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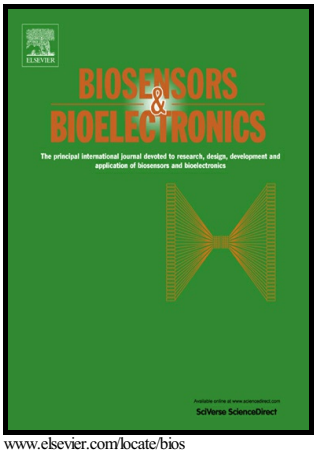

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Reduced graphene oxide-chitosan-aptamer interface as new platform for ultrasensitive detection of human epidermal growth factor receptor 2

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Abstract

The present work describes an ultrasensitive electrochemical aptamer-based assay for detection of human epidermal growth factor receptor 2 protein (HER2) cancer biomarker as a model analyte. Results show that the reduced graphene oxide-chitosan (rGO-Chit) film as a suitable electrode material possesses great favorable properties including high homogeneity, good stability, large surface area and high fraction of amine groups as aptamer binding sites. Various steps of aptasensor fabrication were characterized using microscopic, energy-dispersive X-ray spectroscopy (EDAX), Fourier transform infrared (FTIR) spectroscopy and electrochemical techniques. Using methylene blue (MB) as an electrochemical probe and differential pulse voltammetry (DPV) technique, two linear concentration ranges of 0.5 to 2 ng ml^{-1} and 2 to 75 ng ml^{-1} were obtained with a high sensitivity of 0.14μA ng^{-1} ml and a very low detection limit of 0.21 ng ml^{-1} (very lower than the clinical cut-off). The fabricated aptasensor showed excellent selectivity for detection of HER2 in complex matrix of human serum samples. The sensitive detection of HER2 can be attributed to the multiple signal amplification of MB during its accumulation to the modified electrode surface via both affinity interaction to aptamer molecules and electrostatic adsorption to the HER2 analyte as well as high charge transfer kinetic properties of the applied rGO-Chit film. The rapid and simple preparation of the proposed aptasensor as well as its high selectivity, stability and reproducibility provided a promising protocol for non-invasive diagnosis for various points of care application. The proposed aptasensor showed excellent analytical performance in comparison with current HER2 biosensors.

Keywords: Tumor marker, Human epidermal growth factor receptor 2, Aptamer, Reduced graphene oxide-chitosan, Biosensor, Modified electrode.

1. Introduction

Today, biomarkers provide an interesting approach for the early detection and diagnosis of various types of cancers. Breast cancer is the most common cancer among women throughout the world that consists of about 34% of all cancers in women (Torre et al. 2016). Human epidermal growth factor receptor 2 protein (HER2) is known as a key prognostic biomarker for breast cancer (Mittal et al. 2016). In about 20-30 % of breast cancers, there are a very high number of HER2 receptors on breast cancer cells, which is called HER2 protein overexpression (Riccio et al. 2009). It is important to identify the overexpression of HER2 for effective treatment of HER2-positive breast cancer patients. The extra HER2 receptors stimulate breast cells to grow and divide in an uncontrolled way that is known as HER2-positive breast cancer. Current methods for detection of HER2 are based on a biopsy and taking a tissue sample of the tumor using immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH) methods. Most of these methods are based on cell detection and can not be used for measuring the concentration of free HER2 in serum. Unfortunately, such techniques are limited by invasive biopsies, expensive and complex labeling processes, and time-consuming analysis (Zhu et al. 2013, Mukundan et al. 2009). An alternative approach for overcoming these problems is measuring the concentration of free HER2 proteins that is cleaved and entered into blood serum. The concentration of HER2 in blood of normal individuals is between 2 and 15 ng ml^{-1}, whereas patients with positive breast cancer have an elevated concentration of 15 - 75 ng ml^{-1} of HER2 (Esteva et al. 2005; Ravalli et al. 2015). So, examining the HER2 protein biomarker in blood serum has provided a less invasive method for diagnosis of breast cancer and monitoring the disease recurrence after therapy. Electrochemical-based sensors have attracted considerable attention for analysis of various types of cancer markers due to the advantages of simplicity, ease of miniaturization, high sensitivity, being cost-effective, and their relatively rapid response (Raymundo-Pereira et al. 2016). More recently, various electrochemical biosensors for determination of HER2 biomarker have been developed based on the different nanoparticle-biomolecule hybrid systems (Al−Khafaji et al. 2012; Chun et al. 2013; Mucelli et al. 2008; Patris et al. 2014; Zhu et al. 2012). The aim of the present study is to develop an electrochemical nanobiosensor based on a unique combination of favorable

characteristic properties of reduced graphene oxide-chitosan (rGO-Chit) film, specific HER2 aptamer and HER2 proteins in order to recognize and detect HER2 proteins in human blood serum.

In the last decade, graphene and graphene-based nanocomposites due to high surface area, very good electronic and thermal conductivity, high chemical stability, excellent mechanical strength, and low manufacturing cost, have been widely used for fabrication of highly performed electrochemical biosensors with respect to stability, sensitivity, and precision. The interactions of graphene with various types of molecules and biorecognition molecules have provided effective tools to develop efficient high performance analytical devices (Kuila et al. 2011; Song et al. 2016); and aptamers are highly favorable recognition molecules in this context (Seok Kim et al. 2016). Aptamers can selectively bind to a wide range of targets from small molecules to whole cells and can be used for analysis of various targets such as proteins, small molecules, metal ions, peptides and cells (Ozkan et al. 2002; Seok Kim et al. 2016). Recent literature reviews the bioanalysis application of aptamers (Jeong 2011; S. and 2010; Seok Kim et al. 2016). The use of aptamer-based sensors (aptasensors) for diagnosis of HER2 might offer an alternative means to antibody-based biosensors (immunoassays). Unlike antibodies, aptamers are synthesized by an in vitro chemical process SELEX (systematic evolution of ligands by exponential enrichment). Aptamers are also more stable molecules and can preserve their structures and functionalities even after multiple regeneration steps. Due to these advantages in addition to ease of modification and high binding affinity to a wide range of targets, aptamers are suitable recognition elements for the fabrication of biorecognition devices (Ozkan et al. 2002; Xin et al. 2015). However, the application of aptamers as recognition elements in the generation of electrochemical sensors for diagnosis of HER2 is still in its infancy (Chun et al. 2013).

