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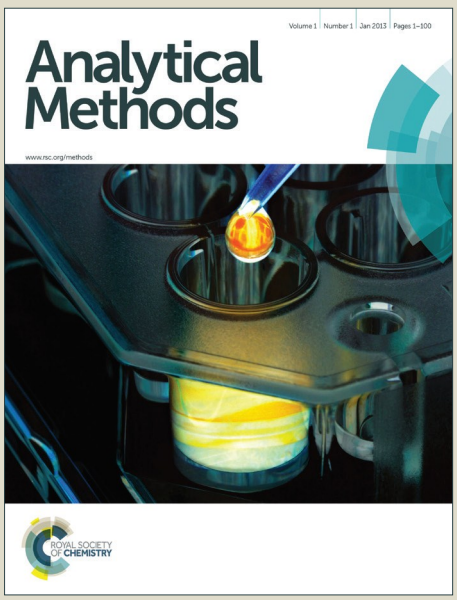

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ARTICLE

An aptamer-based chemiluminescent method for ultrasensitive detection of platelet-derived growth factor by a cascade amplification combining rolling circle amplification with hydroxylamine-enlarged gold nanoparticles

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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An ultrasensitive and selective chemiluminescent (CL) assay system for the detection of platelet-derived growth factor (PDGF)-BB was developed by taking advantage of the powerful signal amplification capability of rolling circle amplification (RCA) and hydroxylamine-amplified gold nanoparticles (Au NPs)-based CL technique. Rabbit anti-human PDGF-BB polyclonal antibody was covalently coupled on the surface of 96-well plate that offers reactive N-oxysuccinimide (NOS) group. In the presence of PDGF-BB, the aptamer-primer oligonucleotides were bound to the surface-captured antigen, resulting in an analyte-specific single-stranded DNA that could serve as the prime for RCA reaction. The captured aptamer-primer was extended through RCA reaction forming a single-stranded tandem repeated biotinylated copy of the circular template. After binding of the RCA product with streptavidin-gold (SA-Au NPs), the assembled Au NPs were enlarged by a NH_2OH-$HAuCl_4$ redox reaction allowing gold metal to be catalytically deposited onto the surface of the Au NPs. After an oxidative gold metal dissolution, a huge number of Au^{3+} ions were released from the enlarged Au NPs and were determined by a simple and sensitive luminol CL reaction. With two successive amplification steps, the proposed CL assay system exhibits not only high sensitivity with the detection limit of PDGF-BB as low as 0.06 pM (corresponding to 3 amol in 50 µL of sample solution), but also high specificity by taking advantage of using aptamer as recognition element. In addition, PDGF-BB has been determined in diluted serum indicating the applicability of this assay.

1. Introduction

Platelet-derived growth factor (PDGF), which is stored in the platelet α-granules and released during the platelet release reaction, is a growth factor protein found in human platelets more than two decades ago.[1] PDGF has at least three isoforms: PDGF-AB, PDGF-AA, and PDGF-BB.[2] Among these forms, PDGF-BB has been found to be frequently over expressed in cancers and acts as a new potent lymphangiogenic factor.[3] For example, high levels of PDGF-BB expression have been found in breast cancer tissues.[4] Lots of findings demonstrate that PDGF-BB plays a critical role in stimulating tumor lymphangiogenesis and lymphatic metastasis.[5] Hence, the sensitive detection of PDGF-BB is of great importance in clinical diagnosis.

In previous, traditional antibody-based radioisotopic methods and enzyme-linked immuno sorbent assay (ELISA) techniques have been developed for the detection of PDGF-BB.[6,7] Nucleic-acid aptamers, which are artificial oligonucleotides selected by systematic evolution of ligands by exponential enrichment (SELEX) technique have specific binding ability to certain targets ranging from small molecules, proteins to whole cells.[8,9] Comparing with traditional antibody, aptamers have salient features such as ease of synthesis, long shelf life, high level of both specificity and affinity, and wide applicability.[10] In consequence, aptamer instead of antibody has been widely employed as recognition element in constructing biosensor for PDGF-BB based on fluorescence,[11] electrochemistry,[12] and colorimetric technology[13] etc. However, these methods suffered from intermediate sensitivity.

