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Ultrasensitive protein detection using an aptamer-functionalized single polyaniline nanowire†

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A highly sensitive, conductometric and label-free biosensor for the detection of immunoglobulin E (IgE) is developed based on the immobilzation of the IgE aptamer onto a single polyaniline nanowire electrochemically synthesized in a facile and controllable way.

Protein-based biomarkers hold great promise as diagnostic markers indicative of disease states.[1–3] It is therefore of great clinical interest to develop biosensors for biomarker detection. Since biomarkers are often present at very low levels in a complex body fluid (blood, interstitial tissue fluid or saliva), the requirements on sensitivity and specificity are extremely high.[4] In the past years, biosensors based on one-dimensional nanomaterials such as nanowires and nanotubes have emerged as a powerful platform for the detection of proteins and other biomolecules.[5–10] Due to their huge surface-to-volume ratio, nanomaterials such as nanotubes, nanoparticles and nanowires are very sensitive to their environments, and their physical or electric properties can vary significantly with changes in electrostatic charges upon surface adsorption/binding of various molecules. With these advantages, nanodevices based on nanomaterials have been used as biosensors with extremely high sensitivity down to the detection range of picomolar[9,11,12] or even femtomolar.[10,13]

Recently, aptamers have been identified as excellent substitutes for antibodies in a number of applications, as they present many advantages compared to antibodies, such as higher stability, higher affinity, and reproducible chemical production.[14] It is thus expected that biosensors combining the advantages of aptamers and one-dimensional nanomaterials will achieve high sensitivity and specificity. While there are a few micro- and nanodevices for protein detection being developed through the immobilization of aptamers on one-dimensional nanomaterials, such as carbon nanotubes,[12,14] Si nanowires,[15,16] and conducting polymer nanotubes[13] and nanowires,[17] the fabrication methods employed for those nanomaterial-based devices are either less controllable[13,17] or require tedious post-processes and have low throughput.[12,14–16]

Here we report an ultrasensitive aptasensor based on a single polyaniline (PANI) nanowire for the detection of IgE, an allergic biomarker. The single PANI nanowire was electrochemically synthesized between two pre-existing Au electrodes through a nanochannel. This process allows easy and precise control over the dimension and location of the formed nanowire.[18] The PANI nanowire possesses many amine groups that can be used for functionalizing different biomolecules including proteins[19–21] and DNAs,[22,23] this makes them ideal for biosensor applications. This biosensor is highly sensitive and selective as it combines the unique properties of single PANI nanowires and aptamers. Furthermore, the PANI nanowire aptasensor achieves label free real time sensing, consumes a tiny amount of samples, and can be easily scaled up to multiplexed arrays, all of which are of great promise for the point-of-care detection of protein biomarkers.

Fig. 1 schematically illustrates a single nanowire fabrication and IgE sensing procedures of the aptasensor. For the direct growth of the single PANI nanowire, a polymethylmethacrylate (PMMA) nanochannel 100 nm wide, 100 nm deep and 5 μm (up to 10 μm) long was created using electron-beam (e-beam) lithography between pre-deposited Au electrodes on an Si/SiO_2 substrate.[24,25] When 0.3 μL solution containing 0.1 M HCl and 0.01 M aniline was dropped onto the nanochannel, the electrolyte solution spread and covered the whole

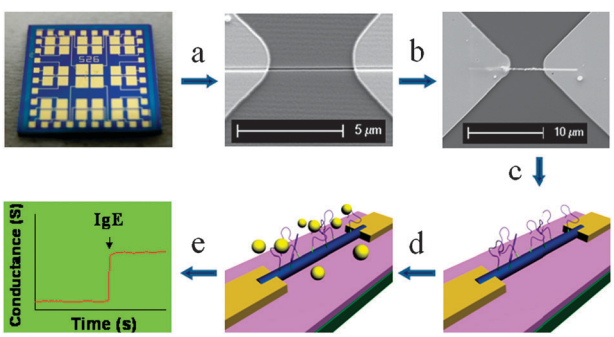

Fig. 1 Schematic representation of the single nanowire fabrication and illustration of the sensing procedure. (a) Magnifying a nanochannel between two microelectrodes; (b) growing a single polyaniline nanowire; (c) functionalizing the nanowire with aptamers; (d) binding of IgE to the immobilized aptamer; (e) generating a sensing signal.

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channel, and connected the two Au electrodes. The single PANI nanowire was electrochemically synthesized by applying a constant current of 500 nA, and the generated potential led to the electropolymerization of aniline in HCl. As a result, PANI grew from the cathode to the anode, through the PMMA nanochannel. When the PANI nanowire was fabricated completely between two Au electrodes (Fig. S1, ESI†), the applied current easily flowed through the nanowire, which resulted in a sharp decrease in the corresponding potential of below 1 mV.