In the present study, by a unique combination of favorable characteristics of rGO-Chit film, configuration change of the HER2 aptamer molecules and electrostatic adsorption of methylene blue (MB) on the surface of HER2 proteins in physiological pH, a novel multiple amplified detection strategy was used for fabrication of a very sensitive and selective HER2 electrochemical aptasensor. The proposed aptasensor was fabricated via covalent coupling of amino-functionalized HER2-specific aptamer on the rGO-Chit film-

modified glassy carbon (GC/rGO-Chit) electrode. To the best of our knowledge, there has been no report on the covalent immobilization of aptamer molecules on the surface of rGO-Chit film via glutaraldehyde (GLA) linker in order to introduce an electrochemical HER2 aptasensor. Also, there is no evidence for the fabrication of HER2 electrochemical aptasensor using the proposed HER2 aptamer sequence. In the present work, the function of rGO-Chit as a novel solid support for the amplification of HER2 analytical signal was effectively demonstrated in comparison with other graphit-based films consisting of graphite-Chit and graphene oxide (GO)-Chit and bare rGO. MB was used to probe biointerfaces events. The increase in the differential pulse voltammetric (DPV) signal of MB in the presence of HER2 was measured as the analytical signal of the aptasensor. Multi amplification in MB signal was achieved during (i) accumulation of MB on the surface via the affinity interaction between this molecule and large number of free guanine bases of the aptamer that adopted its open state structure in the presence of HER2, (ii) electrostatic attraction between positively charged MB and negatively charged HER2 molecules adsorbed on the surface and (iii) High charge transfer kinetic at the surface of applied rGO-Chit film. Various experimental parameters were optimized to determine the best analysis conditions for the proposed aptasensor.

1. Experimental

2.1. Chemicals, apparatus and measurements

All materials and reagents, sequence and structure of aptamer probes, apparatus and measurements are described in the supplementary file in detail.

2.3. Preparation of GO, rGO nanosheets and rGO-Chit composite

Synthesis of GO, rGO and rGO-Chit composite were described in detail in the supplementary file.

2.4. Preparation of the biorecognition surface

The various steps of the fabrication of the aptasensor are described in section 3.2. Briefly, after cleaning the glassy carbon electrode (Supplementary file, section 1.2), 10 µL of the

rGO-Chit composite was placed on the GC electrode surface and allowed to dry at room temperature. For the immobilization of the amino-functionalized HER2 aptamer on the electrode surface, the GC/rGO-Chit electrode was incubated in a 2% GLA solution for 1 h at room temperature, in which GLA was covalently attached to the GC/rGO-Chit surface through its reaction with amine groups of Chit. Then, 10 μL of the HER2 aptamer solution was incubated with the GLA-functionalized electrode surface for 1 h at room temperature. As a result, HER2 aptamer was attached to the rGO-Chit/GLA film through the formation of covalent bonds between the -COH groups of GLA and -NH_2 groups of HER2 aptamer. After washing with PBS, this modified electrode (GC/rGO-Chit/GLA/aptamer electrode) was incubated in 0.5 mg ml^{-1} of BSA for 60 min to block the unreacted sites of the modified electrode surface. This modified electrode was thoroughly rinsed with PBS and denoted as the GC/rGO-Chit/GLA/aptamer/BSA electrode. Each step of modification was investigated by EIS method. The GC/graphite-Chit/GLA/aptamer/BSA and GC/GO-Chit/GLA/aptamer/BSA electrodes were prepared under the same procedure in the presence of graphite and GO, respectively.

2.5. Indicator binding and detection of HER2

The MB was accumulated onto the surface of different modified electrodes by immersing the modified electrodes in a 50 μM MB solution for 1h. The electrodes were thoroughly rinsed with PBS to remove any unadsorbed or weakly-adsorbed MB and denoted as GC/rGO-Chit/GLA/aptamer/BSA/MB, GC/graphite-Chit/GLA/aptamer/BSA/MB and GC/GO-Chit/GLA/aptamer/BSA/MB electrodes. To detect HER2, the signal of the aptasensor in the absence and presence of various concentrations of HER2 was investigated and described in detail in the supplementary file. For the detection of HER2 in real biological matrix, a stock solution of HER2 in human serum sample was prepared, diluted by PBS (pH 7.4) and analyzed by the fabricated aptasensor.

