NANOPARTICLE CRYSTAL BASED NANOFLUIDIC BIOSENSOR AND ITS SIGNAL ENHANCEMENT

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ABSTRACT

Herein we reported an approach utilizing nanoparticle crystal (NPC) as an electrical read-out biosensor to detect the human α-thrombin. We first modified the surface of the 540nm silica nanoparticles with thrombin binding aptamer (TBA1) and the minimum concentration that could be detected was around 5nM. We then used another thrombin binding aptamer (TBA2) to enhance the readout signal. The result indicated that the signal intensity was amplified by about three folds. Being easy to be surface modified, low cost and electrical read-out, the NPC can be a promising biosensing scheme in the micro total analysis system.

KEYWORD

Nanoparticle crystal, biosensor, aptamer, human α-thrombin, signal enhancement

INTRODUCTION

Nanofluidics has been acknowledged as a promising biosensing scheme because of its electrical readout ability and fluidic operation essence[1]. However, nanofluidic biosensors still face some serious challenges from difficulty in surface modification, complexity and costliness in manufacture. Recently, it has been found that NPC exhibited the typical electrokinetics of a single nanochannel, namely the conductance across the single nanochannel or the NPC is proportional to the surface charge density, while insensitive to the bulk ionic concentration, when the ionic concentration of the buffer is low enough[2]. Based on this phenomenon, NPC could be a good alternative of traditional nanofluidic biosensors because of its easy surface modification, simple and cheap fabrication.

Aptamers are synthetic oligonucleotides which can bind a certain target. Compared with antibodies, aptamers have the same or even better high binding affinity and selectivity for their target molecules. What's more, aptamers have several other advantages over antibodies, such as chemical synthesis in vitro, easy to be stored and cost-effective. Two different human α-thrombin binding aptamers (TBA1 and TBA2), which bind distinct epitopes of thrombin, have been extensively studied[3].

So herein we reported a NPC nanofluidic human α-thrombin sensor by packing 540 nm silica particles with surface modified by TBA1 in a prefabricated micropore structure. And we also demonstrated a detection signal enhancement by using TBA2.

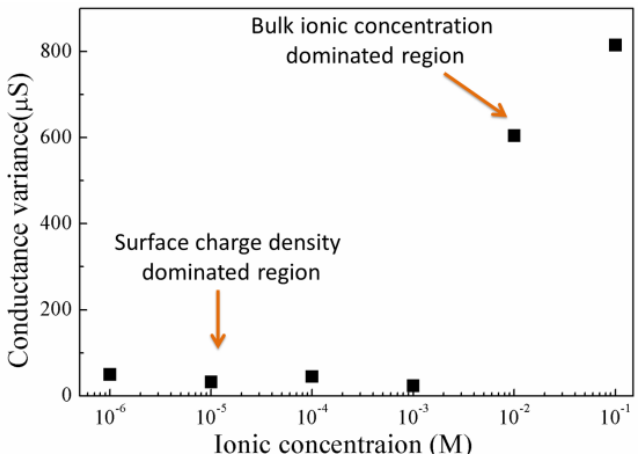

Figure 1. Variation of conductance across the NPC with the buffer ionic concentrations varied from 1 μM to 0.1 M.

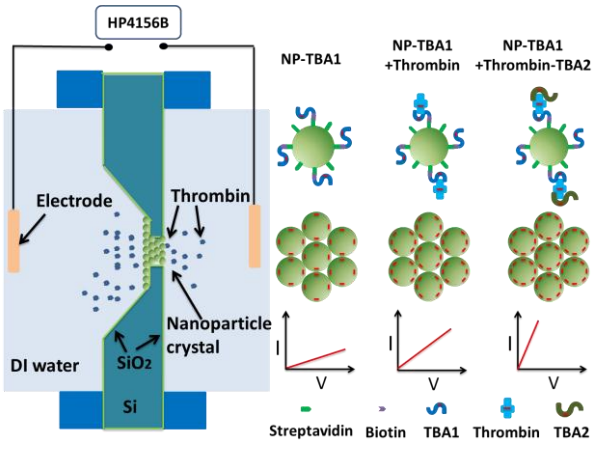

Figure 2. Schematic illustrations of detection and signal enhancement mechanism.

