeled TBA15 for fluorescence signals.

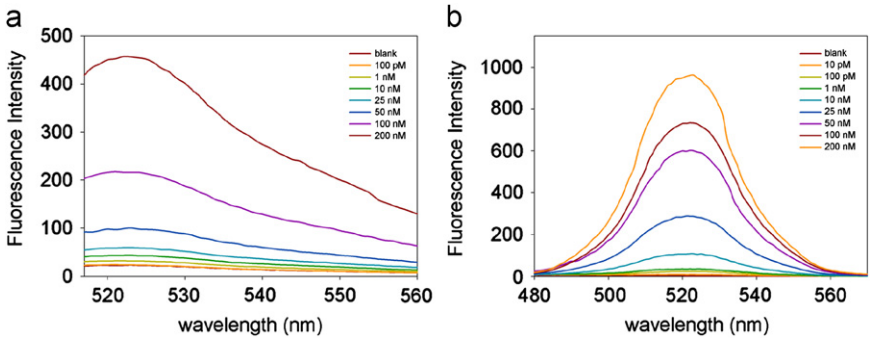


**Fig. 2.** Fluorescence intensities corresponding to different concentrations of thrombin. Dose-dependent thrombin detection by aptasensors using the aptamers-on-nanofiber biosensor system with the use of (a) fluorescein-labeled TBA15 (F-TBA15) and (b) quantum dot (QD)-labeled TBA15 (Q-TBA15) as the signaling probe. The blank was a buffer solution without added thrombin.

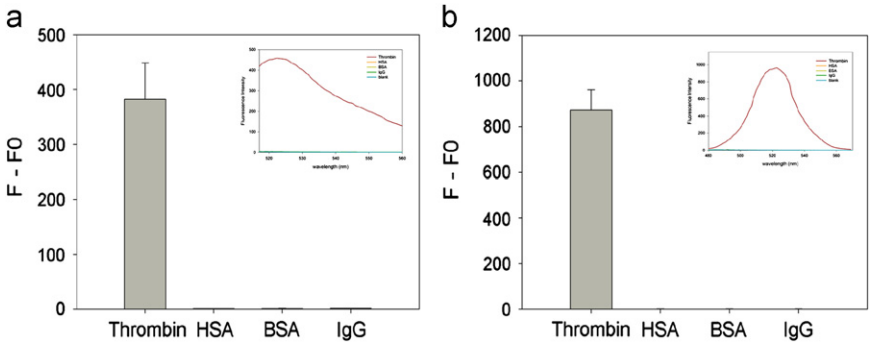


**Fig. 3.** Relative signal intensities (F − F0) obtained from binding assays using 200 nM thrombin and control proteins. Specific detection of thrombin using aptamers-on-nanofiber based aptasensors was confirmed with both (a) fluorescein-labeled TBA15 (F-TBA15) and (b) quantum dot (QD)-labeled TBA15 (Q-TBA15) as the signaling probe. The inset shows the corresponding fluorescence emission of each sample.

intensities increased in proportion to higher thrombin concentrations. From the fluorescence spectra, we determined that the detection limit for thrombin was 1 nM, with a dynamic range of 10–200 nM. These results demonstrate the feasibility of aptamers immobilized on nanofibers as biosensors. Moreover, these assays showed an average of 9.8%CV by calculation of means in all samples tested, demonstrating the reproducibility of the sandwich assays.