3. Results and discussions

3.1. Characterization of the rGO-Chit film

The synthesized rGO nanosheets were first characterized using field emission scanning electron microscopy (FESEM). The layered structure is the major morphology of as-

prepared rGO sheets as shown in the representative FESEM image (Fig. 1.A), which clearly demonstrated the prosperity of rGO synthesis (Zhou et al. 2014). This was further confirmed by the transmission electron microscopy (TEM) measurements (Fig. 1.B). The wrinkled planes of products suggest the thin and flexible feature of the graphene sheets. Furthermore, wrinkles and rough edges on the rGO surface provided more active sites for the electron transfer process and electrochemical reactions. The charge transfer properties of synthesized rGO nanosheets were investigated by EIS experiments. The value of charge-transfer resistance (R_{ct}) for GC and rGO-modified GC electrodes was estimated to be 154.20 Ω and 4.5 Ω, respectively (Fig. S1 and Table S1). These experimental results clearly indicated lower charge-transfer resistance at the GC/rGO interface compared to that of the bare GC electrode. The very low charge transfer resistance at the surface of the rGO film is in good agreement with previous reports (Wang et al. 2016; Yang and Gunasekaran, 2013). After mixing the rGO with Chit, the rGO sheets were covered homogeneously with a thin layer of Chit, as shown in Fig.S2 (The FESEM images of rGO and rGO-Chit films). However, the rGO sheets remained significantly separated after coalescing with chitosan polymer. Furthermore, the dispersibility of the rGO sheets was increased after mixing with chitosan (Fig. S2), as reported previously (Zuo et al. 2013; Wang et al. 2010). Furthermore, the adherence of the rGO-Chit film was increased on the GC electrode surface after mixing with chitosan (Sun et al. 2010), which resulted in a significant increase in the stability of the rGO-Chit film without obvious increasing in the electron charge-transfer resistance (Fig.S.3,Fig S.4 and Table S1). The great advantages of high homogeneity, good stability, large surface area and existence of high fraction of amine groups as aptamer binding sites, make the rGO-Chit film as suitable surface for the immobilization of aptamer molecules, and for the fabrication of the HER2 electrochemical aptasensor.

As-prepared GO, rGO and rGO-Chit films were investigated using Fourier transform infrared (FTIR) spectroscopy (Fig. 1.C). The presence of the typical characteristic peaks of rGO and chitosan in the rGO-Chit FTIR spectrum, the appearance of a new absorption peak and an absorption band shift in the rGO-Chit spectrum, provided a preliminary proof for the fabrication of the rGO-Chit film. The details are reported in the supplementary section.

Here Fig. 1

3.2. Characterization of the biorecognition surface

The various steps of the fabrication of the aptasensor are illustrated in Fig. 2.A and characterized using EIS method. Fig. 2.B shows typical EIS complex plane plots of the bare GC, GC/rGO-Chit, GC/rGO-Chit/GLA, GC/rGO-Chit/GLA/aptamer and GC/rGO-Chit/GLA/aptamer/BSA electrodes. The quantitative values of R_{ct} and more kinetic parameters (R_s, and n) were obtained for each electrode from the fitting of the EIS data to the modified Randles model (the inset of Fig. 2. B); and the results are given in Table S1. The results show that the R_{ct} value of the bare GC electrode (curve a) decreased upon its modification with the rGO-Chit film (curve b). This trend can be a result of two effects: (i) high electronic conductivity of rGO as well as its large surface area that resulted in the facilitation of charge transfer process of the redox probe at the electrode interface and (ii) electrostatic attraction between anionic probe and positively charged amino acid groups of the chitosan, leading to an increase in mass transport of redox probe to the surface. The R_{ct} increased slightly by incubation of the GC/rGO-Chit electrode in glutaraldehyde solution, due to the attachment of glutaraldehyde on the electrode surface. With further immobilization of aptamer strand on the surface, R_{ct} increased dramatically due to (i) the blocking effect of immobilized DNA backbone that impede charge transfer at the electrode surface, and (ii) electrostatic repulsion between negatively charged phosphate groups of DNA and $[Fe(CN)_6]^{3-/4-}$ probe (curve c). Finally, in the last step, accumulation of BSA on the surface of the electrode effectively blocked the surface (curve d) that caused in an increase in the R_{ct}. These trends in the data can be seen clearly in the semicircle part of the impedance spectra. The obtained data are in good agreement with other pervious works (Erdem et al. 2014; Tahmasebi and Noorbakhsh 2016). Also the EDAX spectra of rGO-Chit and rGO-Chit/GLA/Aptamer were investigated (Fig. 2 D). The presence of phosphorus element in the EDAX spectra of the GO-Chit/GLA/aptamer modified electrode revealed the immobilization of ss-DNA molecules on the surface of the modified electrode (Supplementary file, section 2.3)

Here Fig. 2

3.3. Detection of HER2

For determination of HER2, the change in the DPV signal of the MB as a redox probe in the absence and presence of HER2 was investigated (Fig. 2C and supplementary file, section 2.4 and Fig. S5). MB interacts with DNA via its specific interaction with free guanine bases of DNA strand, electrostatic interaction and intercalation (Ozkan et al. 2002; Tahmasebi and Noorbakhsh 2016). Fig. 2.C illustrates the sensing strategy of the present aptasensor for the detection of HER2. As is shown, the HER2 aptamer has adopted a stem–loop conformation (also known as hairpin loop) (Kim and Jeong 2011). This conformation is self-generated due to the intramolecular base paring of the terminal parts of the aptamer strand, resulting in the formation of a double-stranded structure within the same aptamer strand. In the presence of analyte HER2 molecule, the aptamer interacted with HER2 protein which resulted in the formation of aptamer/HER2 complex, and thereby opened the hairpin. As a result, the electrochemical signal of MB increased due to its interaction with free guanine bases that are present in significantly larger number of copies per open state aptamer compared to that per hairpin state aptamer (Ozkan et al. 2002; Tahmasebi and Noorbakhsh 2016). Furthermore, positively charged MB molecules have a large tendency to electrostatically interact with HER2 molecules that are negatively charged at physiological pH (isoelectric point of HER2 = 5.8), which resulted in an additional increase in the amount of MB accumulated onto the electrode surface. (Lakshmi ; S. and 2010). So, dual signal amplification is achieved in the presence of HER2 molecules. The increase in the electrochemical signal of MB in the presence of HER2 was measured as the analytical signal of the aptasensor (Fig. S5).

