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Modification-free amperometric biosensor for the detection of wild-type p53 protein based on the *in situ* formation of silver nanoparticle networks for signal amplification

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ABSTRACT

Sensitive and accurate quantification of wild-type p53 protein is of great importance for biological research and clinical diagnosis. Herein, a modification-free amperometric biosensor was proposed for sensitive detection of wild-type p53 protein by the signal amplification of silver nanoparticles (AgNPs) networks formed *in situ* on electrode surface. Double-stranded DNA (dsDNA) probe containing two consensus sites was immobilized on gold electrode surface to capture wild-type p53 protein. The cysteine thiol and amine groups on the exterior of the protein allowed for the attachment of bare AgNPs through the Ag—S or Ag—N interactions. Meanwhile, benzene-1,4-dithiol (BDT) molecules in solution triggered the assembly of more AgNPs on electrode surface through the Ag—S interactions, thus leading to the *in situ* formation of AgNPs networks for signal amplification. The target at the concentration as low as 0.1 pM can be readily determined. This method was further applied to determine wild-type p53 protein in spiked human serum and cell lysates with satisfactory results. Moreover, the biosensor is regenerative and does not require the modification of AgNPs with recognition element for signal readout. The modification-free strategy can potentially be applied to develop novel biosensors for detection of other biological macromolecules.

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1. Introduction

p53 protein, a tumor suppressor and transcription factor encoded by *TP53* gene, plays important roles in multiple central cellular processes (*e.g.* DNA repair, cell cycle control and apoptosis) [1]. Concentration of wild-type p53 protein in normal cells is usually ultralow [2,3]. Mutation or inactivation of p53 gene in more than 50% of human cancers can cause the decrease of wild-type p53 protein and the accumulation of mutated p53 protein [4]. Following the death and destruction of tumor cells, p53 protein is released from cancer cells and circulated in the blood of cancer patients. Serum levels of p53 can accurately indicate tissue alteration at the gene and/or protein level [5]. Therefore, it is of great significance to achieve sensitive detection of wild-type p53 protein at ultralow concentration in human serum for clinical research and early diagnosis of cancers.

The classic methods for detection of p53 protein, such as enzyme-linked immunosorbent assay (ELISA) [6], electrophoretic mobility shift assay [7], immunostaining [8] and immunohistochemistry [9], generally involve in semi-quantification, low sensitivity and tedious experiment steps. In order to meet the increasing demand for sensitive detection of p53 protein, researchers have focused considerable efforts to establish novel analytical methods, including electrochemistry [10–25], electrochemiluminescence [26], photoelectrochemistry [27], localized surface plasmon resonance (LSPR) [28], surface enhanced Raman scattering (SERS) [29,30] [31], and immunochromatographic test strip [3]. Among them, electrochemical biosensors have attracted considerable attention because of their intrinsic advantages of simple operation, high sensitivity, speed response and low cost. However, the biosensors mainly based on the specific antibody-antigen interaction cannot distinguish the wild-type p53 and mutant p53. Human p53 protein composing of 393 amino acid residues is functionally divided into three distinct domains: the transactivation domain at the N-terminus, the central DNA-binding domain, and the oligomerization domain near the C-terminus. It has been revealed that four wild-type p53 molecules can assembly into a tetramer in solution by their C-termini. The resultant p53 tetramer can bind to sequence-specific sites in the consensus double-stranded DNA (dsDNA) but the mutant p53 protein loses the binding ability [32]. Several groups have investigated the dsDNA/p53 interaction and developed a few biosensors with the consensus dsDNA as the acceptor for selective detection of wild-type p53 protein [33–43]. Herein, we attempt to develop a simple and sensitive electrochemical biosensor for specific detection of wild-type p53 protein using the consensus dsDNA receptor.

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To meet the urgent demand of ultrasensitive detection of biomarkers, electrochemical sensing strategies can be integrated with signal amplification to enhance the detection sensitivity. With the striking development of nanotechnology, various nanomaterials have been synthesized and used for designing of electrochemical biosensors with improving sensitivity, including noble metal nanoparticles (NPs), carbon nanostructures, metal-organic framework, metal oxide/sulfide NPs and other novel two dimensional (2D) nanomaterials [44–46]. The nanomaterials are usually employed as electrode modifiers to accelerate electron transfer and increase electrode surface area, as the carriers to load large numbers of enzyme labels or electroactive species, and/or as the reporters to amplify the electrochemical signal. For example, silver nanoparticles (AgNPs) modified with specific recognition element have been extensively utilized as the redox reporters to develop sensitive electrochemical biosensors for the analysis of cancer cells, DNA, microRNAs, proteins, drugs and metal ions because of their excellent electrochemical properties [47–57]. Wild-type p53 proteins in solution can form tetrameric oligomer. The tetramer can bind to dsDNA at the C-terminus with high binding affinity and each p53 monomer contains five surface accessible cysteine thiols on the exterior [38,58]. For this view, we developed an electrochemical biosensor for the detection of wild-type p53 protein based on the interaction of cysteine thiols and AgNPs. The signal was further amplified on the basis of benzene-1,4-dithiol (BDT)-induced *in situ* formation of AgNPs networks on electrode surface. The method has the advantages of high sensitivity and simplicity and does not require the modification of AgNPs nanolabels with recognition element for signal readout. Note that metal-free cysteine thiol and amine groups in most of proteins can attach onto the bare Au/Ag surface but dsDNA hybrids exhibit no interaction with bare Au/Ag nanoparticles [59]; the method may be extended to design novel biosensors by matching sequence-specific dsDNA acceptors.