Even greater sensitivity of this aptamers-on-nanofiber biosensor was achieved (detection limit of 10 pM) by using QD-conjugated TBA15 (Q-TBA15; Fig. 2b). When Q-TBA15 was used, we observed a broad detection range from 100 pM to 50 nM. In addition, the detection sensitivity increased 100-fold compared to that of the fluorescein-labeled aptamer (F-TBA15). The average CV was calculated to be 11%, verifying the reproducibility. This improved sensitivity could be explained by the high quantum yield, unique optical properties, and excellent photostability of QDs (Michalet et al., 2005). A previous study demonstrated a dynamic range of 2.7–27 nM in an aptamer-based sandwich assay for thrombin detection using QDs and magnetic beads on microfluidic chips (Tennico et al., 2010). The analytical sensitivity and broad working range obtained from this aptasensor system using an aptamers-on-nanofiber biosensor were superior to those of methods employed in previous reports, in which QDs were used as fluorescence signaling molecules in sandwich type thrombin detection assays, and were also clinically meaningful (Zain et al., 2000).

### 3.3. Specificity test for the aptamers-on-nanofiber based sandwich assay

In order to confirm the specificity of the aptamers-on-nanofiber based thrombin binding assays, control experiments were carried out using proteins that are commonly present in serum, such as HSA, BSA, and human IgG. Fluorescence signals were obtained from thrombin-only samples in both F-TBA15 and Q-TBA15 sandwich type binding assays on nanofibers, with corresponding fluorescence intensities of $382.25 \pm 66.29$ (CV, 17%) and $869.52 \pm 89.20$ (CV, 10%), respectively, while control samples showed negligible fluorescence intensities ($-1.5$ to 1.36; CV, 9.6%), similar to blank samples (Fig. 3). These control experiments demonstrated the specific detection of thrombin by target-specific aptamers-on-nanofiber aptasensors and suggested that SA-coated nanofibers can significantly minimize the adsorption of other nonspecific proteins (Kim et al., 2011a).

### 3.4. Visualization of thrombin binding using aptamers–nanofiber complexes

The formation of TBA29–thrombin–TBA15 sandwiches was visualized with CLSM (Fig. 4). The nanofibers exhibited uniform fluorescence emissions along the entire length of the fiber due to the binding of QDs or fluorescein-labeled TBA15 aptamers to the nanofibers immobilized with TBA29 aptamer thrombin complexes. The fluorescence intensity of Q-TBA15-nanofiber complexes (Fig. 4a–c) was stronger than that of F-TBA15-nanofiber complexes (Fig. 4d–f), and fluorescent signals were consistent with the fluorescence emission spectra observed during the detection of thrombin using our novel aptamers-on-nanofibers biosensors (Fig. 2).

### 3.5. Enhanced sensitivity of the aptamers-on-nanofiber based sandwich assay system

The increased sensitivity of thrombin detection by our novel aptamers-on-nanofiber system using Q-TBA15 aptamers was

