Sensitive determination of NT-proBNP for diagnosing abdominal aortic aneurysms incidence on interdigitated electrode sensor

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Abstract

Abdominal aortic aneurysm (AAA) is a vascular disease found to have progressive growth in the area of aorta. Rupturing of aorta causes excessive bleeding that leads to health-related issues, which can be fatal sometimes. Therefore, it becomes important to make early diagnosis of AAA and its condition and start immediate treatment. Blood-based biomarker helps to diagnose AAA and to monitor the condition after AAA surgery. N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a hormone produced in the heart in small quantities and increased when the heart needs to work harder. NT-proBNP was proved to be strongly linked with AAA incidence. Moreover, quantifying the level of NT-proBNP helps to determine the risk factors on cardiovascular system after the surgery. This work is quantifying the NT-proBNP on interdigitated electrode sensor by using NT-proBNP binding aptamer. The detection limit of NT-proBNP was calculated as 1 pg/mL on a linear regression curve [$y = 0.2148x + 0.8849$; $R^2 = 0.9049$]. The linear range with dose-dependent analysis was from 0.01 until 100 ng/mL. Moreover, the control experiment with complementary aptamer sequence did not show the current signal, specifying the detection of NT-proBNP. This research benefits to identify the heart condition of patient after the removal of AAA. Volume 0, Number 0, Pages 1–6, 2020

Keywords: *abdominal aortic aneurysm, vascular disease, biosensor, dielectrode*

1. Introduction

Abdominal aortic supplies the blood containing oxygen to all over the body including abdomen and lower portion of the body. Abdominal aortic aneurysm (AAA) is the bulge in the area of abdomen aorta, which blocks or reduces the flow of blood to other parts of the body [1]. Various reasons such as age, hypertension, diabetes, cholesterol, and smoking are the main causes for AAA [2]. Rupture in AAA causes excessive bleeding and leads to life-threatening issues including death [3]. Early diagnosis followed by timely removal of AAA is mandatory to save the lives of patients. In general, imaging such as computerized tomography scan has been used to diagnose AAA. Along with the imaging, blood-based biomarker is helpful for AAA identification and follow-up treatment after the surgery. In addition, AAA has been identified accidently in most of the cases, while scanning for other complications. To overcome these issues, N-terminal pro-brain natriuretic peptide (NT-proBNP) is the hormone that is highly linked with the increasing risk of AAA can be considered as early AAA diagnosing biomarker. In particular, higher concentration of NT-proBNP

Abbreviations: *AAA, abdominal aortic aneurysm; APTES, (3-aminopropyl)triethoxysilane; IDE, interdigitated electrode; GNR, gold nanorod; NT-proBNP, N-terminal pro-B-type natriuretic peptide; RT, room temperature.*

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Highlights

Abdominal aortic aneurysm (AAA) is a vascular disease found to have progressive growth in the area of aorta and rupturing makes it urgent to create smart sensor. N-terminal pro-B-type natriuretic peptide is an AAA biomarker, quantified on interdigitated electrode sensor by aptamer–gold nanorod complex. The detection limit was 1 pg/mL on a linear regression with dose dependency from 0.01 until 100 ng/mL. This research helps to identify the heart condition of patient specifically with post-AAA operation.

is correlated with AAA [4]. Moreover, patient undergoing AAA surgery needs a follow-up treatment, especially focused on the side effects such as cardiovascular risk. As NT-proBNP is also found as one of the efficient biomarkers for cardiovascular-related problems, quantifying the level of NT-proBNP helps much with the AAA cases [5]. This research is targeted to determine NT-proBNP level by its specific aptamer on interdigitated electrode (IDE).

Aptamer is an oligonucleotide, either RNA or DNA, that generates from the mixed pool of library by using the steps with binding, separation, and amplification [6–8]. Aptamers are known as an artificial antibody, which can be used for various applications to replace antibody [9,10]. In addition, aptamers have higher sensitivity than antibody with their target and are widely utilized in various fields including biosensor [11–13]. Aptamers bind to their targets in small portion, which helps to discriminate the closely related biomolecules [14–16]. Several aptamers have been generated against various targets and utilized for different purposes. In this work, aptamer interaction with NT-proBNP was utilized to determine AAA progression.