2. Experimental

2.1. Chemicals and reagents

Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), BDT, 6-mercapto-1-hexanol (MCH), tris-(hydroxymethyl)aminomethane hydrochloride (Tris–HCl), IgG, thrombin and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (Shanghai, China). p53 ELISA kits were provided by SenBeiJia Biological Technology Co., Ltd. (Nanjing, China). HPLC-purified oligonucleotides were ordered from Sangon Biotechn. Co., Ltd. (Shanghai, China). Their sequences were listed as follows: the thiolated single-strand DNA (ssDNA): 5′-HS $(CH_2)_6$-TTT TTA GAC ATG CCC AGA CAT GCC C-3′ and the complementary DNA: 5′-GGG CAT GTC TGG GCA TGT CT-3′. Each p53 monomer binds to one-quarter site of the dsDNA with sequence of (A/T)(G/C)(A/T)(C/G)(A/T)(T/A)(G/C)(C/G)(C/G)(C/G). The DNA solution was prepared using TE buffer (20 mM Tris–HCl, 1 mM EDTA, pH 7.4). Prostate specific antigen (PSA) was provided by Linc-Bio Science Co. Ltd. (Shanghai, China). Serum sample was provided by Anyang Maternal and Child Health Care Hospital (Anyang, China). Silver nitrate ($AgNO_3$), ethylene diamine tetraacetic acid (EDTA), trisodium citrate and other reagents were obtained from Aladdin Reagent Company (Shanghai, China). All chemicals were of analytical grade and used without further purification. All solutions were prepared with ultrapure water (18.2 MΩ) obtained from a Milli-Q water purifying system (Milli-Q, Millipore).

2.2. Instruments

All electrochemical measurements were conducted on a CHI 660 electrochemical workstation (Shanghai CH instrument Inc., China). The conventional three-electrode system comprised the reference electrode (Ag/AgCl), the auxiliary electrode (platinum wire) and the work electrode. Scanning electron microscopy (SEM) images were taken with a SU8010 scanning electron microscope (Hitachi, Japan).

2.3. Preparation of citrate-capped AgNPs

Citrate-capped AgNPs was synthesized according to our previously reported procedure [56]. In brief, 1 mL of 10 mM $AgNO_3$ solution and 1 mL of 10 mM trisodium citrate solution were added to 36.8 mL of ultrapure water under vigorous stirring. This is followed by addition of 1.2 mL of 10 mM freshly prepared $NaBH_4$. The solution color turned to yellow, indicating the formation of AgNPs. After stirring for additional 10 min, the solution was stored at 4 °C for use.

2.4. Preparation of sensor electrode

Prior to modification, the gold electrode (AuE) with 2 mm diameter was polished with alumina suspensions down to 0.05 μm, followed by cleaning ultrasonically the electrode with ethanol and water. After that, the cleaned electrode was transferred into the TE buffer solution containing 50 μM thiolated ssDNA probe, 0.1 M NaCl and 100 μM TCEP for 12 h at room temperature. The probes were immobilized on the electrode surface through the Au—S interactions. Subsequently, the modified electrode was rinsed thoroughly with water and soaked in a 1 mM MCH solution for 30 min to block the unmodified region of electrode and remove non-specifically adsorbed ssDNA (Fig. S1). Before the assay, the probe-covered electrode was incubated with 10 μM complementary DNA for 2 h in the TE buffer solution. Finally, the consensus dsDNA modified electrode was rinsed with TE buffer and used for the capture of wild-type p53 protein.

2.5. p53 protein detection

10 μL of wild-type p53 protein at a given concentration in phosphate buffer (10 mM, pH 7.2) was casted onto the consensus dsDNA-modified electrode surface for 20-min incubation. Then, the electrode was washed thoroughly with the buffer and subsequently exposed to 25 μL of AgNPs suspension in a homemade plastic cell. After incubation for 5 min, 25 μL of BDT solution was added to the AgNPs suspension and incubated for 15 min again. After being rinsed with water, the electrode was placed in 1 M KCl solution for linear-sweep voltammetry (LSV). After each assay, the sensor electrode was regenerated with 10 mM NaOH.

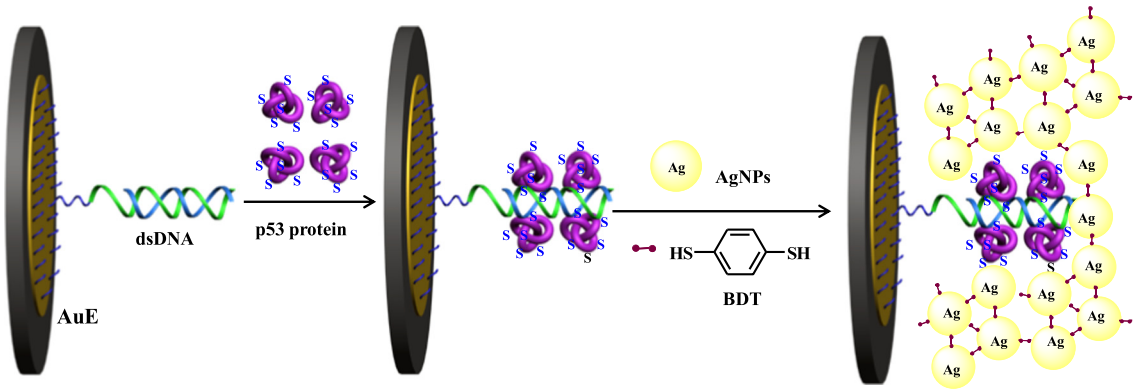
2.6. Analysis of cell lysates

The procedure for the preparation of cell lysates followed the previous report [38]. Briefly, MCF-7 and HeLa cancer cells and PC12 nerve cells were incubated in DMEM (Dulbecco's Modified Eagle Medium) for 24 h. The living cells were washed three times with ice-cold phosphate buffer and then lysed with lysis buffer. The lysed cells were collected and centrifuged at 4 °C for 10 min. The supernatants were diluted by 10-fold with phosphate buffer and then analyzed immediately.