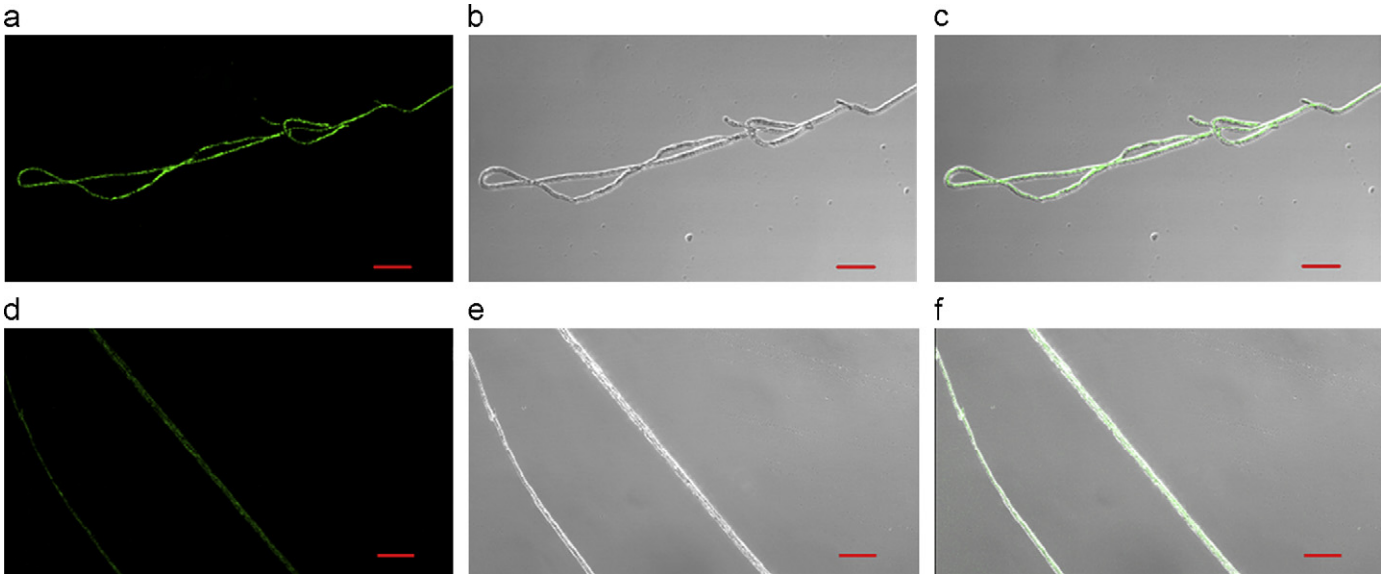


**Fig. 4.** Fluorescence composite images of thrombin binding assays using the aptamers-on-nanofiber based biosensor with (a–c) quantum dot (QD)-labeled aptamers and (d–f) fluorescein-labeled aptamers for fluorescence signaling. (b, e) Bright field images; (c, f) final composite of image of fluorescence and bright field nanofiber images. The thrombin concentration was 100 nM, and the scale bar was 20 μm.

confirmed by performing sandwich assays for thrombin detection in a conventional 96 microwell plate assay. Thrombin concentrations ranging from 0 to 200 nM were incubated with TBA29 aptamers immobilized on the microwells. F-TBA15/Q-TBA15 aptamers were added as described for conjugation with nanofibers (identical binding buffers, reaction times, washing buffers, and washing frequencies/times). The measured fluorescence signals increased with higher thrombin concentrations, similar to the results of binding assays performed on nanofibers. However, the fluorescence intensities of assays on the 96 microwell plate were markedly lower than assays performed using nanofibers. In terms of detection sensitivity, the limit of detection was 50 nM when F-TBA15 was used (CV, 6.9%). Even with Q-TBA15, signals were increased only 2-fold (detection limit, 25 nM; CV, 22%). Dose-dependent fluorescence intensities comparing thrombin detection by aptamers-on-nanofiber and 96 well plates are shown in Fig. 5. The *y*-axis represents the intensity of each sample minus the intensity of the blank (without thrombin).

These results suggested that the large surface area of nanofibers, allowing for immobilization of capturing probes, was more effective at target binding than the use of fluorophores. In addition, the PS–PSMA nanofiber can be easily produced through electrospinning methods and is cost-effective. Nanofiber composites are easy to handle compared with most other nanomaterials used in the biosensor field, which often include solution-based materials that require delicate handling.