3.4. Optimization of analytical parameters

To achieve the best analysis conditions, experimental parameters that could affect the analytical performance of the aptasensor were optimized. The results are described in supplementary file in detail (Section 2.5, Fig. S6 to Fig. S11). In the present work aptamer immobilization time of 60 min, rGO to Chit ratio of 1:2 (w/w%), aptamer

concentration of 5μm, HER2 reaction time of 60 min and MB incubation time of 60 min were obtained as the optimum experimental conditions for this aptasensor. Under these optimum conditions, the analytical performances of the proposed aptasensor were investigated.

3.5 . Comparison of HER2 determination at bare rGO, rGO-Chit, GO-Chit and graphite-Chit based modified electrodes

In a control experiment, the function of rGO-Chit as a favorable solid support for the amplification of HER2 analytical signal was adequately demonstrated. For this purpose, four graphite-based films, consisting of bare rGO, graphite-Chit, GO-Chit and rGO-Chit, were employed for fabrication of the HER2 aptasensor, and then, the analytical signals of corresponding fabricated aptasensors were compared for the same concentration of HER2 using the DPV technique (Fig. S12- S15). As can be shown in Fig. 3.A, the fabricated aptasensor using the rGO-Chit film exhibited much larger response to HER2 compared to the electrodes fabricated using bare rGO, graphite-Chit and GO-Chit films. Also, control experiments were carried out without using aptamer molecules (supplementary file, section 2.6, Fig. S16 and Fig.S17). Our experiments clearly revealed that the signal of the electrochemical biosensor was greatly amplified in the presence of the rGO-Chit film. These results indicated the good performance of the rGO-Chit film for fabrication of the proposed aptasensor. The existence of numerous edges and edge-plane-like defective sites in rGO nanosheets can accelerate the charge transfer process in the electrode-solution interface (Zhou et al. 2009). Also high surface area of rGO nanosheets and the existence of high fraction of amine groups as aptamer binding sites provided large number of sites for the immobilization of aptamer probes on the electrode surface (Zhang et al. 2013).

3.6. Analytical performance of the aptasensor

Under the optimized conditions, the proposed aptasensor was used to detect HER2 at various concentrations. For this purpose the aptasensor was treated with various concentrations of 0.5 to 125 ng ml^{-1} HER2 and the aptasensor responses were recorded. As Fig. 3.B shows, increasing the concentration of HER2 led to an increase in the current response. The electrochemical response of the aptasensor toward HER2 is linear in two

ranges of 0.5 to 2 ng ml^{-1} and 2 to 75 ng ml^{-1} according to the equations (1) and (2), respectively.

$I(\mu A) = 0.1401\ [HER2]\ (ng\ ml^{-1}) + 0.0985\ (\mu A)$ $R^2 = 0.9991$ (1)

$I(\mu A) = 0.0162\ [HER2]\ (ng\ ml^{-1}) + 0.3676\ (\mu A)$ $R^2 = 0.9932$ (2)

From the equation (1), the sensitivity of 0.14μA ng^{-1} ml was determined for the detection of HER2 at the GC/rGO-Chit/GLA/aptamer/BSA electrode, with an impressive limit of detection of 0.21 ng ml^{-1} (S/N=3) (Supplementary file, section 2.7). This aptasensor offers a favorable analytical performance compared to the current HER2 aptasensors and most of immonusensors described in the literature (Table 1).

A relative standard deviation of 3.9 and 4.3% (n=4) was obtained for the detection of 0.5 and 25 $ngml^{-1}$ HER2, respectively, representing good reproducibility of the aptasensor. The developed aptasensor showed good stability toward the detection of HER2. After 4 days storage in 0.05 M PBS (pH 7.4) at 4 °C, the aptasensor retained more than 94% of its initial response current to 25 $ngml^{-1}$ of HER2. The remarkable stability of the modified electrode may be attributed to the high stability of the rGO-Chit film on the GC electrode surface and strong binding of aptamer to the rGO-Chit supporting film via covalent attachment, which resulted in the formation of a stable assembly on the electrode surface. Great overall performance of the fabricated aptasensor can be attributed to (i) the multiple MB signal amplification strategy which used in the proposed aptasensor (ii) high homogeneity, high stability and large surface area of the rGO-Chit nanocomposite with suitable fraction of amine groups that provided a very favorable environment for the covalent immobilization of aptamer molecules on the electrode surface and (iii) high selectivity of the applied HER2 aptamer molecules.

Here Fig. 3

3.7. Selectivity of the biosensing system

The potential of the presented aptasensor for selective detection of HER2 was investigated by performing control experiments. The DPV analytical response of the aptasensor was recorded and compared for HER2 and some other species such as

adenosine and guanosine as small molecules and BSA and HSA as large proteins. As shown in Fig. 4A, compared to the control species, only a significant response was observed for HER2, indicating the good selectivity of the proposed aptasensor for the detection of HER2. The high selectivity of the aptasensor is attributed to the highly specific interaction of aptamer with HER2.