3. Results and discussion

3.1. Detection principle of the method

The modification-free amperometric biosensor for detection of wild-type p53 protein was developed based on the high affinity of wild-type p53 protein toward consensus dsDNA. The detection principle is illustrated in Scheme 1. The mixed dsDNA/MCH self-assembled monolayers (SAMs) are performed on the surface of gold electrode as the bioreceptor to capture wild-type p53 protein and eliminate the non-specific adsorption. After the formation of the p53 protein/dsDNA complex, the five metal-free cysteine thiols and amine groups at the surface



Scheme 1. Schematic representation of the biosensor for detection of wild-type p53 protein by the *in situ* formation of AgNPs networks for signal amplification.

of wild-type p53 protein allow for the capture of AgNPs through the Ag—S or Ag—N interactions. The signal was then amplified by BDT-induced assembly of AgNPs *via* the formation of Au—S bonds. In brief, the surface-anchored AgNPs allow for the attachment of BDT molecules through the Ag—S interactions. Then, the anchored BDT molecules facilitate the capture of more AgNPs through the same Ag—S interactions. Thus, BDT and AgNPs in solution circularly recruit more dissociative AgNPs on the electrode surface, leading to the *in situ* formation of the AgNPs-based networks. Finally, the network architecture of AgNPs produced an amplified electrochemical signal by the solid-state Ag/AgCl process. Note that dsDNA cannot adsorp onto the bare Au/Ag surface [59]; the dsDNA/MCH-covered electrode will not capture AgNPs, thus decreasing the nonspecific adsorption of signal labels and improving the detection specificity.

3.2. Feasibility

Electrochemical impedance spectroscopy (EIS) is sensitive to small charge change at the solid-liquid interface. The stepwise fabrication process of sensor electrode was first characterized by EIS technique. The Nyquist curves corresponding to the modification of electrode surface at different steps were presented in Fig. 1A. The semicircle diameter in high-frequency region was equal to the electron-transfer resistance (R_{et}), displaying the electron transfer dynamics of $[Fe(CN)_6]^{3-/4-}$ on the electrode surface. The bare gold electrode showed a relatively small R_{et} (curve a). After immobilization of ssDNA onto the electrode surface, the R_{et} increased greatly (curve b), which is due to the repulsion between negatively charged ssDNA backbone and $[Fe(CN)_6]^{3-/4-}$. When the ssDNA-modified electrode was covered with MCH, a

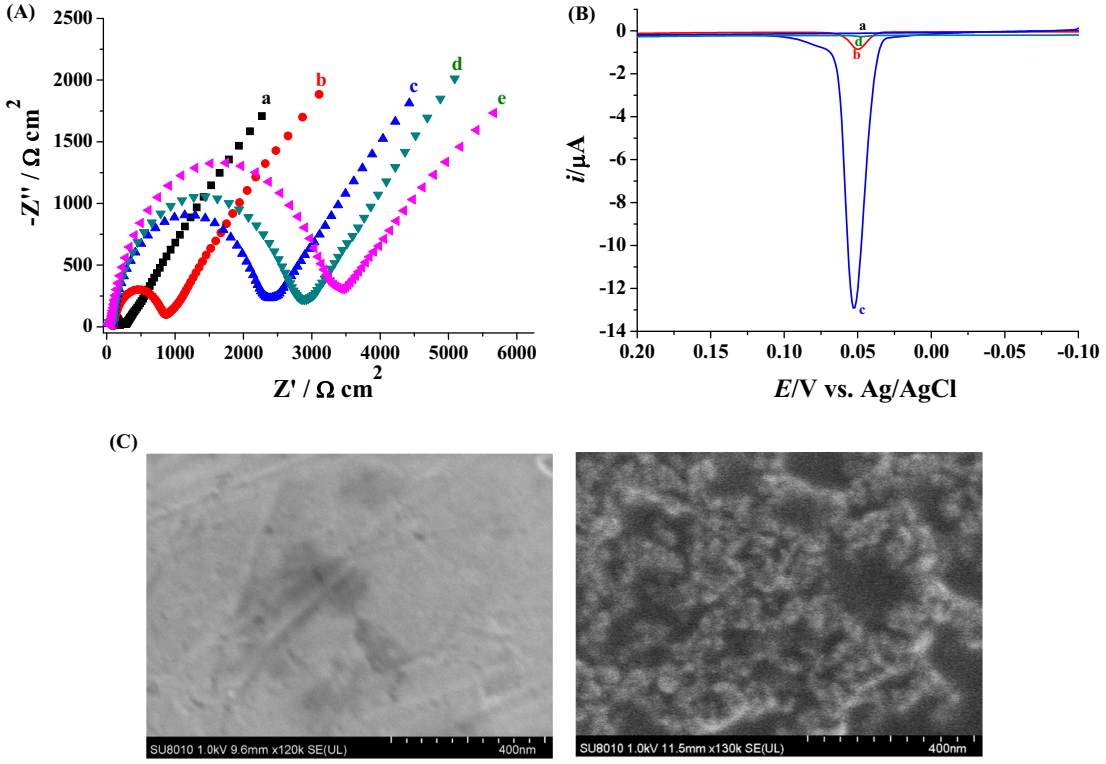


Fig. 1. (A) EIS of AuE (a), AuE/ssDNA (b), AuE/ssDNA/MCH (c), AuE/dsDNA/MCH (d) and AuE/dsDNA-p53/MCH (e). (B) LSV responses acquired at the sensor electrode after incubation with various solutions: AgNPs/BDT (a), wild-type p53 + AgNPs (b), and wild-type p53 + AgNPs/BDT (c). Curve d corresponds to that acquired at AuE/ssDNA/MCH after successive incubation with wild-type p53 protein and AgNPs/BDT. (C) SEM images of the p53-captured electrode before (left) and after (right) incubation with AgNPs/BDT.

significant increase of R_{et} (curve c) was observed. The result is attributed to the steric hindrance and electrostatic repulsion by the compact ssDNA/MCH SAMs. Hybridization of complementary DNA to form the consensus dsDNA on electrode surface led to a further increase in R_{et} (curve d). In addition, the R_{et} increased after the dsDNA/MCH-covered electrode was incubated with wild-type p53 protein, indicating that the target was captured by the sensor electrode.