RCA, an isothermal amplification method, has been commonly used to increase sensitivity in DNA quantitation and array-based sandwich immunoassays.[14] In a typical RCA process, the circular templates are synthesized from a padlock probe in which the 5'- and 3'- termini hybridize with primer and are ligated by a DNA ligase. The resulting circular templates are copied into linear concatamers by DNA polymerases under isothermal conditions generating thousands of tandem repetitive single stranded DNA strands that are complementary to the circular template. Lately, several RAC-based approaches have been developed for sensitive determination of PDGF-BB.[15-17] For example, Li group[18] reported a colorimetric assay system for PDGF-BB detection employing DNA aptamer coupled to RCA. Moreover,

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Savran group[19] performed a control experiment to verify that RCA assisted with the sensitivity of PDGF-BB detection. Their results showed that 1 nM PDGF-BB did not produce noticeable signal without RCA, while 10 pM PDGF-BB could be quantitively detected after RAC reaction. The results confirmed that RCA reaction plays an important role in signal amplification. However, we notice that RCA-based assay system for PDGF-BB using chemiluminescence (CL) technique has not been reported yet.

In previous, our group found that $HAuCl_4$-NH_2OH redox reaction could be used as powerful amplification strategy for constructing sensitive CL biosensors. For example, the hydroxylamine-amplified gold nanoparticles-based CL method for the detection of sequence-specific DNA showed 1000-fold of improvement in sensitivity than that without hydroxylamine amplification.[20] Hence, in the present study, a cascade amplification strategy for ultrasensitive CL detection of PDGF-BB was developed based on RCA and hydroxylamine-amplified gold nanoparticles. The strategy takes advantage of using aptamer as recognition element, and combining inherent signal amplification of RCA reaction with $HAuCl_4$-NH_2OH redox reaction–based catalytic enlargement of assembled Au NPs. The enlarged Au NPs were then dissolved by an acid dissolution and the released gold ions were detected by a sensitive luminol CL reaction. The proposed CL detection system exhibits extraordinary sensitivity and high specificity. The optimization and attractive performance characteristics of the new CL detection of PDGF-BB are reported in the following sections.

2. Experimental

2.1 Materials and chemicals

All chemicals were of analytical reagent grade and were used as received. Distilled water (18.2 MΩ cm^{-1}) was used throughout the work. Rabbit anti-human PDGF-BB polyclonal antibody was purchased from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Recombinant human PDGF-BB, PDGF-AA, and PDGF-AB were obtained from Peprotech Inc. (New Jersey, USA). T4 DNA ligase, phi29 DNA polymerase, dATP, dCTP, dGTP, biotin-11-dUTP were obtained from Thermo Fisher Scientific (China) Co., Ltd. Exonuclease I and Exonuclease III were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Bovine serum albumin (BSA), cytochrome C, dithiothreitol (DTT), and human serum were obtained from Puboxin Biotechnology Co., Ltd. (Beijing, China). Hemoglobin, calmodulin were purchased from Sigma-Aldrich (St. Louis, MO). Hydroxylamine hydrochloride (NH_2OH·HCl), hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4$·$4H_2O$), Tween 20 and other chemical reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Citrate-stabilized Au NPs (15 nm diameter) were prepared according to the methods described in the literature.[21,22] Streptavidin-modified gold nanoparticles (SA-Au NPs) were prepared according to our previous literature.[20] ATP and all oligonucleotides were acquired from Sangon Biological Engineering Technology & Service Co., Ltd. (Shanghai, China). A 96-well plate coated with a layer of reactive N-oxysuccinimide esters, referred to as the NOS group surface, was purchased from Corning Inc. (New York, United States). All oligonucleotides were purified by high-performance liquid chromatography (HPLC) and are listed in Table 1.

Table 1 Oligonucleotides used in the present study[a]

Name	Sequence
Primer Probe	5′-TGTCCGTGCTAGAAGGAAACAGTTACCA-3′
Aptamer-primer	5′-TACTCAGGGCACTGCAAGCAATTGTGGTCCCAATG GGCTGAGTATTTTTTTTTGTCCGTGCTAGAAGGAAACA GTTACCA-3′
Padlock Probe	5′-PO4-*TAGCACGGAC*ATATATGATGGTACCGCAGTA TGAGTATCTCCTATCACTACTAAGTGGAAGAAA*TGGT AACTGTTTCCTTC*-3′

[a]The underlined sequences in aptamer-primer can hybridize with primer probe and italic part of the padlock probe, respectively.