3.8. In vitro detection of HER2

We also applied the developed aptasensor for the in vitro detection of HER2 in human serum samples. HER2 samples with the concentration of 75 ng ml^{-1} were prepared both in standard solution and two human serum samples (Serum 1 and Serum 2). These samples were analyzed using the proposed aptasensor (Fig. 4B). The results represented average good recovery values of 99.1, 98.1% and 98.7% for HER2, in standard, serum 1 and serum 2 samples, respectively(n=3), indicating a very good selectivity of the fabricated aptasensor and showed that the complex matrix of the serum do not interfere with the determination of HER2. Also, the fabricated aptasensor was validated by comparing with a commercial ELISA Kit (Supplementary file, section 2.8). The response of the proposed aptasensor and the ELISA method for the determination of various concentrations of 0, 1, 7, 15 and 50 ng ml^{-1} HER2 in human serum samples are shown in the Table 2. No obvious difference between two methods was observed. The recoveries were obtained in the range of 98-107% and 96-105% for the ELISA and the present aptasensor, respectively. This data demonstrate that the proposed aptasensor is reliable and has very good accuracy for determination of HER2 for clinical applications.

Here Fig. 4

4. Conclusion

In summary, sensitive aptamer-based electrochemical assay for the detection of HER2, as a model analyte, was developed based on the unique properties of the rGO-Chit composite and multiple signal amplification strategy. The developed aptasensor showed very favorable analytical performance such as high sensitivity, high selectivity, good stability, good reproducibility and very low detection limit far below the clinical cut-off

for HER2 biomarker. These features of the proposed aptasensor are believed to be due to the excellent charge transfer properties, homogeneous structure, very good stability and remarkable ability of the rGO-Chit supporting film for the immobilization of ss-DNA molecules as well as multiple signal amplification strategy of the proposed biointerface. The developed aptasensor exhibited excellent applicability, reliability and accuracy for the detection of HER2 in complex matrix of serum samples, which was achieved through the selective and high-affinity interaction between aptamer and HER2. Considering the analytical performance and simplicity of preparation, the proposed aptasensor is favorably comparable or better than the most sensitive protocols currently reported in the literature for breast cancer diagnosis. The developed method of construction of the present assay can be expanded for fabrication of various sensitive aptamer based biosensors.

Acknowledgements

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Figure Caption

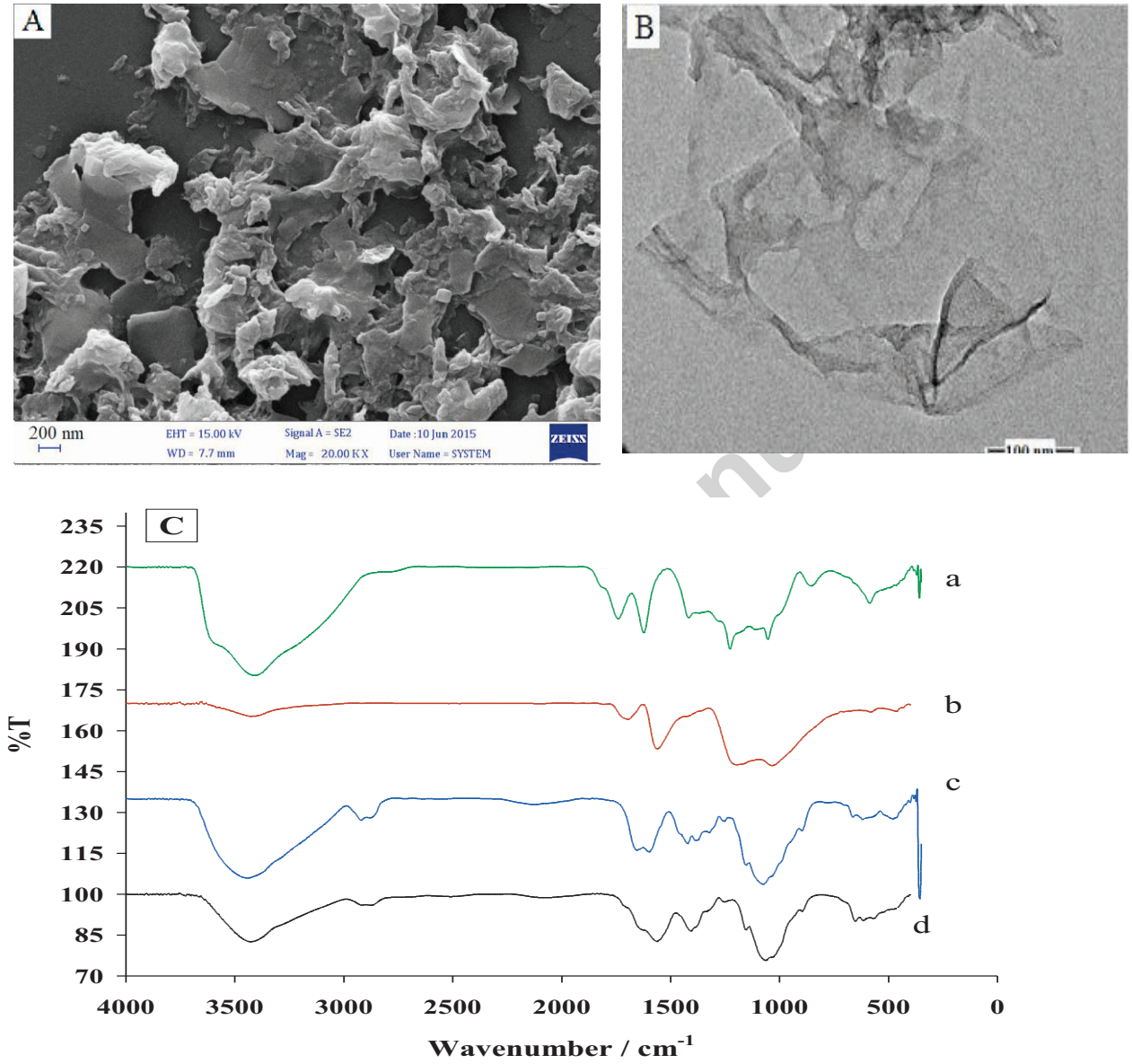
Fig. 1. (A) FESEM image and (B) HRTEM image of rGO. (C) FTIR spectra of (a) GO, (b) rGO, (c) Chit and (d) rGO-Chit.