Efficient probe immobilization on the sensor is mandatory to improve the detection limit. Electrostatic, chemical, and physical methods are commonly performed to attach the probe on the sensing surface [12,17–19]. Recently, usage of nanoparticles is also focusing on the biosensor field for probe immobilization due to its high biocompatibility [20–22]. Moreover, higher number of probes can immobilize through nanoparticle, which leads to the lowering of detection limit. Among other metals, gold nanoparticle is a well-established metal for the application of biosensor to improve the detection due to its optical behavior and stability. In addition, nanomaterial-conjugated biomolecules are more stable on the sensing surface and attract more analyte molecules, which enhance the limit of detection [23–25]. Gold is one of the potential nanomaterials and behaves better than other metallic particles because of its inertness, biocompatibility, easy availability in varied tailored sizes, and ability to get chemically modified easily. These characteristics make gold nanomaterial suitable for biological and nonbiological applications [16,26]. This research utilized gold nanorod (GNR) to conjugate the aptamer on the sensing surface in order to enhance the detection of NT-proBNP.

2. Materials and Methods

2.1. Biomolecules and reagents

Phosphate-buffered saline with pH 7.4, (3-aminopropyl) triethoxysilane (APTES), aluminum (Al)-etching solution, Al-coil, and ethanolamine were received from Sigma–Aldrich, Shanghai (MI). NT-proBNP was brought from Sino Biological (Beijing, China). GNR (length 700 nm; diameter 25 nm) was purchased from Nanopartz Inc. (Loveland, CO). Silica wafer was ordered from Mallinckrodt Baker Inc. (Philipsburg, NJ). Positive photoresist and resist developer was purchased from Futurrex Inc., Shanghai (NJ). Following aptamer sequences were commercially synthesized from the local supplier (Apical Scientific Oligo, Kuala Lumpur, Malaysia). The predicted secondary structure of aptamer by mfold program with the sequence 5′-ATACGGGAGCCAACACCAGATGCGAGGACGGTGGGTGGG AGGGTGGAGGT CTCGAGAGCAGGTGTGACGGAT-3′ (72 mer) [27].

2.2. Al-IDE sensing surface fabrication

The silica wafer (IDE substrate) was first cleaned with RCA2 and RCA1 to remove the unnecessary substance on the wafer. And then, at 500 °C, thermal oxidation was performed for 1 H to form the silicon dioxide. On the oxidized wafer, Al was deposited by using the Al-coil and thermal evaporator. The electrode pattern was made by this Al-substrate, and conventional photolithography technique was used. For that, using the spin coater, positive photoresist was applied on Al-surface, and then soft-baked (1 Min at 90 °C) to clear the stationary wave and the moisture on silicon dioxide surface. Ultraviolet light was exposed to the surface for transferring the pattern of IDE onto the photoresist. The area of unexposed wafer was removed by immersing in the photoresist developer. The developed surface was hard-baked (1 Min at 110 °C) to eliminate the moisture. Finally, the IDE was immersed in etchant liquid containing Al until the unexposed Al layer was completely etched. The fabricated surface was cleaned by acetone followed by distilled water and the surface immobilization was performed to detect the target.

2.3. Conjugation of aptamer on GNR

Aptamer was conjugated on GNR through the thiol linker. For that, 1 μM of diluted aptamer was mixed with one optical density of GNR and the mixture was rested for 1 H at room temperature (RT). And then, the bound aptamers with GNR were recovered by using the centrifugation (10,000g for 10 Min). The complex was washed with distilled water and again recovered the aptamer–GNR by centrifugation and kept in the refrigerator for future use.