2.2 Apparatus

CL measurements were performed with a BPCL chemiluminescence analyzer (Beijing, China). The Au NPs were characterized using ultraviolet visible spectrophotometry (Agilent, China) and Zetasizer Nano ZS system (Malvern Instruments Ltd., Worcestershire, UK).

2.3 Preparation of Au NPs

An aqueous solution of HAuCl4 (50 mL, 0.01% [w/v]) was brought to the boil with stirring, and then 1% trisodium citrate solution (2.5 mL) was added rapidly, which resulted in a change of solution color from pale yellow to wine red (15 nm). Upon color change, the solution was refluxed for an additional 15 min, and then cooled to room temperature. The colloidal gold suspensions were stored in amber glass vessels at 4 °C until needed.

2.4 Preparation of SA-Au NPs

Streptavidin (10% more than the minimum amount, which was determined using a flocculation test) was added to a 1 mL Au NPs suspension (pH 7.0, adjusted by 0.1 M K_2CO_3) followed by incubation at room temperature for 30 min. The conjugates were centrifuged at 4 °C (25 000g, 60 min). After carefully removing the supernatant, the red soft sediment was resuspended in 1 mL of BA buffer (20 mM Tris-HCl and 0.5 M NaCl, pH 8.0). The addition of BSA with a final concentration of 1% allowed storage of SA-Au NPs at 4 °C for several days.

2.5 Preparation of circular template

Circular template was prepared according to the methods described in literature.[23,24] Briefly, 20 μL of ligation reaction solution consisting of 1×T4 ligase buffer (40 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP, pH 7.8), 0.5 μM padlock probe, and 1.5 μM primer probe was incubated at 37 °C for 30 min. 1 U T4 DNA ligase was then added, and the reaction mixture was incubated at 16 °C overnight. After ligation, 2 U Exonuclease I and 20 U Exonuclease III were added to the

mixture, and the mixture was incubated at 37 °C for 30 min. Finally, the reaction mixture was inactivated at 90 °C for 10 min and purified by ultrafiltration device with molecular weight cut off 10 000. The resulting product was stored at -20 °C with an estimated concentration of 1 μM.

2.6 Assay procedure of CL detection of PDGF-BB

In a typical experiment, rabbit anti-human PDGF-BB polyclonal antibody was diluted to 5 ng μL^{-1} using coupling buffer (0.05 M Na_2HPO_4-NaH_2PO_4, pH 8.5) and the diluted antibody was allocated into the wells of 96-well plate (50 μL per well) which offers reactive NOS group at plate surface. Following incubation for 60 min with gentle shaking at 37 °C, the wells were rinsed with washing buffer (7 mM Tris, 0.17 M NaCl and 0.05% Tween 20, pH 8.0) three times. After removing the solution, 200 μL of Tris (10 mM in coupling buffer) was added into each well of 96-well plate and incubated at 37 °C for another 60 min to block the remaining NOS group, and then the wells were rinsed with washing buffer three times. After discarding the solution, 50 μL of various concentrations of PDGF-BB antigen in reaction buffer (20 mM Tris, 0.14 M NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 2 mM $MgCl_2$, pH 7.0) or diluted human serum was applied to the antibody-modified plate surfaces, and incubated at 37 °C for 60 min. After washing with PBSM buffer (0.01 M phosphate-buffered saline, 0.14 M NaCl, 3 mM KCl and 2 mM $MgCl_2$, pH 7.4), 50 μL of 30 nM aptamer-primer in PBSM buffer was applied to the wells and incubated at 37 °C for 30 min. (Immediately before use, the aptamer-primer solution was heated to 95 °C for 5 min and chilled on ice.) After the wells were washed with 2×SSC solution (0.03 M sodium citrate, 0.3 M NaCl and 0.05% Tween 20, pH 7.0) twice, 50 μL of 16 nM circular template was added to the wells and incubated at 37 °C for 30 min. Then, the RCA reaction was performed at 37 °C for 45 min in 33 mM Tris-acetate buffer (pH 7.9) containing 10 mM $Mg(Ac)_2$, 66 mM KAc, 1 mM DTT, 1 mM dATP, dCTP and dGTP each, 1 μM biotin-11-dUTP, and 2 U phi29 DNA polymerase. The wells were then washed three times with washing buffer. 50 μL of SA-Au NPs (0.815 M in BA buffer containing 1% BSA) was added and incubated at 37 °C for 30 min. The wells were washed three times with washing buffer, and the Au NPs assembled on the surface of the 96-well plate were catalytically enlarged in the presence of 0.75 mM NH_2OH and 0.1 mM $HAuCl_4$ at room temperature for 12 min. Then, the wells were washed three times with the washing buffer. After carefully removing the rinsing solution, 100 μL of gold metal oxidative solution (the final concentration was 0.25 mM Br_2-5 mM HCL-1.25 mM NaCl) was pipetted into the wells and made to react for 10 min. The resultant mixture was placed in the water bath at 62.5 °C for 19 min. Finally, 90 μL of the solution was transferred into 14×40 mm glass tubes containing 100 μL of 10^{-5} M luminol (in 0.75 M NaOH) and the CL signal was then displayed in the CL analyzer (*n*=3).