Fig. 2. (A) Schematic of the step-wise fabrication of aptasensor. (B) (A) EIS complex plane plots obtained on (a) GC, (b) GC/rGO-Chit, (c) GC/rGO-Chit/GLA, (d) GC/rGO-Chit/GLA/aptamer and (e) GC/rGO-Chit/GLA/aptamer/BSA electrodes in 0.3 M KCl electrolyte solution containing 10 mM $Fe(CN)_6^{3-/4-}$. Scan rate: 100 mV s^{-1}. EIS measurements were performed at an applied potential of 0.22 V and a frequency range of 0.1 to 10 kHz. Symbols and trend lines show the experimental and the fitted data, respectively (See table S1 for further informations). Inset: the equivalent circuit models used to approximate EIS data. (C) Schematic illustration of aptasensor sensing strategy. (D) EDAX spectra of (a) rGO-Chit film and (b) rGO-Chit/GLA/Aptamer.

Fig. 3. (A) DPV responses of the different GC/graphite-Chit/GLA/aptamer/BSA, GC/GO-Chit/GLA/aptamer/BSA, GC/rGO/GLA/aptamer/BSA, GC/rGO-Chit/GLA/aptamer/BSA, electrodes to 50 ng ml^{-1} HER2 in PBS (pH 7.4) at the scan rare of 10 mV s^{-1}. (B) Plot of the aptasensor response versus different concentrations of 0.5, 1, 2, 5, 15, 25, 50, 75 and 125 ng ml^{-1} of HER2.

Fig. 4. (A) Comparison between the aptasnsor responses to 75 ng ml^{-1} HER2 with other interfering agents: including 1000 ng ml^{-1} adenosine, 1000 ng ml^{-1} guanosine 1000 ng ml^{-1} HSA and 1000 ng ml^{-1} BSA (e), in PBS (pH 7.4) at the scan rare of 10 mV s^{-1}. (B) Aptasensor responses to 75 ng ml^{-1} HER2 spiked in various sample including: PBS, pH 7.4 (standard solution), 25% human blood serum (Serum 1) and 50% human blood serum (Serum 2).

Table 1. Electrochemical HER2 aptasensors and immunosensors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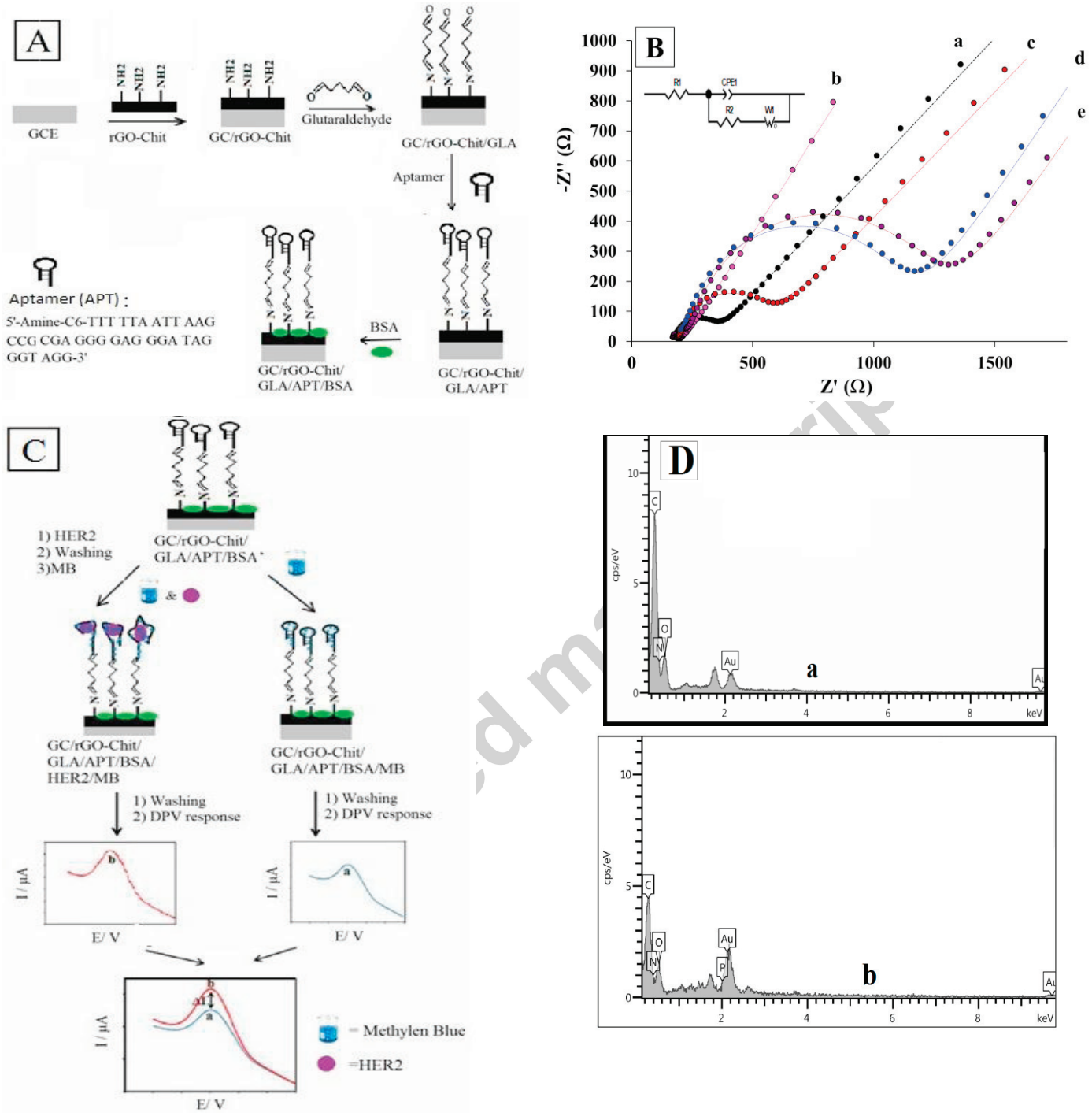
200 nm
EHT = 15.00 kV
WD = 7.7 mm
Signal A = SE2
Mag = 20.00 K X
Date :10 Jun 2015
User Name = SYSTEM
ZEISS
B
100 nm
C
%T
235
220
205
190
175
160
145
130
115
100
85
70
4000
3500
3000
2500
2000
1500
1000
500
0
Wavenumber / cm-1
a
b
c
d