Critical for the construction of the aptasensor is the immobilization of aptamers onto the PANI nanowire surface. The aptamer, a single strand DNA chain, needs to fold and form unique secondary and tertiary structures so as to bind to its target.[26,27] Therefore, the immobilization of the aptamer should not restrict its freedom of conformational change. Here, the aptamers are covalently attached through their 5′ ends to the PANI nanowire in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS). The phosphate groups in the 5′ ends of aptamers react with the amine groups on the surface of the PANI nanowire with the assistance of EDC and NHS,[22,23] thus covalent bonds will be formed between the aptamers and the PANI nanowire. Since the immobilized aptamers were only fixed by the very apex of their 5′ ends to the PANI nanowire, their folding is less hampered.

To verify the successful immobilization, aptamers labeled with a fluorophore 6-carboxyfluorescein (6-FAM) to their 3′ ends were immobilized on the PANI nanowire surface and characterized with a fluorescence microscope (Axioskop 2 MAT, Carl Zeiss). As shown in Fig. S2 (ESI†), green fluorescence resulting from the 6-FAM labeled aptamer is only observed in the positions where the PANI was electrodeposited, indicating that the 6-FAM labeled aptamers are exclusively attached to the PANI. Further tests were performed by soaking the PANI nanowire with 6-FAM labeled aptamer solution without EDC/NHS, and no green fluorescence was observed. This indicates that the observed green fluorescence was from the covalently attached 6-FAM labeled aptamers on the PANI nanowire surface, but not the physically adsorbed ones.

The biosensing performance of the aptamer functionalized PANI nanowire was tested by applying IgE and other non-specific proteins. To prevent the possible nonspecific adsorption, the aptamer-functionalized PANI nanowire was soaked in phosphate buffer saline (PBS, 10 mM, pH 7.4) containing 2 mg mL^{-1} BSA for 30 minutes to block the free sites on its surface. It is expected that the IgE will specifically bind to the immobilized aptamer and cause a conductance change of the aptamer-functionalized PANI nanowire. Fig. 2 clearly shows that the conductance of the aptamer-functionalized PANI nanowire is rapidly increased to a constant value upon addition of IgE at a concentration as low as 10 pg mL^{-1}. The addition of non-specific proteins such as BSA and IgG, which is similar to IgE structurally,[28] showed no significant change in conductance for the aptamer-functionalized PANI nanowire. In addition, no conductance change related to the addition of IgE to the non-modified PANI nanowire was observed. These observations confirmed that the change of conductance in the

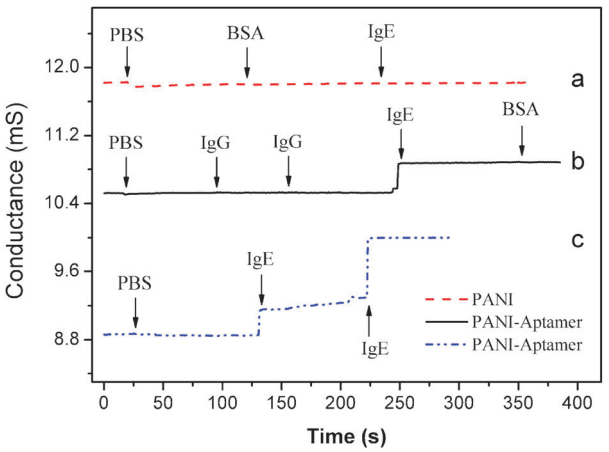

Fig. 2 Electrical responses of single PANI nanowires without (a) and with (b, c) aptamer upon the injection of different proteins at various concentrations. Different injections: (a) 50 ng mL^{-1} BSA, 670 pg mL^{-1} IgE; (b) 10 ng mL^{-1} IgG, 10 ng mL^{-1} IgG, 50 pg mL^{-1} IgE, 20 ng mL^{-1} BSA; (c) 10 pg mL^{-1} IgE, 66.7 pg mL^{-1} IgE.

nanowire aptasensor was due to the specific binding of IgE to the immobilized anti-IgE aptamer on the single PANI nanowire. The sensing mechanism of this PANI nanowire biosensor, where the nanowire is a p-type, may be explained by charge accumulation or depletion in the nanowire depending on the charge of targets. When negative charges (the IgE proteins are negatively charged in pH 7.4 PBS) are applied to a p-type nanowire, its conductance will increase (resistance decrease) with accumulation of the major carrier in the nanowire. In addition to its high specificity, another advantage of the aptasensor is the fast real-time response. As can be seen in Fig. 2, the aptasensor exhibited a real-time response towards the addition of IgE, and the response time was normally within 5 s.