3. Results and discussion

3.1 CL mechanism of luminol reaction

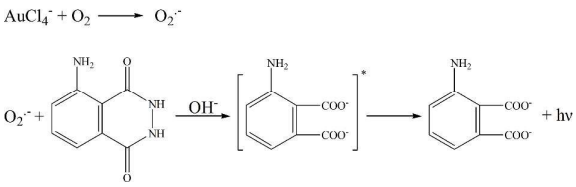
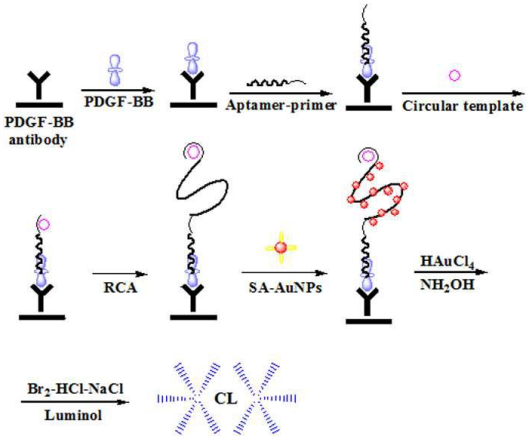


Fig. 1 CL mechanism of luminol reaction.

Br_2-NaCl-HCl was used as the dissolution solution of Au NPs. Br_2 can be hydrolyzed in dilute solution into bromhydric and hypobromic acids. Bromhydric and hypobromic acids serve as strong oxidative agents to transform Au into Au^{3+} ion. The chloride anion assists the gold dissolution in coordinating the generated Au^{3+} ion with formation of a strong complex $AuCl_4^-$. The formed $AuCl_4^-$ can catalyze the dissolved oxygen into $O_2^{\cdot-}$. Under alkaline conditions, the generated $O_2^{\cdot-}$ oxidates luminol to an excited state and when the excited state returns to the ground state, light emits (Fig. 1).

3.2 Scheme for detection of PDGF-BB

Scheme 1 depicts the assay protocol of the hydroxylamine-enlarged Au NPs-based CL immunosensor for PDGF-BB by taking advantage of aptamer and RCA technique. Rabbit anti-human PDGF-BB polyclonal antibody was covalently coupled on the surface of 96-well plate which offers reactive NOS group and then reacted with the analyte PDGF-BB in sample solution. The aptamer-primer oligonucleotide was subsequently bound to the surface-captured antigen resulting in an analyte-specific single-stranded DNA which could serve as prime for the RCA reaction. After hybridization with circular template, RCA reaction was initiated producing a single-stranded tandem repeated biotinylated copy of the circular probe. After binding SA-Au NPs on the biotinylated RCA product, the assembled Au NPs were enlarged in the presence of NH_2OH and $HAuCl_4$. Finally, the enlarged Au NPs were dissolved in an oxidative NaCl-HCl-Br_2 solution resulting in the release of gold ions into solution phase. The released gold ions were quantitatively measured by the gold ion-catalyzed luminol CL reaction. The CL signal is proportionally correlated to the concentration of PDGF-BB in the standard or sample.



Scheme 1 The schematic diagram of RCA-based method for CL detection of PDGF-BB.