Fig. 1B shows the LSV responses of the dsDNA/MCH-covered electrode after incubation with various solutions. Without capture of target p53 protein, no apparent LSV peak was detected even if the electrode was incubated with AgNPs/BDT (curve a), indicating that no or undetectable AgNPs were attached on the electrode surface. This is acceptable since dsDNA cannot interact with AgNPs or BDT. However, a small LSV peak was observed when the electrode was successively incubated with target p53 protein and AgNPs only (curve b), demonstrating that a few AgNPs were bound to the sensor electrode. Interestingly, when the p53-captured electrode was incubated with mixture of AgNPs and BDT, an intensive and well-defined LSV peak at 50 mV was observed (curve c). The sharp peak is generated from the highly characteristic solid-state Ag/AgCl reaction from AgNPs since no LSV peak was observed in the absence of AgNPs. The peak current is approximately 16-fold higher than that incubated with AgNPs only (curve b), indicating that the signal was amplified by the formation of AgNPs-based networks. The result was also confirmed by SEM (Fig. 1C): obvious AgNPs networks were found when the sensor electrode was incubated with target p53 protein and AgNPs/BDT. To prove that the binding of target is dsDNA-dependent, the ssDNA-modified electrode was incubated with p53 protein and the mixture of AgNPs and BDT. As a result, no discernible peak was observed (curve d). This confirmed that the nonspecific interaction between ssDNA and wild-type p53 protein was negligible. Therefore, the distinct and amplified LSV responses enabled the proposed electrochemical biosensor with the potential ability to quantify wild-type p53 protein.

3.3. Optimization of experiment conditions

BDT as the linker can trigger the assembly of metal nanoparticles by the metal-S interactions. The BDT concentration may has a great influence on the AgNPs assembly and the performance of the biosensor. Thus, we first investigated the influence of BDT concentration on the peak current. As shown in Fig. S2, the anodic peak current (i_{pa}) increased with the increasing concentration of BDT and reached to the maximum value at 5 μM. When the BDT concentration was too low, the signal was small, demonstrating that only few AgNPs network architecture was produced on the electrode surface. However, when the concentration was higher than 5 μM, i_{pa} began to fall down. This can be attributed to the competition between free BDT and cysteine thiols of p53 protein to bind with AgNPs. The incubation time for BDT-induced assembly of AgNPs was also optimized. As shown in Fig. S3, the current was intensified with the increase of incubation time and began to level off beyond 15 min, which is indicative of the completion of the assembly of AgNPs on electrode surface.

The dsDNA hybrid and dsDNA/p53 complex are susceptible to denaturation and disassembly at low pH. By washing the electrode with acidic solution, we found that the electrode remained over 90% of the initial response after ten regeneration cycles. This suggested that the sensor electrode can be conveniently regenerated. Thus, multiple samples can be measured at one electrode. The relative standard deviations (RSDs) for assays of each sample at three chosen electrodes are all below 10.7%, indicating that the proposed method has acceptable reproducibility. Thus, multiple electrodes can also be prepared in parallel for assays of different samples.

3.4. Sensitivity

The normal concentration of p53 protein in serum circulatory system is very low. Thus, high sensitivity is required for the practical application of biosensor for detection of wild-type p53 protein. To evaluate the sensitivity and detection limit of the method, LSV responses for the detection of various concentrations of target protein were collected. As shown in Fig. 2A, the peak current increased with the increase of target protein from 0 to 5 nM, demonstrating that the attachment of AgNPs on electrode was dependent upon p53 protein concentration. Fig. 2B displays the dependence of i_{pa} on the concentration of target protein. The linear regression equation was fitted as $i_{pa} = 33.4[p53]\,(nM) + 0.094$ in the range of 0.1–100 pM. The target at the concentration as low as 0.1 pM was readily measured. The value is lower than that achieved by surface plasmon resonance (SPR) (1.06 pM) [39], chemiluminescence (3.8 pM) [41], fluorescence (8 pM) [43], electrochemistry (2.2 pM) [38], and enzyme-linked electrochemical amperometric immunosensor (14 pM) [16]. The sensitivity is comparable to (or even lower than) that of sandwich-type immunoassays of p53 proteins by different signal amplification strategies (Table 1). The high sensitivity can be attributed to the low background current, the well-defined silver stripping peak and the signal amplification of AgNPs-based networks. Moreover, the method does not require the pre-labeling of nanolabels with recognition element for signal readout, thus simplifying the test steps, reducing the detection complexity and decreasing the assay cost.

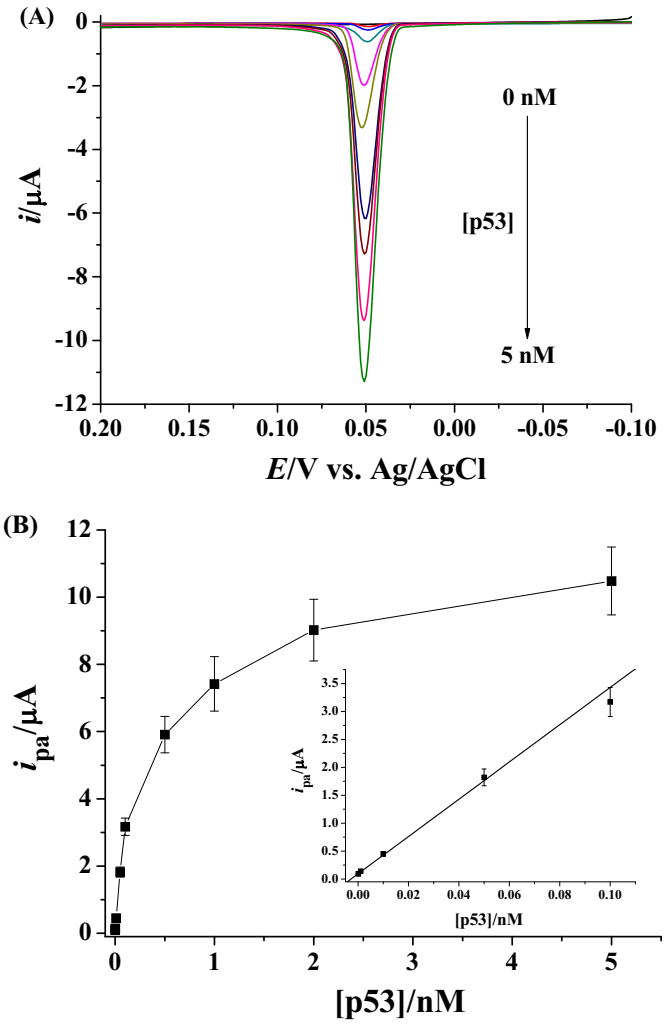


Fig. 2. (A) LSV responses for detection of various concentrations of wild-type p53 protein (0, 0.0001, 0.001, 0.01, 0.05, 0.1, 0.5, 1, 2 and 5 nM). (B) Dependence of i_{pa} on p53 concentration. The inset shows the linear portion of the curve.

