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A highly sensitive aptamer-immunoassay for vascular endothelial growth factor coupled with portable glucose meter and hybridization chain reaction

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ABSTRACT

In this paper, coupled with hybridization chain reaction and portable personal glucose meter, a highly sensitive aptamer-based immunoassay for vascular endothelial growth factor (VEGF) is fabricated. The antibody immobilized on the microplate captured the target protein, which would be sandwiched by the formation of aptamer/VEGF/antibody complex in the presence of aptamer. And then, an 18-nt elongated strand at the 3′ end of aptamer, with the assist of invertase-functionalized auxiliary probe, triggered the hybridization chain reaction to generate invertase-concatamers. Vast invertase catalyzed sucrose to glucose, which is monitored through personal glucose meter. Finally, this portable sensing system can achieve the detection of limit of 1.2 pg/mL with good stability and selectivity. Meanwhile, VEGF in the serum would also be assayed with satisfied result.

1. Introduction

Vascular endothelial growth factor (VEGF), a key regulator of angiogenesis, stimulates vascular endothelial cell growth, survival, and proliferation [1,2]. Meanwhile, VEGF is a signaling protein, which has been used as a serum biomarker for various diseases, including cancer [3,4], rheumatoid arthritis [5], psoriasis [6] and Parkinson's disease [7]. The rapid growth and metastasis the tumors need independent blood supplies for oxygen and nutrients, resulting in the overexpression of VEGF [8,9]. VEGF in human blood averages from <100 pg/mL among healthy individuals to 434 pg/mL among cancer patients [10,11]. Even in different clinical stages, VEGF levels are different (198 pg/mL in stage I–II and 955 pg/mL in stage III–IV) [12]. Therefore, the development of highly sensitive and selective assay method for VEGF play an important role in disease diagnosis and subsequent therapy monitoring. Immunoassay techniques, including enzyme-linked immunosorbent assays (ELISAs) [13] and immunohistochemistry [14], are most common analytical methods for VEGF. In these classical immunoassays, the large size and complexity of antibody usually influence the modification process and limit bind kinetics.

Alternatively, aptamers are artificial oligonucleotides isolated by systematic evolution of ligands by exponential enrichment (SELEX). Owing to smaller in size and complexity, easy to manufacture, stable during storage, and high selectivity and affinity [15,16], aptamers have been applied as the succedaneum of antibody for the aptamer-based immunoassay. Considering the well-designed of the aptamer owns high affinity to the heparin-binding domain of VEGF [17,18], various amplification methods have been introduced to develop highly sensitive aptamer-based immunosensor. Recently, nucleic acid amplification strategies have attracted more attention for the immunosensor. For example, immuno-polymerase chain reaction (IPCR) is an immunoassay with classic PCR process [19], resulting in the amplification of about 100 ~ 10000 times compared with conventional ELISA. In addition, rolling circle amplification (RCA), an isothermal nucleic acid amplification technology [20], has also been applied to develop various immuno-RCA (IRCA) sensors [21–24]. Although they have excellent sensitivity, there are still several drawbacks. Firstly, because these

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nucleic acid amplifications require either strict thermal cycling or expensive enzyme, the whole process needs complex operations. In addition, large-scale and expensive instruments are essential for the data collection, which limits their application to the public and point-of-care test (POCT) [25,26]. Therefore, it is urgently desired to develop an affordable, sensitive, specific, user-friendly, fast, robust, equipment-free, and deliverable (ASSURED) immunoassay for the end users.

Different from the enzyme-assisted DNA amplifications, in recent decade, enzyme-free DNA self-assembly has paid more widespread attention. Among them, hybridization chain reaction (HCR) as a classic self-assembly technology has been firstly reported by Dirks and Pierce [27]. During HCR process, using a single-strand DNA (ssDNA) as the primer, linear double-strand DNA structures can be self-assembled by the free energy of base pair, which does not need complex operations and costly polymerase [28,29]. The generated DNA concatamers have been used as signal amplification for the detection of various targets [30–35]. Moreover, in order to meet the demand of the POCT or ASSURED, the portable personal glucose meter (PGM) is used as the transducer to obtain the detection signal, which has several features, including "pocket" size, low cost, and simple operation. In this research, coupled with HCR and the portable PGM, we design an IHCR method for the detection of VEGF with the detection limit of 1.2 pg/mL. Meanwhile, the sensor is applied to assay VEGF in the complex matrix, such as the serum. This assay method not only exhibits high sensitivity, but also does not need any large and complex instruments, which is suitable in POCT field. Therefore, it is envisioned that this highly sensitive assay method with the portable PGM could be used to assay more targets by choosing different aptamers.