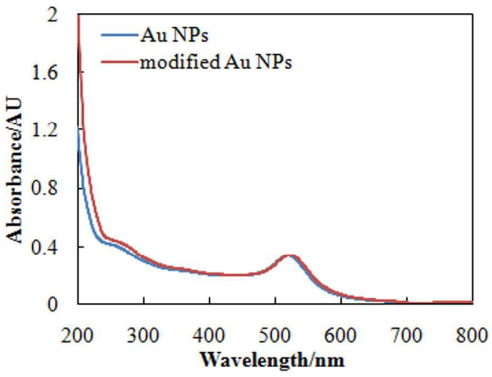


Fig. 2 The resonance absorption spectrum of Au NPs before and after modification.

3.3 Characterizations of Au NPs and SA-Au NP

Citrate-stabilized Au NPs was characterized using ultraviolet visible spectrophotometry and Zetasizer Nano ZS system. The resonance absorption spectrum of Au NPs was showed in Fig. 2. The characteristic absorption peak of the prepared AuNPs is 520 nm. And the particle size is 15nm by using Zetasizer Nano ZS system. The SA-Au NP was characterized using ultraviolet visible spectrophotometry (Fig. 2). The characteristic absorption peak of the streptavidin-modified AuNPs is 522 nm. The result indicated that the concentration of Au NPs after modification was the same as that before modification.

3.4 Optimization of assay conditions for the determination of PDGF-BB

Several parameters were investigated systematically in order to establish optimal conditions for the CL detection of PDGF-BB, including the hydroxylamine amplification conditions such as the concentration of NH_2OH and the time of reduction reaction (T_R), RCA conditions such as the time of RCA reaction and the amount of phi29 DNA polymerase, and other experimental condition such as the concentration of SA-Au NPs.

NH_2OH was employed as the reduction agent in the second signal amplification step where gold metal was catalytically deposited onto the surface of the assembled Au NPs. While NH_2OH is thermodynamically capable of reducing Au^{3+} ions to bulk metal, the reaction is dramatically accelerated by Au NPs. As a result, no new particle nucleation occurs in solution and all the added Au^{3+} ions go into the production of larger Au particles. The concentration of NH_2OH and T_R affected the quantity of gold atoms deposited on the surface of gold labels. Firstly, both sample signal and blank monotonously increased with the increase of the concentration of NH_2OH (Fig. 3A) indicating more gold atoms were deposited on the surface of Au NPs with higher concentration of NH_2OH. However, we found that when concentration of NH_2OH was above 0.75 mM, the blank signal increased dramatically with the increase of NH_2OH concentration. The high blank signal was not benefit to

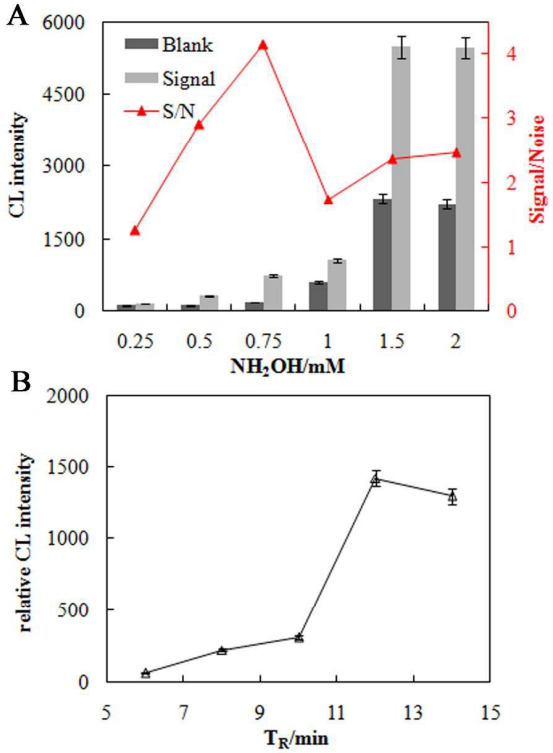


Fig. 3 (A) The effect of NH_2OH on the CL intensity of the PDGF-BB sensing system, experimental conditions: antibody, 0.5 μg; PDGF-BB, 60 pM; aptamer-primer, 1.5 pmol; circular template, 0.8 pmol; dATP/dCTP/dTTP, 1 mM each; biotin-dUTP, 1 μM; phi29 DNA polymerase, 2 U; RCA time, 30 min; SA-Au NPs, 0.582 nM; $HAuCl_4$, 0.1 mM; reduction time, 12 min. (B) The effect of reduction time on the relative CL intensity of the PDGF-BB sensing system, experimental conditions: NH_2OH, 0.75 mM; other experimental conditions were the same as (A).