2.4. Aptamer–GNR immobilization on IDE surface

Aptamer–GNR was attached on as fabricated Al-IDE through chemical surface functionalization. APTES was used as a linker between the aptamer and the IDE surface. Before this step, IDE was treated with 1% of KOH to enhance the APTES

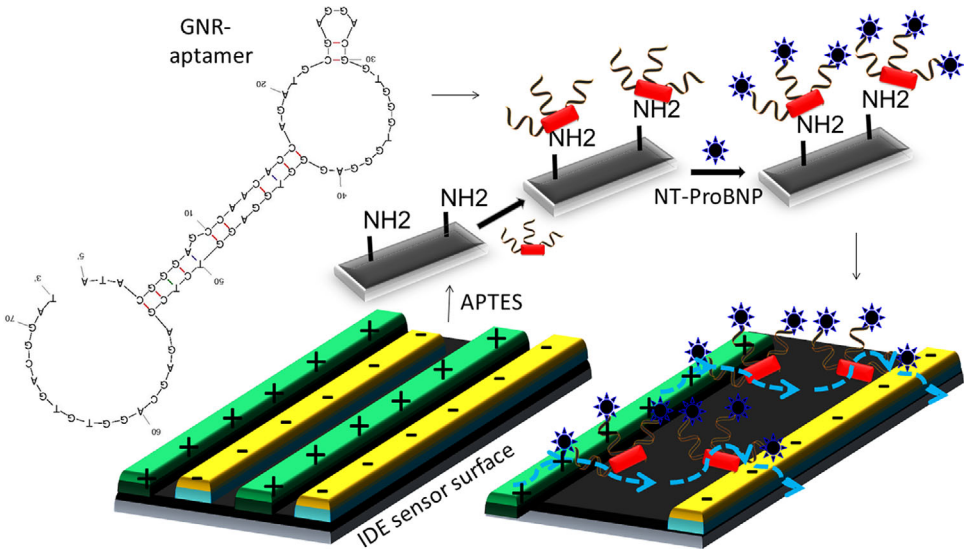

FIG. 1 *Schematic representation for detecting NT-proBNP on aptamer-modified IDE surface. Aptamer-conjugated gold nanorod was immobilized on IDE through amine linker and detected NT-proBNP. The predicted secondary structure of aptamer by mfold program and the surface mechanism on IDE surface are shown as insets. Dipole moment is displayed.*

immobilization. After washing the surface with distilled water, APTES at the dilution of 2% (diluted in 30% of ethanol) was added on IDE and incubated for 2 H followed by washing with 30% ethanol to remove the excess APTES. After that, 1 μM of aptamer–GNR complex was dropped on APTES-modified surface and kept 1 H at RT. After the washing steps, the aptamer-modified surface was used to determine NT-proBNP. For each step, before and after immobilization, the levels of current increments were recorded for comparison.

2.5. Interaction of NT-proBNP with aptamer

The interaction of NT-proBNP with its aptamer was evaluated on aptamer-modified IDE surface. Before that, aptamer-modified surface was blocked by 750 nM of ethanolamine to reduce the binding of other biomolecules. After that, NT-proBNP at the concentration of 100 ng/mL was interacted on the aptamer-immobilized surface for 5 Min. Before and after dropping of NT-proBNP, the current levels were recoded to find the interaction of NT-proBNP and aptamer.

2.6. Limit of detection of NT-proBNP

To find the minimum interaction of NT-proBNP with its aptamer, NT-proBNPs with different concentrations were diluted and dropped individually on aptamer-immobilized surfaces. NT-proBNPs from the concentrations of 1 pg/mL until 10 ng/mL by 10-order dilutions were prepared and added separately on the aptamer-modified IDE. The changes of current were noticed before and after the addition of NT-proBNP. The difference in current was plotted in an excel sheet to find the detection limit of NT-proBNP by the aptamer.

2.7. Control experiments: specificity analysis

To find the specific interaction of NT-proBNP with aptamer, the control experiments were executed with complementary sequences and the condition with no aptamer immobilization. For that, instead of specific aptamer, complementary aptamer sequences were conjugated with GNR and then immobilized on APTES-modified IDE surface. And then, 100 ng/mL of NT-proBNP was interacted and current changes were noted to find the specific binding of NT-proBNP by its aptamer.