EXPERIMENTAL PRINCIPLE

For a given straight nanochannel, if the diameter is larger than the Debye length, the conductance across the nanochannel will be proportional to the bulk ionic concentration. On the contrary, if the feature size of this nanochannel is equal to or smaller than the Debye length, then the electric double layers will overlap inside the channel and the ionic conductance will be proportional to the surface charge density while not insensitive to the bulk ionic concentration. Similarly, the close packed NPC also enjoys this electrokinetics, as shown in Fig 1. From this

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figure, we can see that the conductance across the NPC has changed little when the ionic concentration is low where the Debye length is larger than the interstices inside the NPC. This lays the foundation for the NPC to be used for biosensing. In this scenario, as shown in Fig 2, the silica nanoparticle used in this experiment was originally modified with streptavidin. Then the nanoparticle could easily bind the TBA1 with biotin modification at its 5'-ternimal. This TBA1-binding particle then was packed inside a micropore to form the NPC. In thrombin solution, when the TBA1 inside the NPC captured the thrombin, which was negative charged under this condition, the surface charge density of the nanoparticle would increase correspondingly, so as the conductance across the NPC. The readout signal intensity varied with the different concentrations of thrombin and then we could reverse deduced the concentration of thrombin based on the readout signal intensity.

In signal enhancement, the TBA2, which bind a distinct epitope of thrombin, was also negative charged. So the surface charge density of the nanoparticle would increase more as the TBA2 bind the thrombin captured by TBA1. Then the intensity of readout signal would be amplified.

EXPERIMENT

TBA1 with fluorescent molecule FAM modification at 3'-ternimal was first used to confirm that TBA1 could bind the silica nanoparticle through biotin-streptavidin reaction. The result indicated that the binding efficiency increased as the binding reaction time increased. (The result was not shown here) To optimize an assay to be as both quick and efficient as possible, a 1-h incubation time was used for the binding in the following experiment. To get the NPC, 10μl TBA1-binding nanoparticles in DI water (50 mg/ml) was loaded into a pre-fabricated micropore with edge length of 20μm and depth of 50μm (Fig. 3A). After being kept overnight, nanoparticles were assembled in the micropore to form the NPC (Fig. 3B). A home-made gadget was used for chip fixing during the electrical measurement (HP 4156B, Agilent), as shown in Fig. 3C.

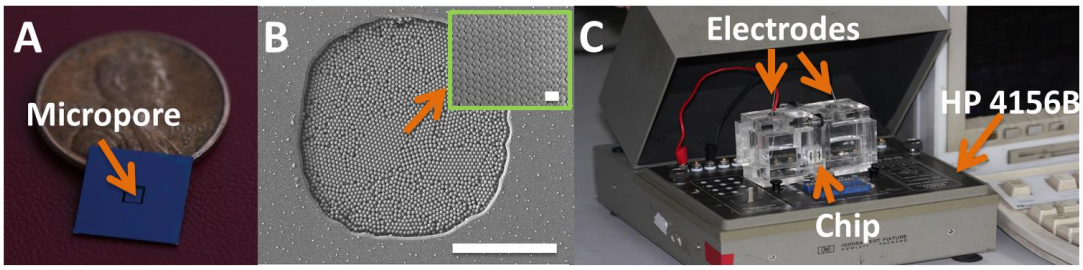

Figure 3. A) Chip with the micropore; B) SEM photo of NPC inside the micropore and the inserted photo showed detailed packed particles. The scale bars were respectively 10μm and 1μm; C) Photo of electrical measurement devices.

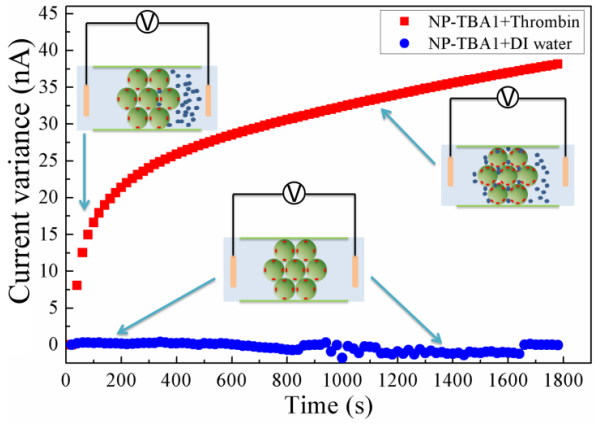

Figure 4.The electrophoresis current varied with the time in both thrombin solution and DI water.