Table 1
Analytical performances of sandwich-type electrochemical biosensors for p53 protein detection.

Electrode modifier	Signal label	Detection limit	Linear range	Ref.
SAMs	Fc-AuNPs	2.2 pM	Non-linear	[38]
AuNPs/GO	HPR	0.03 pM	0.2–200 pM	[10]
Au NPs	GO-HPR	10 pM	20–2000 pM	[12]
Nano-SPEC	AP	50 pM	200–50,000 pM	[18]
MWCNT-PA6	HRP	0.02 pM	0.04–40 pM	[19]
Graphene–CHI	HRP	2 pM	4–200 pM	[60]
SAMs	AgNPs	0.1 pM	0.1–100 pM	This work

Abbreviations: Fc-AuNPs, ferrocene-capped gold nanoparticles; GO, graphene oxide; HRP, horseradish peroxidase; Nano-SPEC, nanostructured screen-printed carbon electrode; AP, alkaline phosphatase; MWCNT, multi-walled carbon nanotube; PA6, doped nylon 6; CHI, chitosan.

3.5. Selectivity and anti-interference

Selectivity is a critical factor to evaluate the applicable capability of electrochemical biosensors. To demonstrate the selectivity of the proposed method, we challenged the sensing system with four common proteins (IgG, thrombin, PSA and BSA) instead of wild-type p53 protein under the same experimental conditions. As shown in Fig. 3, the current caused by the tested proteins are close to background level, even at the concentration of at least 100-fold higher than that of target protein. Meanwhile, when introducing these non-target proteins as interferences to the detection system, there was no significant difference in the current value by comparison to that of target p53 protein only. The high selectivity and good anti-interference ability should be attributed to the specific interaction between wild-type p53 protein and dsDNA and the high antifouling ability of the dsDNA/MCH SAMs toward AgNPs.

3.6. Real sample analysis

To indicate the accuracy and amenability of the biosensor for real sample analysis, we determined the concentration of wild-type p53 protein in a serum sample. As a result, no or undetectable target protein was found. Thus, standard protein samples at the final concentrations of 1, 10 and 50 pM were spiked to the serum and then analyzed by the method. According to the above calibration curve, the concentrations of target protein were deduced to be 0.9 pM, 10.3 and 49.1 pM, respectively. The found contents are close to those of the known values and the recoveries vary in the range of 90–103%, indicating that the method is reliable for analysis of p53 protein in biological matrix sample. Thus,

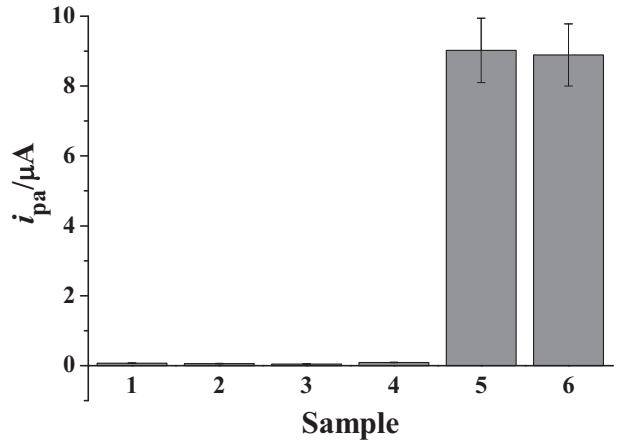


Fig. 3. Selectivity and anti-interference. Bar 1, IgG; bar 2, thrombin; bar 3, PSA; bar 4, BSA; bar 5, p53; bar 6, the mixture of 1–5. The concentration of wild-type p53 protein is 2 nM and that of other proteins is 0.1 μg/mL.

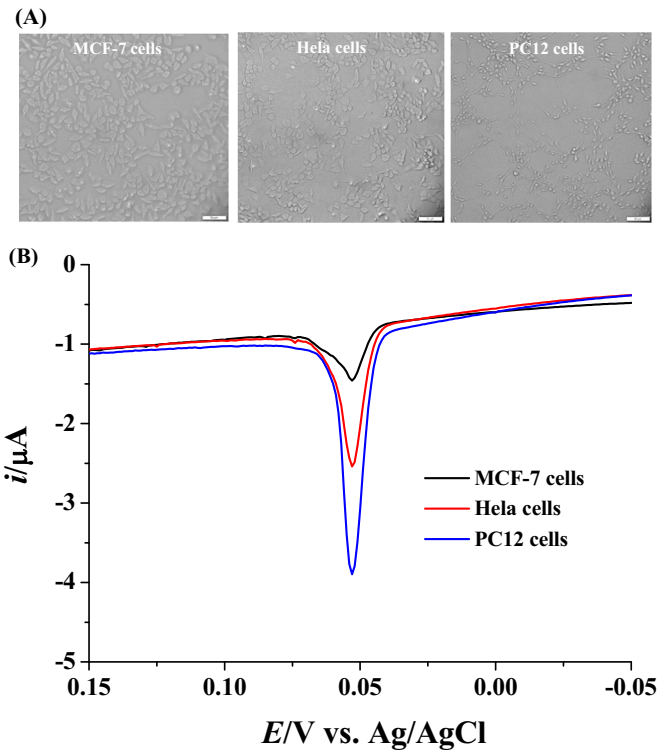


Fig. 4. (A) Confocal images of living cells and (B) LSV responses for analysis of cell lysates.

this method was further used to detect wild-type p53 protein in different cell lysates, including cancer cells of MCF-7 and Hela and normal nerve PC12 cells. Fig. 4A depicts the confocal images of these living cells. Fig. 4B shows the LSV responses for detection of wild-type p53 protein in the supernatants of various cell lines. The found concentration of wild-type p53 protein in the cancer cells was much lower than that in the normal cells (Table 2). Meanwhile, the contents of total p53 proteins (wild-type and mutant combined) were also determined by ELISA kits with a calibration curve of $OD_{450} = 0.01 + 0.004[p53]$ (pM) (Fig. S4). It was found that the total p53 protein concentration in the cancer cells is higher than that in the normal cells. From the deduced difference between wild-type and total p53 proteins, the content of mutant p53 protein in the cancer cells was greatly higher than that in the normal cells. Note that the ratio between wild-type and mutant p53 proteins is a preferred criterion for cancer diagnosis; thus, the biosensor may be used as a promising tool for diagnosis of cancer development by measuring the level of wild-type p53 protein.