2. Experimental section

2.1. Materials and reagents

Monoclonal human VEGF antibodies, high-binding polystyrene 96-well microtiter plates, and VEGF standards were purchased from Shanghai and shanghai Zhen Biotechnology Co., Ltd. All the synthetic oligonucleotides used in this study are purchased from Shanghai Sangon Biotechnology Co. (Shanghai, China). The sequences of oligonucleotides are as follows:

Anti-VEGF Aptamer [36]:

5′-TGTGGGGGTGGACGGGCCGGGTAGA *ACTAAAAGGGTCTGAGGG*-3′

Auxiliary probe 1 (A1):

5′-SH-TTTTTTT**ACTCCCCCAGGTGC** CCCTCAGACCCTTTTAGT-3′

Auxiliary probe 2 (A2):

5′-SH-TTTTTT**GCACCTGGGGGAGTA** *ACTAAAAGGGTCTGAGGG*-3′

Invertase from baker's yeast (S. cerevisiae), sulfosuccinimidylz-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), and Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) are bought from Sigma. Other chemicals employed are all of analytical grade. All the solutions are prepared with double-distilled water, which has been purified with a Milli-Q purification system. Buffer A for the functionalization of DNA contains 0.1 M NaCl, and 0.1 M sodium phosphate buffer solution (pH 7.3). Buffer B for the hybridization of DNA contained 10 mM Tris-HCl (pH 7.4) and 0.3 M NaCl.

2.2. Preparation of invertase-functionalized auxiliary probe

Invertase-conjugated DNA was prepared using sulfo-SMCC as the linkers according to the previous report [37]. Firstly, 2 μL of 1 mM TCEP was introduced into the solution containing 9 μL of 100 μM thiol-probe and 2 μL of 1 M sodium phosphate buffer at pH 5.5 in the dark at room temperature for 1 h to active the thiol groups. Next, 400 μL of 20 mg/mL invertase in Buffer A was mixed with 1 mg of sulfo-SMCC, and then placed on a shaker for 1 h at room temperature. The mixture was then centrifuged and the insoluble excess sulfo-SMCC was removed. The solution of sulfo-SMCC-activated invertase was mixed with the above solution of the deprotected probes to keep at room temperature for 48 h. The mixture was purified by Amicon–100 K for 3 times using Buffer A to remove unreacted thiol-DNA.

2.3. HCR-based immunoreaction

High-binding polystyrene 96-well microtiter plates were coated with 50 μL of monoclonal VEGF antibodies at the concentration of 10 μg/mL overnight at 4 °C. On the following day, the microwells were incubated with 300 μL bovine serum albumin (BSA) for 1 h at 37 °C with shaking. Next, 50 μL of VEGF standard in different concentrations were introduced into the microwells and incubated for 30 min at 37 °C. 50 μL of 1 μM anti-VEGF aptamer was added into the above plate for 1 h at 37 °C. For the HCR process, the above well was soaked in Buffer B containing 0.8 μM A1 and A2 for 1 h at 37 °C. Notice: during each reaction, the microwells should be covered with adhesive plastic plate sealing films to prevent evaporation; after the modification, the microwells should be rinsed with washing buffer to eliminate the physical adsorption.

2.4. The detection of VEGF using PGM

20 μL of 1 M sucrose (0.01 M PBS, pH = 7.0) was added into the above microplates and incubated for 15 min at room temperature. 10 μL of resulted solution was detected using the PGM as the transducer.

2.5. Assay VEGF by traditional ELISA

Different concentrations (50 μL) of VEGF were introduced into respective microwell covered by VEGF antibody and incubated for 1 h at 37 °C. Next, 50 μL of horseradish peroxidase (HRP)-labeled detecting antibody was added to each microwell and incubated at 37 °C for 1 h. TMB enzyme substrate (50 μL) was added to each well, and the plates were incubated at room temperature on a plate shaker. Color development was stopped by addition of 2 M sulfuric acid (50 μL). The optical density was read at 450 nm with a microplate reader within 15 min after stopping the reaction. Notice: during each reaction, the microwells should be covered with adhesive plastic plate sealing films to prevent evaporation; after the modification, the microwells should be rinsed with washing buffer to eliminate the physical adsorption.

3. Result and discussion

3.1. The working principle of this sensor

The sensing process of sensor consists of four steps: a) the recognition of target VEGF; c) the binding of aptamer; c) the initiation of HCR; d) the detection via the PGM, as shown in Fig. 1. In step a, target VEGF can attach onto the VEGF antibody-covered microwells by the immunoreaction. In step b, the well-designed anti-VEGF aptamer is introduced, which contains an 18-nt elongated strand for HCR and a 25-nt aptamer for VEGF. After binding with VEGF, a sandwich structure of aptamer/VEGF/antibody is constructed on the microwell. In step c, the self-assembly process of HCR is initiated. Two auxiliary probes (A1 and A2) with invertase at 5′ end are designed, both of which included two fragments: the italic fragment at the 3′ end of A1 is complementary with the italic one of A2; while the bold fragment at the 5′ end of A2 can hybridize with the bold one of A1.