3. Results and Discussion

Abdominal aortic aneurysm is the vascular progressive disease in the area of abdominal aorta and the subsequent rupture with a significant mortality exceeding with a time [28]. AAA with a diameter >55 mm represents the highest risk for rupturing and research has found that higher level of NT-proBNP correlates with AAA incidence [4]. Apart from that, monitoring the level of NT-proBNP is mandatory after the open chest repair of AAA, in order to avoid cardiac arrest. In this research, the level of NT-proBNP was quantified on IDE surface by using the specific aptamer selected against NT-proBNP. Figure 1 displays the schematic representation for the detection strategy with NT-proBNP. GNR-conjugated aptamer was immobilized on amine-modified surfaces and detected the NT-proBNP. Figure 1 also represents the secondary structure of aptamer and the molecular immobilized surface operates with dipole moment as explained earlier [29].

3.1. Surface functionalization on IDE surface

Surface immobilization of aptamer–GNR on IDE surface was confirmed by voltammetry analysis. As shown in Fig. 2A, only the bare Al-IDE shows the electric current flow as 5.3E−08 A. After APTES coating, the current flow was increased to

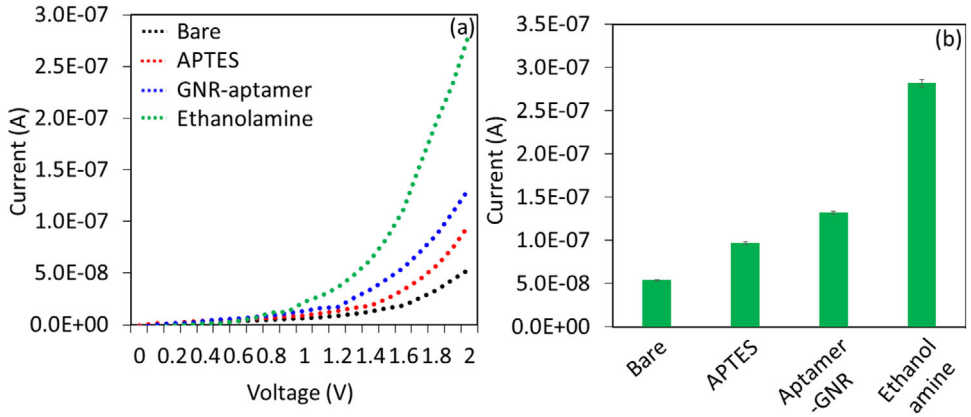

FIG. 2 *(A) Immobilization process of aptamer–GNR on IDE surface. After adding each biomolecule, current was increased gradually. (B) Graphical representation for current levels. Changes with each molecular immobilization are shown. Aptamer–GNR shows the clear current changes confirming the attachment of aptamer on IDE. Data were averaged by the number of replicates (n = 3).*

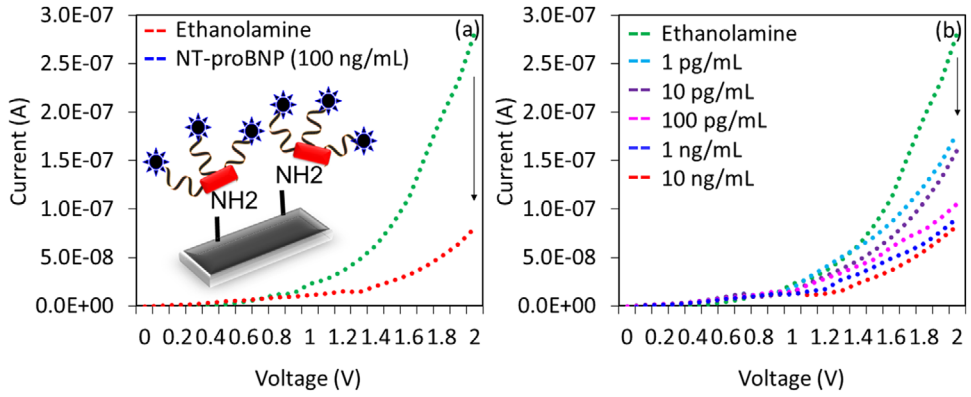

FIG. 3 *(A) Detection of NT-proBNP on aptamer-modified surface. NT-proBNP (100 ng/mL) was interacted on aptamer-immobilized surface. Clear current changes were noted that confirm the interaction of aptamer with NT-proBNP. Figure inset is displaying the graphical representation. (B) Binding affinity of different concentrations of NT-proBNP with its aptamer. Clear current changes were noted after interacting NT-proBNP on aptamer surfaces.*