Fig. 1


A
GCE
rGO-Chit
GC/rGO-Chit
Glutaraldehyde
GC/rGO-Chit/GLA
Aptamer
Aptamer (APT) :
5'-Amine-C6-TTT TTA ATT AAG CCG CGA GGG GAG GGA TAG GGT AGG-3'
BSA
GC/rGO-Chit/ GLA/APT/BSA
GC/rGO-Chit/ GLA/APT
B
-Z'' (Ω)
Z' (Ω)
a
b
c
d
e
C
1) HER2
2) Washing
3)MB
GC/rGO-Chit/ GLA/APT/BSA
GC/rGO-Chit/ GLA/APT/BSA/ HER2/MB
GC/rGO-Chit/ GLA/APT/BSA/MB
1) Washing
2) DPV response
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=HER2
D
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keV
C
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N
Au
P

Fig. 2

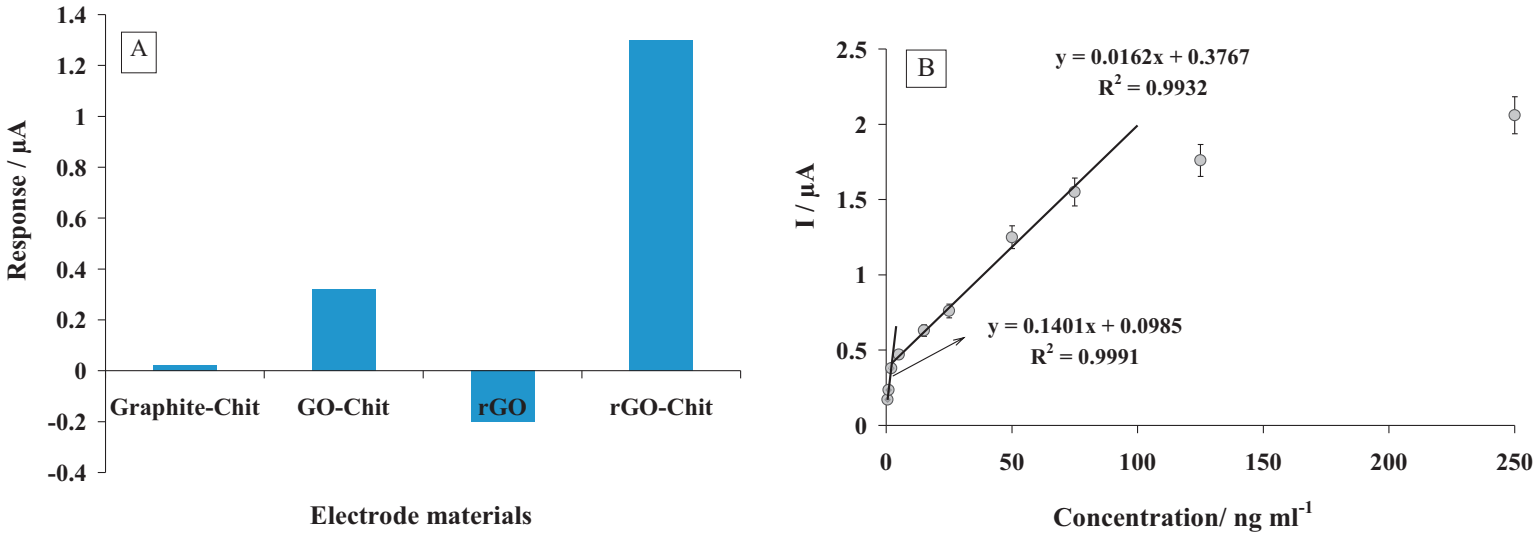

Fig. 3

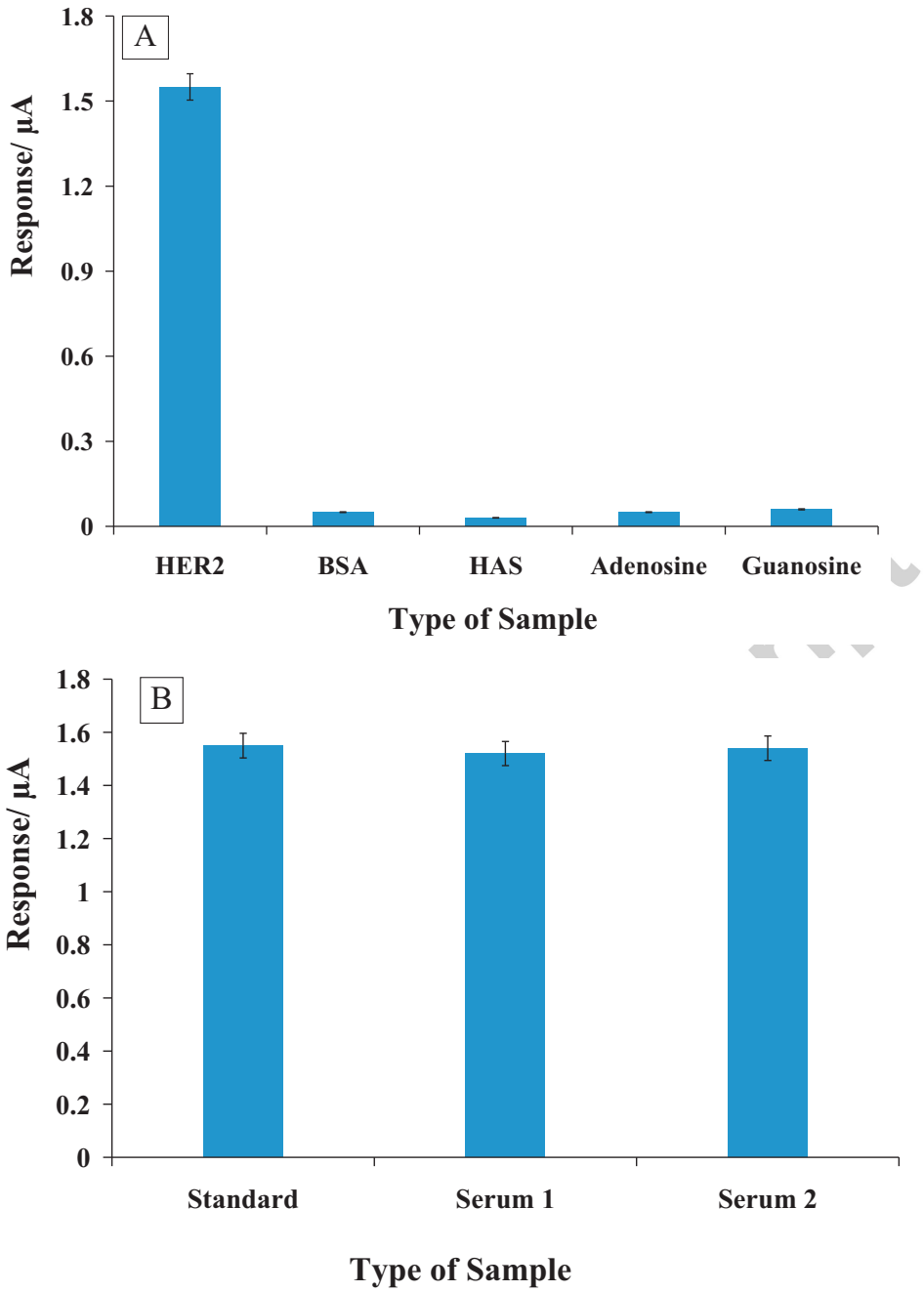

Fig. 4.

Table1. Electrochemical HER2 aptasensors and immunosensors

Type of Sensor	Technique	Detection limit	Linear range	Ref.
Electrochemical immunosensor	EIS[1]	7.4 ng ml^{-1}	10-110 ng ml^{-1}	(Arkan et al. 2015)
Electrochemical immunosensor	CV[2]	40 ng ml^{-1}	-	(Mucelli et al. 2008)
Electrochemical immunosensor	LSV[3]	4.4 ng ml^{-1}	15-100 ng ml^{-1}	(Marques et al. 2014)
Electrochemical immunosensor	DPV	6 ng ml^{-1}	0-30 ng ml^{-1}	(Al‐Khafaji et al. 2012)
Electrochemical immunosensor	DPV	1 μg ml^{-1}	1-200 μg ml^{-1}	(Patris et al. 2014)
Electrochemical affisensor	EIS	6 ng ml^{-1}	0-15 ng ml^{-1}	(Ravalli et al. 2015)