improving detection sensitivity. As the concentration of NH_2OH increased, the signal/noise ratio increased rapidly from 0.25 to 0.75 mM, and then dicreased slightly. And the signal/noise ratio maintained almost the same when the concentration of NH_2OH was above 1.0 mM. Thus, 0.75 mM NH_2OH was selected for the following experiments. Secondly, the relative CL intensity (refers to sample signal minus blank) increased rapidly with the extension of reduction time in the range from 6 to 12 min, and then decreased slightly which is attributed to a significantly enhanced background (Fig. 3B). Considering a significant increased background would be obtained with T_R above 12 min, T_R was set at 12 min.

In principle, the more complementary copies of the circular template are produced, the stronger CL intensity will be obtained. Therefore, the effects of phi29 DNA polymerase amount and RCA reaction time on CL intensity were examined. First, the relative CL intensity increased with the extension of RCA reaction time in the range from 15 to 45 min (Fig. 4A) indicating more complementary copies of the circular template were produced. However, the relative CL intensity decreased when the RCA reaction time is above 45 min. We speculated that maybe the sandwich-type conjugates were not stable during the RCA process, and shorter reaction time may be

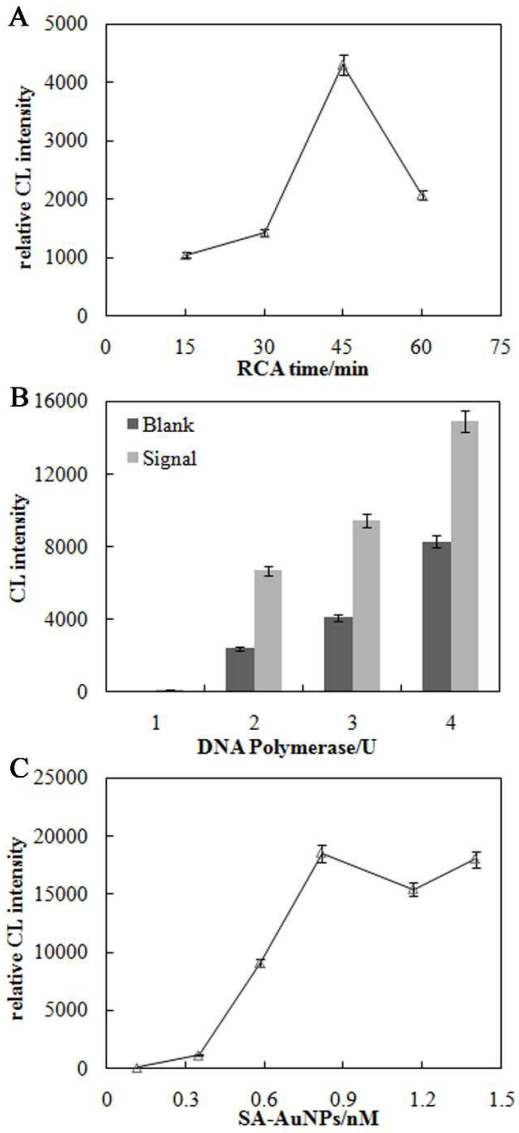


Fig. 4 (A) The effect of RCA time on the relative CL intensity of the PDGF-BB sensing system, experimental conditions: reduction time, 12 min; other experimental conditions were the same as Fig. 3B. (B) The effect of phi29 DNA polymerase amount on the CL intensity of the PDGF-BB sensing system, experimental conditions: RCA time, 45 min; other experimental conditions were the same as (A). (C) The effect of SA-Au NPs on the relative CL intensity of the PDGF-BB sensing system, experimental conditions: phi29 DNA polymerase, 2 U; antibody, 0.25 μg; other experimental conditions were the same as (B).

benefit to improving the sensitvity and reproducibility of PDGF-BB detection. Thus, the RCA reaction time was set at 45 min.

Secondly, an important factor for the success of this method is the unique nature of phi29 DNA polymerase: This single subunit, proof reading DNA polymerase, is able to incorporate >70 000 nt per binding event. It is very stable, with linear reaction kinetics at 30°C for over 12 h. Here both the blank and sample signal increased with the increase of phi29 DNA polymerase from 1 to 4 U (Fig. 4B). The blank signal increased dramatically when the amount of phi29 DNA polymerase is above 2 U. Hence, 2 U of phi29 DNA polymerase was selected for the following experiments.