4. Conclusion

In summary, we developed a modification-free electrochemical biosensor for sensitive detection of wild-type p53 protein. AgNPs networks formed *in situ* on the sensor electrode surface were utilized as the electroactive reporters for signal readout. The well-defined electrochemical signal originated from the solid-state Ag/AgCl reaction of AgNPs-based networks. The biosensor exhibited a wide linear range and a detectable concentration as low as 0.1 pM. In contrast to other nanomaterials-based strategies for signal amplification, the biosensor

Table 2
Results for analysis of p53 protein in cell lysates.

Cell lysates	Wild-type p53 (pM)	Total p53 (pM)
MCF-7 cells	20.8 ± 0.21	102.4 ± 7.36
Hela cells	49.5 ± 0.31	127.8 ± 6.52
PC12 cells	83.2 ± 6.82	85.5 ± 2.65

is simple and cost-effective and obviates the modification of nanolabels for molecular recognition. We believe that the method has promising applications in early clinical diagnosis and prognosis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2020.04.271.

References

[1] A.J. Levine, p53, the cellular gatekeeper for growth and division, Cell 88 (1997) 323–331.
[2] N.D. Smith, J.N. Rubenstein, S.E. Eggener, J.M. Kozlowski, The p53 tumor suppressor gene and nuclear protein: basic science review and relevance in the management of bladder cancer, J. Urol. 169 (2003) 1219–1228.
[3] T. Jiang, Y. Song, D. Du, X. Liu, Y. Lin, Detection of p53 protein based on mesoporous Pt—Pd nanoparticles with enhanced peroxidase-like catalysis, ACS Sens 1 (2016) 717–724.
[4] M. Toshiyuki, J.C. Reed, Tumor suppressor p53 is a direct transcriptional activator of the human bax gene, Cell 80 (1995) 293–299.
[5] K. Husgafvel-Pursiainen, A. Kannio, P. Oksa, T. Suitiala, H. Koskinen, R. Partanen, K. Hemminki, S. Smith, R. Rosenstock-Leibu, P.W. Brandt-Rauf, Mutations, tissue accumulations, and serum levels of p53 in patients with occupational cancers from asbestos and silica exposure, Environ. Mol. Mutagen. 30 (1997) 224–230.
[6] M. Wu, C. Mao, Q. Chen, X.W. Cu, W.S. Zhang, Serum p53 protein and anti-p53 antibodies are associated with increased cancer risk: a case-control study of 569 patients and 879 healthy controls, Mol. Biol. Rep. 37 (2010) 339–343.
[7] Y. Adachi, W. Chen, W.H. Shang, T. Kamata, Development of a direct and sensitive detection method for DNA-binding proteins based on electrophoretic mobility shift assay and iodoacetamide derivative labeling, Anal. Biochem. 342 (2005) 348–351.
[8] F.S. Pardo, D.W. Hsu, R. Zeheb, J.T. Efird, P.G. Okunieff, D.M. Malkin, Mutant, wild type, or overall p53 expression: freedom from clinical progression in tumours of astrocytic lineage, Brit. J. Cancer 91 (2004) 1678–1686.
[9] B. Vojtesek, C.J. Fisher, D.M. Barnes, D.P. Lane, Comparison between p53 staining in tissue sections and p53 proteins levels measured by an ELISA technique, Brit. J. Cancer 67 (1993) 1254–1258.
[10] H. Afsharan, B. Khalilzadeh, H. Tajalli, M. Mollabashi, F. Navaeipour, M.-R. Rashidi, A sandwich type immunosensor for ultrasensitive electrochemical quantification of p53 protein based on gold nanoparticles/graphene oxide, Electrochim. Acta 188 (2016) 153–164.
[11] D. Du, J. Wang, D. Lu, A. Dohnalkova, Y. Lin, Multiplexed electrochemical immunoassay of phosphorylated proteins based on enzyme-functionalized gold nanorod labels and electric field-driven acceleration, Anal. Chem. 83 (2011) 6580–6585.
[12] D. Du, L. Wang, Y. Shao, J. Wang, M.H. Engelhard, Y. Lin, Functionalized graphene oxide as a nanocarrier in a multienzyme labeling amplification strategy for ultrasensitive electrochemical immunoassay of phosphorylated p53 (S392), Anal. Chem. 83 (2011) 746–752.
[13] A. Chen, Y. Bao, X. Ge, Y. Shin, D. Du, Y. Lin, Magnetic particle-based immunoassay of phosphorylated p53 using protein cage templated lead phosphate and carbon nanospheres for signal amplification, RSC Adv. 2 (2012), 11029. .
[14] Y. Luo, A.M. Asiri, X. Zhang, G. Yang, D. Du, Y. Lin, A magnetic electrochemical immunosensor for the detection of phosphorylated p53 based on enzyme functionalized carbon nanospheres with signal amplification, RSC Adv. 4 (2014) 54066–54071.
[15] X. Ge, A. Zhang, Y. Lin, D. Du, Simultaneous immunoassay of phosphorylated proteins based on apoferritin templated metallic phosphates as voltammetrically distinguishable signal reporters, Biosens. Bioelectron. 80 (2016) 201–207.
[16] M. Giannetto, M.V. Bianchi, M. Mattarozzi, M. Careri, Competitive amperometric immunosensor for determination of p53 protein in urine with carbon nanotubes/gold nanoparticles screen-printed electrodes: a potential rapid and noninvasive screening tool for early diagnosis of urinary tract carcinoma, Anal. Chim. Acta 991 (2017) 133–141.
[17] M. Pedrero, F.J.M. de Villena, C. Munoz-San Martin, S. Campuzano, M. Garranzo-Asensio, R. Barderas, J.M. Pingarron, Disposable amperometric immunosensor for the determination of human p53 protein in cell lysates using magnetic microcarriers, Biosensors 6 (2016) E56.
[18] O. Amor-Gutiérrez, E. Costa-Rama, N. Arce-Varas, C. Martínez-Rodríguez, A. Novelli, M. Teresa Fernández-Sánchez, A. Costa-García, Competitive electrochemical immunosensor for the detection of unfolded p53 protein in blood as biomarker for Alzheimer's disease, Anal. Chim. Acta 1093 (2020) 28–34.
[19] X. Wang, C. Gao, G. Shu, Y. Wang, X. Liu, The enzyme electrocatalytic immunosensor based on functional composite nanofibers for sensitive detection of tumor suppressor protein p53, J. Electroanal. Chem. 756 (2015) 101–107.