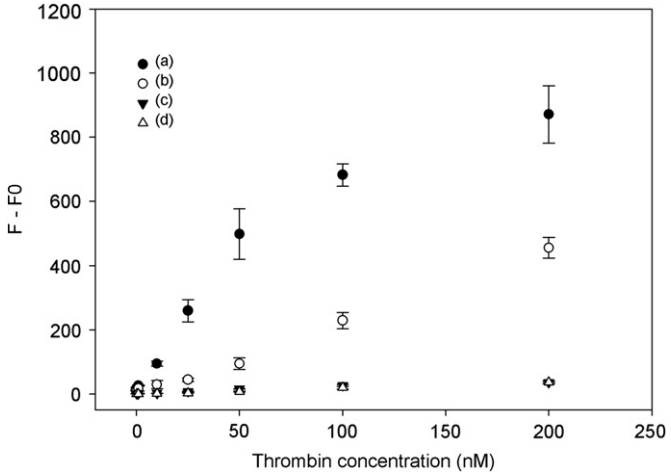


**Fig. 5.** Comparison of the relative signal intensities (F − F0) obtained in dose–response binding assays for aptamer/nanofiber complexes and commercial 96 well plates. Q-TBA15 and F-TBA15 were used for aptamer/nanofiber based assays (a and b, respectively) and 96-well plate assays (c and d, respectively). Dynamic ranges were observed only in binding assays using aptamer/nanofiber complexes (a: 0.1–50 nM, $R^2$ = 0.99; b: 10–200 nM, $R^2$ = 0.99), and the fluorescence intensities obtained from the 96 well plate assay were negligible compared to those obtained in (a) and (b).

### 3.6. *Analytical performance of aptamer/nanofiber based aptasensors in human serum samples*

The ability of the aptamers-on-nanofiber based aptasensors to capture thrombin in biologically complex matrices was evaluated using 10-fold diluted human serum spiked with exogenous thrombin. The human serum was diluted to reduce the strength of and potential interference from abundant proteins in the serum (Zhang et al., 2009). Fig. 6a shows the real fluorescence spectra following dose-dependent binding of thrombin (0–200 nM) to the aptamers-on-nanofiber complexes. In terms of sensitivity, the limit of detection was 10 pM, consistent with the addition of thrombin to nonserum buffers, indicating that the abundance of proteins in human serum did not disturb the detection of target proteins by aptamer–nanofiber complexes. The average CV was 13%, confirming the reproducibility of the analytical performance. However, this assay had a relatively narrow working range (0.1–25 nM) compared to detection of thrombin in buffer solution. Taken together, these results demonstrated that application of aptamers-on-nanofiber based sensor platforms could efficiently detect target proteins, even in complex serum samples.

## 4. Conclusions

The described aptamers-on-nanofibers sandwich biosensor system represents a novel aptasensor platform for protein detection. This biosensor system was found to be compatible with both fluorescein dye and QDs as fluorescence signal reporters. Among

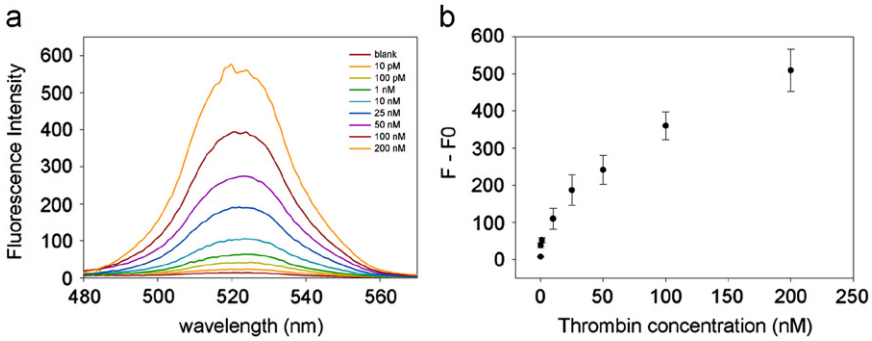


**Fig. 6.** Dose-dependent thrombin binding assays using human serum samples. (a) Fluorescence spectra obtained from concentration–response assays using aptamers-on-nanofiber biosensors with Q-TBA15 as the signaling probe and (b) relative fluorescence signal intensities (F − F0) of thrombin detection. The dynamic range was 0.1–25 nM ($R^2$ = 0.99).

the advantages of nanofibers, high loading of aptamers on a large surface area, easy manipulation compared to other nanomaterials, and the consistency are the main benefits of this sensor platform. High sensitivity for thrombin (up to 10 pM) in both buffer and diluted human serum samples could be obtained by using QDs with high fluorescence intensities. This reported nanofiber-based biosensor can be applied for the detection of various targets using dual aptamers or antibody–aptamer sandwich assays.

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