To obtain a maximum response using a minimal concentration of SA-Au NPs, the concentration of SA-Au NPs was optimized by investigating the CL intensity of the CL biosensor with different concentrations of SA-Au NPs (Fig. 4C). The relative CL intensity increased with the increase of the concentration of SA-Au NPs from 0.116 to 0.815 nM and then leveled off between 0.815 and 1.399 nM. Thus, 0.815 nM SA-Au NPs was selected for the following experiments.

3.5 Assay performance of detecting PDGF-BB

According to the protocol described in Scheme 1, the quantitative behavior of the CL assay was assessed with different concentrations of PDGF-BB under the optimized conditions. Fig. 5 shows the CL intensity of the sensing system upon addition of increasing concentration of PDGF-BB. The CL intensity increased remarkably with the increasing concentration of PDGF-BB from 0.2 to 200 pM. A calibration graph in the concentration range of 0.2-200 pM showed a linear correlation (R^2 = 0.9947) between the concentration of PDGF-BB (pM) and the CL intensity, represented by $I = 193.39C + 1113.1$. Based on the 3σ rule, as low as 0.06 pM of target PDGF-BB could be sensitively detected using this method, which exhibited 1000 times enhancement of sensitivity over previous aptameric CL detection of PDGF-BB without RCA reaction.[25] A series of seven repetitive measurements with PDGF-BB (60 pM) was used for estimating the reproducibility of the proposed method, and yielded reproducible signals with a relative standard deviation of 4.01%.

Although the binding between aptamer and its target protein is of high specificity, the non-specific adsorption of other proteins coexisting in the complex sample may possibly cause an interfering signal. Hence, the detection selectivity of the aptamer-based CL system is an important criterion for its actual application. To evaluate the selectivity of the proposed aptamer-based CL system for PDGF-BB, control experiments were performed using some potential interfering proteins including BSA, cytochrome C, hemoglobin, calmodulin, ATP,

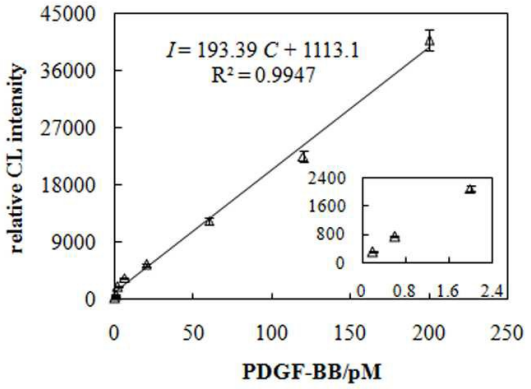


Fig. 5 Correlation between the results measured by the proposed method for PDGF-BB detection. It displays the linear relationship between relative CL intensity and PDGF-BB concentration in the range from 0.2 to 200 pM.

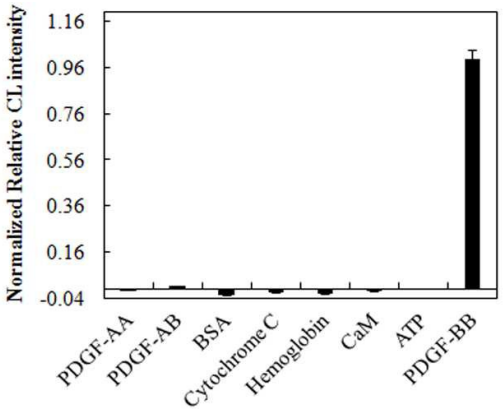


Fig. 6 Selectivity of the CL sensing system for PDGF-BB compared to other possible interfering targets. The concentration of PDGF-BB, PDGF-AA and PDGF-AB is 60 pM. The concentration of BSA, cytochrome C, hemoglobin, calmodulin and ATP is 6 nM. Other experimental conditions were the same as denoted in Fig. 5.