[20] Z.-H. Liu, G.-F. Zhang, Z. Chen, B. Qiu, D. Tang, Prussian blue-doped nanogold microspheres for enzyme-free electrocatalytic immunoassay of p53 protein, Microchim. Acta 181 (2014) 581–588.
[21] E.B. Aydın, M. Aydin, M.K. Sezginturk, Electrochemical immunosensor based on chitosan/conductive carbon black composite modified disposable ITO electrode: an analytical platform for p53 detection, Biosens. Bioelectron. 121 (2018) 80–89.
[22] M. Aydın, E.B. Aydin, M.K. Sezginturk, A disposable immunosensor using ITO based electrode modified by a star-shaped polymer for analysis of tumor suppressor protein p53 in human serum, Biosens. Bioelectron. 107 (2018) 1–9.
[23] J. Yeo, J.Y. Park, W.J. Bae, Y.S. Lee, B.H. Kim, Y. Cho, S.M. Park, Label-free electrochemical detection of the p53 core domain protein on its antibody immobilized electrode, Anal. Chem. 81 (2009) 4770–4777.
[24] M. Hasanzadeh, H.N. Baghban, A. Mokhtarzadeh, N. Shadjou, S. Mahboob, An innovative immunosensor for detection of tumor suppressor protein p53 in unprocessed human plasma and cancer cell lysates, Int. J. Biol. Macromol. 105 (2017) 1337–1348.
[25] M. Hasanzadeh, H.N. Baghban, N. Shadjou, A. Mokhtarzadeh, Ultrasensitive electrochemical immunosensing of tumor suppressor protein p53 in unprocessed human plasma and cell lysates using a novel nanocomposite based on poly-cysteine/graphene quantum dots/gold nanoparticle, Int. J. Biol. Macromol. 107 (2018) 1348–1363.
[26] H. Afsharan, F. Navaeipour, B. Khalilzadeh, H. Tajalli, M. Mollabashi, M.J. Ahar, M.-R. Rashidi, Highly sensitive electrochemiluminescence detection of p53 protein using functionalized Ru-silica nanoporous@gold nanocomposite, Biosens. Bioelectron. 80 (2016) 146–153.
[27] Y.-C. Zhu, N. Zhang, Y.-F. Ruan, W.-W. Zhao, J.-J. Xu, H.-Y. Chen, Alkaline phosphatase tagged antibodies on gold nanoparticles/TiO_2 nanotubes electrode: a plasmonic strategy for label-free and amplified photoelectrochemical immunoassay, Anal. Chem. 88 (2016) 5626–5630.
[28] W. Zhou, Y. Ma, H. Yang, Y. Ding, X. Luo, A label-free biosensor based on silver nanoparticles array for clinical detection of serum p53 in head and neck squamous cell carcinoma, Int. J. Nanomedicine 6 (2011) 381–386.
[29] A.R. Bizzarri, I. Moscetti, S. Cannistraro, Surface enhanced Raman spectroscopy based immunosensor for ultrasensitive and selective detection of wild type p53 and mutant p53R175H, Anal. Chim. Acta 1029 (2018) 86–96.
[30] F. Domenici, A.R. Bizzarri, S. Cannistraro, Surface-enhanced Raman scattering detection of wild-type and mutant p53 proteins at very low concentration in human serum, Anal. Biochem. 421 (2012) 9–15.
[31] L. Wu, Z. Wang, S. Zong, H. Chen, C. Wang, S. Xu, Y. Cui, Simultaneous evaluation of p53 and p21 expression level for early cancer diagnosis using SERS technique, Analyst 138 (2013) 3450–3466.
[32] W.S. El-Deiry, S.E. Kern, J.A. Pietenpol, K.W. Kinzler, B. Vogelstein, Definition of a consensus binding site for p53, Nat. Gene. 1 (1992) 45–49.
[33] Y. Han, J.M. Chabu, S. Hu, L. Deng, Y.N. Liu, S. Guo, Rational tuning of the electrocatalytic nanobiointerface for a "turn-off" biofuel-cell-based self-powered biosensor for p53 protein, Chem. Eur. J. 21 (2015) 13045–13051.
[34] K. Nemcova, L. Havran, P. Sebest, M. Brazdova, H. Pivonkova, M. Fojta, A label-free electrochemical test for DNA-binding activities of tumor suppressor protein p53 using immunoprecipitation at magnetic beads, Anal. Chim. Acta 668 (2010) 166–170.
[35] K. Nemcova, P. Sebest, L. Havran, P. Orsag, M. Fojta, H. Pivonkova, Electrochemical detection of DNA binding by tumor suppressor p53 protein using osmium-labeled oligonucleotide probes and catalytic hydrogen evolution at the mercury electrode, Anal. Bioanal. Chem. 406 (2014) 5843–5852.
[36] V. Tichy, P. Sebest, P. Orsag, L. Havran, H. Pivonkova, M. Fojta, Protein p53 binding to cisplatin-modified DNA targets evaluated by modification-specific electrochemical immunoprecipitation assay, Electroanalysis 29 (2017) 319–323.
[37] E. Palecek, H. Cernocka, V. Ostatna, L. Navratilova, M. Brazdova, Electrochemical sensing of tumor suppressor protein p53-deoxyribonucleic acid complex stability at an electrified interface, Anal. Chim. Acta 828 (2014) 1–8.
[38] J. Wang, X. Zhu, Q. Tu, Q. Guo, C.S. Zarui, J. Momand, X.Z. Sun, F. Zhou, Capture of p53 by electrodes modified with consensus DNA duplexes and amplified voltammetric detection using ferrocene-capped gold nanoparticle/streptavidin conjugates, Anal. Chem. 80 (2008) 769–774.
[39] Y. Wang, X. Zhu, M. Wu, N. Xia, J. Wang, F. Zhou, Simultaneous and label-free determination of wild-type and mutant p53 at a single surface plasmon resonance chip preimmobilized with consensus DNA and monoclonal antibody, Anal. Chem. 81 (2009) 8441–8446.
[40] N. Xia, L. Liu, X. Yi, J. Wang, Studies of interaction of tumor suppressor p53 with apo-MT using surface plasmon resonance, Anal. Bioanal. Chem. 395 (2009) 2569–2575.
[41] X. Chen, C. He, Z. Zhang, J. Wang, Sensitive chemiluminescence detection of wild-type p53 protein captured by surface-confined consensus DNA duplexes, Biosens. Bioelectron. 47 (2013) 335–339.
[42] R. Qian, Y. Cao, Y.-T. Long, Dual-targeting nanovesicles for in situ intracellular imaging of and discrimination between wild-type and mutant p53, Angew. Chem. 128 (2016) 729–733.
[43] Q. Xu, K. Liang, R.Y. Liu, L. Deng, M. Zhang, L. Shen, Y.N. Liu, Highly sensitive fluorescent detection of p53 protein based on DNA functionalized Fe3O4 nanoparticles, Talanta 187 (2018) 142–147.