It is also observed that the conductance change of the aptasensor upon the addition of IgE increased with the increasing IgE concentration over a broad range. Fig. 3 shows the conductance change ratio (change of conductance over

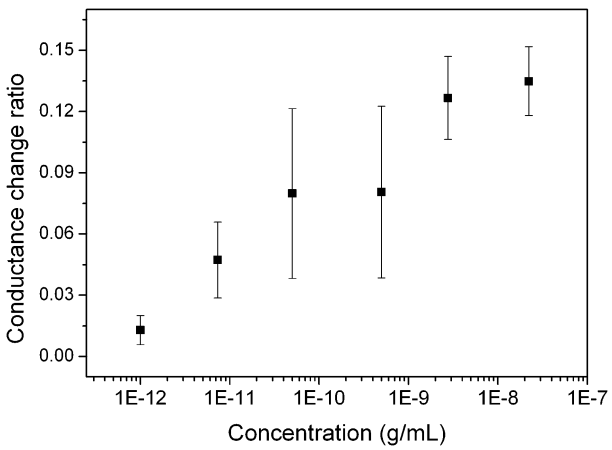

Fig. 3 The conductance change ratio of the aptasensor as a function of the corresponding IgE concentration. Each data point represents the average of three measurements, and the error bars represent the standard deviation.

original conductance) as a function of the IgE concentration. Due to the variation of some synthesized nanowires, the measured data exhibit large error bars. Nevertheless, a trend of conductance change increase over concentration can be observed. Future work will involve further optimizing and strictly controlling the synthesis conditions for more uniform nanowire production. As we can see, the aptasensor exhibits a wide dynamic sensing range from 1.0 pg mL^{-1} to 22.0 ng mL^{-1}. The detection limit of the IgE aptasensor was calculated to be 0.56 pg mL^{-1}, based on a signal-to-noise ratio of 3. This detection limit corresponds to 2.8 fM, considering the molecular weight of IgE being about 200 000, it is much lower than that of the aptasensor based on carbon nanotube field-effect transistors (250 pM).[12]

This ultrahigh sensitivity should be ascribed to the combination of unique properties of the aptamer and single PANI nanowire. The electrical property of the single PANI nanowire is very sensitive to its local environment. The changes of charge on the PANI nanowire surface will greatly affect its conductance. As the aptamer is very tiny in size compared to antibodies, the single PANI nanowire with a diameter of about 100 nm can be functionalized with aptamers at a high density, offering many binding sites for IgE. Meanwhile, the IgE aptamer has a very high affinity to its target IgE with a dissociation constant of about 10 nM.[29] As a result, the aptamer-functionalized PANI nanowire surface has a high efficiency in binding IgE. Considering the strong negative charge (the isoelectric point of IgE is 5.2–5.8) and the considerably big size (about 10 nm)[12] of IgE, it is expected that significant changes in the local environment of the single PANI nanowire can be induced due to the binding of IgE even at a very low level. In principle, a single nanowire-based sensor is sensitive enough to detect single molecules.[30,31]

Another advantage of the aptasensor is the reusability. Many aptamer–IgE conjugates will be formed after sensing due to the binding of IgE to the immobilized aptamer, which will block subsequent binding for further detection. In order to regenerate the used aptasensors, they were soaked in 0.1 M HCl to dissociate the conjugates and release the bound IgE from the aptamers. After a thorough rinse with PBS, the regenerated aptasensors were used to detect IgE again. Among the six independent aptasensors, four could be used repeatedly four times without a significant trend of losing sensitivity. Two were reused three times followed by nanowire breakage due to repeated soaking and washes. Therefore, the aptasensor shows reusability. If integrated with microfluidic systems where the washing process is more gentle and controllable, the reusability of the aptasensor can be further improved.

In conclusion, we described a nanosensor for label-free and real-time detection of protein biomarkers based on the aptamer functionalized single PANI nanowire. The combination of aptamer properties (small size, high affinity and stability) and single PANI nanowire attributes (simple and controllable synthesis and functionalization, ultrasensitivity) yield an aptasensor that has excellent specificity and ultrasensitivity. The detection limit of the aptasensor for IgE can reach a few fM, which surpasses the requirements for the detection of most protein biomarkers. Due to the excellent stability and reversible conformational change of the aptamers, these aptasensors can be repeatedly regenerated and reused. This highly reproducible nanosensor design shows great promise for the creation of reusable and sensitive microdevices for the point-of-care detection of protein biomarkers.

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