PDGF-AA, and PDGF-AB. As shown in Fig. 6, a high CL response was observed when 60 pM PDGF-BB was tested, whereas equal concentration of PDGF isoforms and 100-fold higher concentration of other interfering proteins including BSA, cytochrome C, hemoglobin, calmodulin and ATP showed negligible signals that were similar to blank signal. Compared to previous reference in which a PDGF-B aptamer was used alone for the determination of PDGF,[26] our method exhibits higher specificity to PDGF-BB. PDGF-AA and PDGF-AB will not interfere with the CL detection of PDGF-BB, which may be attributed to the combination of specific PDGF-B aptamer and anti-human PDGF-BB polyclonal antibody. The results suggest that the non-specific proteins do not interfere with the CL detection of PDGF-BB obviously. The excellent selectivity indicates that the proposed method holds promise for future success in determination of PDGF-BB in complex samples.

3.6 Analysis of PDGF-BB in serum sample

Finally, the applicability of the proposed CL system to real samples was verified by using a standard addition method. A series of samples were prepared by adding known concentration of PDGF-BB to 100-fold-diuted human serum. The CL responses were recorded under the optimized proposed RCA-based CL method for the clinical detection of PDGF-BB in serum samples. experimental conditions. The recoveries of PDGF-BB from serum samples spiked with 2, 20, 100 pM were calculated based on the calibration graph using PDGF-BB in reaction buffer at comparable levels (n=3). The results are shown in Table 2 that indicates the recoveries of CL detection of PDGF-BB ranging from 86.9% to 103.3%. The result demonstrated very little interference of complex sample matrices in the designed strategy indicating the practicality of using the the proposed RCA-based CL method for the clinical detection of PDGF-BB in serum samples.

Table 2 Recovery of PDGF-BB spiked into human serum samples

Amount added (pM)	Amount measured (pM)	Recovery (%)
2	1.738 ± 0.06	86.9 ± 2.8
20	20.66 ± 1.06	103.3 ± 5.3
100	100.2 ± 7.12	100.2 ± 7.1

4. Conclusions

A versatile and ultrasensitive aptamer-based sensing system for CL detection of PDGF-BB was constructed by taking advantage of the powerful signal amplification capability of RCA and hydroxylamine-enlarged Au NPs. The proposed CL method exhibited a broad linear range over 5 orders of magnitude and high sensitivity with a detection limit of 0.06 pM with RCA which was superior to most of the reported methods (Table 3). In addition, by combining the use of aptamer and polyclonal antibody the proposed method demonstrated extraordinary specificity towards PDGF-BB and could distinguish the target PDGF-BB from PDGF isoforms. Theproposed CL method was applied to detecting PDGF-BB in diluted human serum samples, and the recovery ranged from 86.9% to 103.3%. From the analytical chemistry point of view, the proposed method can be easily generalized for other target proteins by simply changing the corresponding aptamer. We believe the method demonstrated here holds great potential in becoming a powerful tool for protein detection due to the wide availability of aptamer for numerous proteins.

Table 3 Comparison of sensitivity for different PDGF-BB assay methods

Analytical method	Label	Detection limit	Linearity range	Method
CL (this study)	Au NPs	0.06 pM	0.06-200 pM	Aptamer-based; RCA
CL[27]	Label-free	0.68 pM	1.0-10000 pM	Cascade autocatalytic recycling
CL[28]	FITC	50 pM	0.1-100 nM	Aptamer-based
ECL[29]	Au NPs	0.017 pM	0.1-500 pM	Aptamer-based
Fluorescence[30]	Label-free	0.1 pM	0.5-10000 pM	Aptamer-based
Fluorescence[31]	dsDNA-Cu NPs	4 nM	0-50 nM	Aptamer-based
Fluorescence[32]	FAM	167 pM	167-1167 pM	Aptamer-based
Electrochemistry[33]	Ferrocene	0.41 pM	0.82-8.2 pM	Aptamer-based
Colorimetry[34]	Au NPs	1.1 nM	2.0-80 nM	Strand displacement amplification
SERS[35]	Au NPs	0.5 pM	1-50 pM	Aptamer-based
Flatbed scanner[36]	Ag NPs	64 pM	64-412 pM	Aptamer-based

Acknowledgements

This study was supported by National Natural Science Foundation of China (21105071), National Basic Research Program (973 program) of China "The fundamental and frontier studies of the new topology of molecular functional carbon materials" (nos. 2015CB856500), Tianjin Research Program of Application Foundation and Advanced Technology (15JCQNJC11200) and Collaborative Innovation Center of Chemical Science and Engineering (Tianjin).

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