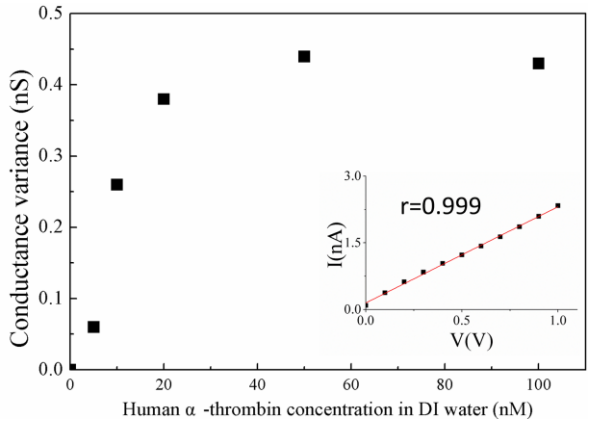

Figure 5. Variation of conductance with concentrations of thrombin dissolved in DI water varied from 0nM to 100nM. And the inserted photo showed the typical I-V curve.

After loading the thrombin in the reservoir, a 10 V-bias was first applied for 10min to electrically drive the thrombin molecules through the NPC for capturing. Variation of the electrophoresis current with the time was shown in Fig. 4. I-V curves across the NPC were then measured with bias varied from 0 V to 1 V. The preliminary experimental results, as exhibited in Fig. 5, indicated that the conductance increments could be used to trace back the thrombin concentration down to around 5nM.

Another thrombin binding aptamer, namely TBA2, was used to further enhance the surface charge variation, so as to enlarge the electrical readout signal intensity of the present biosensor. In this experiment, the target thrombin molecules were first incubated with TBA2 for 2 hours before being added into the reservoirs. Considering more

bases may provide more charges, TBA2 with sequences of 10 and 20 thymine bases (TBA2-10T, TBA2-20T) modifications at its 5'-terminal were also tested as signal-enhancement molecules. Interestingly, only TBA2 worked as expected and amplified the readout signal intensity considerably, as shown in Fig. 5, say in 50nM thrombin solution, the signal intensity was amplified more than 3 times. The reason why TBA2-10T or TBA2-20T didn't work here may be that the extra bases (10T and 20T) prevented TBA2 from forming the specific three-dimensional structure, which we know is the key for TBA2 to bind thrombin. That is to say that the extra modification of TBA2 has a negative influence on the binding between TBA2 and thrombin.

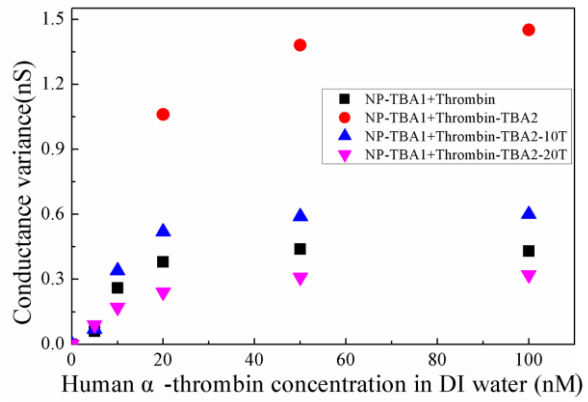

Figure 6. Conductance variance with the thrombin concentration in the signal enhancement experiment

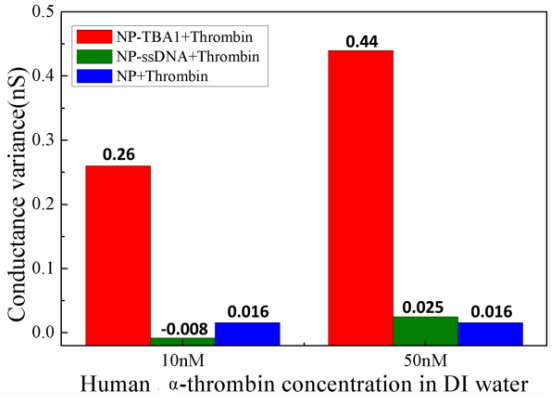

Figure 7. Conductance variance with the thrombin concentration in controlled experiment

Non-specific absorption of thrombin molecules on the nanoparticles could be neglected as neither random ssDNA probed nor naked NPCs had statistic responses to the thrombin concentration variation, shown in Fig. 7.

CONCLUSIONS

Briefly, this work reported a NPC-based nanofluidic biosensor for thrombin detection with sensitivity of 5 nM. A sandwich-type signal enhancement was demonstrated for the first time, which is believed useful for ultralow concentration sensing.

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