[44] Z. Farka, T. Jurík, D. Kovář, L. Trnková, P. Skládal, Nanoparticle-based immunochemical biosensors and assays: recent advances and challenges, Chem. Rev. 117 (2017) 9973–10042.
[45] J. Shen, Y. Li, H. Gu, F. Xia, X. Zuo, Recent development of sandwich assay based on the nanobiotechnologies for proteins, nucleic acids, small molecules, and ions, Chem. Rev. 114 (2014) 7631–7677.
[46] W. Wen, X. Yan, C. Zhu, D. Du, Y. Lin, Recent advances in electrochemical immunosensors, Anal. Chem. 89 (2017) 138–156.
[47] H. Chai, P. Miao, Bipedal DNA walker based electrochemical genosensing strategy, Anal. Chem. 91 (2019) 4953–4957.
[48] H. Li, Z. Sun, W. Zhong, N. Hao, D. Xu, H.Y. Chen, Ultrasensitive electrochemical detection for DNA arrays based on silver nanoparticle aggregates, Anal. Chem. 82 (2010) 5477–5483.
[49] P. Miao, K. Han, H. Sun, J. Yin, J. Zhao, B. Wang, Y. Tang, Melamine functionalized silver nanoparticles as the probe for electrochemical sensing of clenbuterol, ACS Appl. Mater. Interfaces 6 (2014) 8667–8672.
[50] P. Miao, F. Meng, B. Wang, X. Zhu, Y. Tang, Highly sensitive microRNA quantification with zero background signal from silver nanoparticles, Electrochem. Commun. 51 (2015) 89–92.
[51] P. Miao, B. Wang, X. Chen, X. Li, Y. Tang, Tetrahedral DNA nanostructure-based microRNA biosensor coupled with catalytic recycling of the analyte, ACS Appl. Mater. Interfaces 7 (2015) 6238–6243.
[52] P. Miao, B. Wang, F. Meng, J. Yin, Y. Tang, Ultrasensitive detection of microRNA through rolling circle amplification on a DNA tetrahedron decorated electrode, Bioconjug. Chem. 26 (2015) 602–607.
[53] W. Song, H. Li, H. Liang, W. Qiang, D. Xu, Disposable electrochemical aptasensor array by using in situ DNA hybridization inducing silver nanoparticles aggregate for signal amplification, Anal. Chem. 86 (2014) 2775–2783.
[54] Y. Tang, Y. Dai, X. Huang, L. Li, B. Han, Y. Cao, J. Zhao, Self-assembling peptide-based multifunctional nanofibers for electrochemical identification of breast cancer stem-like cells, Anal. Chem. 91 (2019) 7531–7537.
[55] T. Wei, T. Dong, Z. Wang, J. Bao, W. Tu, Z. Dai, Aggregation of individual sensing units for signal accumulation: conversion of liquid-phase colorimetric assay into enhanced surface-tethered electrochemical analysis, J. Am. Chem. Soc. 137 (2015) 8880–8883.
[56] N. Xia, X. Wang, B. Zhou, Y. Wu, W. Mao, L. Liu, Electrochemical detection of amyloid-beta oligomers based on the signal amplification of a network of silver nanoparticles, ACS Appl. Mater. Interfaces 8 (2016) 19303–19311.
[57] J. Zhang, H. Chen, Y. Cao, C. Feng, X. Zhu, G. Li, Design nanoprobe based on its binding with amino acid residues on cell surface and its application to electrochemical analysis of cells, Anal. Chem. 91 (2019) 1005–1010.
[58] Y. Wang, J.F. Schwedes, D. Parks, K. Mann, P. Tegtmeyer, Interaction of p53 with its consensus DNA-binding site, Mol. Cell. Biol. 15 (1995) 2157–2165.
[59] D. Vilela, M.C. Gonzalez, A. Escarpa, Sensing colorimetric approaches based on gold and silver nanoparticles aggregation: chemical creativity behind the assay. A review, Anal. Chim. Acta 751 (2012) 24–43.
[60] Y. Xie, A. Chen, D. Du, Y. Lin, Graphene-based immunosensor for electrochemical quantification of phosphorylated p53 (S15), Anal. Chim. Acta 699 (2011) 44–48.