Capacitance aptasensor	n-FIS[4]	0.2 ng ml^{-1}	0.2-2 ng ml^{-1}	(Qureshi et al. 2015)
Electrochemical aptasensor	EIS	5 ng ml^{-1}	10^{-5}-10^{2} ng ml^{-1}	(Chun et al. 2013)
Electrochemical aptasensor	DPV	0.22 ng ml^{-1}	0.5-2 ng ml^{-1} 2-75 ng ml^{-1}	This work

1: Electrochemical impedance spectroscopy; 2: Cyclic voltammetry; 3: Linear sweep voltammetry; 4: non-Faradic impedance spectroscopy

Table 2: HER2 determination by the fabricated HER2 aptasensor and ELISA method.

Human serum sample	Spiked HER2 (ng ml^{-1})	Mean recovery % (HER2 aptasensor)	Mean recovery % (ELISA)
1	0	Not detectable	Not detectable
2	1	105	Not detectable
3	7	104	98
4	15	96	107
5	50	99	102

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Highlights

A novel and highly sensitive electrochemical aptasensor for detection of HER2 cancer marker was fabricated.

The rGO-Chit film provided a very favorable environment with high homogeneity and stability for the immobilization of aptamer molecules

Multiple MB signal amplification strategy, favorable properties of the rGO-Chit film and high selectivity of the applied HER2 aptamer resulted in very good analytical performances of the aptasensor.

The proposed HER2 aptasensor can be used for determination of HER2 in human serum samples.