9.65E−08 A. This increment of electric current flow confirmed that APTES modification on Al-IDE occurred. When 1 μM aptamer–GNR with was further added, the current level further increased to 1.31E−07 A, which clearly shows the attachment of aptamer–GNR on APTES-modified surface. As stated elsewhere, gold has attraction to the amine group by the electrostatic interaction. The similar strategy was used in the current study to attach GNR on the APTES-modified silica surface. This complex has been evidenced with no notable aggregation and enhances the plasmonic effect [30,31]. Finally, when 750 nM of was ethanolamine added, the surface electric flow was drastically increased to 2.81E−08 A. After each immobilization, the current change clearly shows the increment, indicating the immobilization of aptamer–GNR on IDE through the chemical modification (Fig. 2B).

3.2. NT-proBNP detection on aptamer–GNR-modified surfaces

On aptamer–GNR-modified surface, NT-proBNP was detected. As shown in Fig. 3A, 100 ng/mL of NT-proBNP interaction with immobilized aptamer was clearly noticed. The current flow was changed from 2.82E−07 (green line) to 8.01E−08 A (red line). The changes of current were noticed as 2.01E−07 A. This drastic change in current confirms the binding of NT-proBNP on aptamer-immobilized IDE surface.

3.3. Detection limit of NT-proBNP

To find the detection limit of NT-proBNP with aptamer, different dilutions of binding affinity of NT-proBNP with fixed 1 μM concentration of aptamer were evaluated. In Fig. 3B, NT-proBNP ranges from 1 pg/mL to 10 ng/mL interactions with aptamer were displayed. Ethanolamine blocked surface shows the current flow as 2.82E−07 A. After adding 1 pg/mL, it changed into 1.78E−07 A. This change of current shows the evident binding of NT-proBNP with its aptamer. Further increment with the concentrations to 10 pg/mL, 100 pg/mL, 1 ng/mL, and 10 ng/mL, the current levels were noticed as 1.6E−07, 1.05E−07, 9E−08, 8.29E−07, and 8.01E−07 A, respectively. This result clearly shows that with increasing

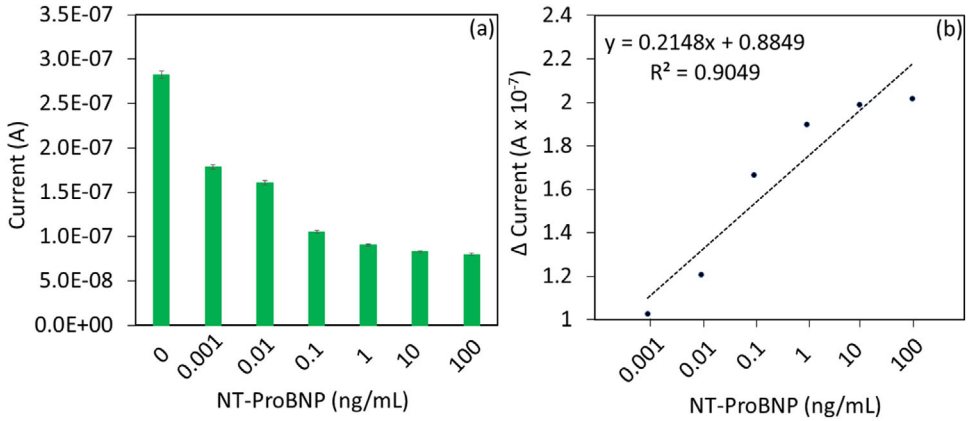

FIG. 4 *(A) Current level with binding of different concentrations of NT-proBNP with aptamer. With increasing NT-proBNP concentrations, current levels were gradually decreased. (B) A linear regression analysis. Difference in current changes upon NT-proBNP binding with aptamer. Differences are plotted in an excel sheet and the limit of NT-proBNP detection was calculated as 1 pg/mL. Data were averaged by the number of replicates (*n = *3).*

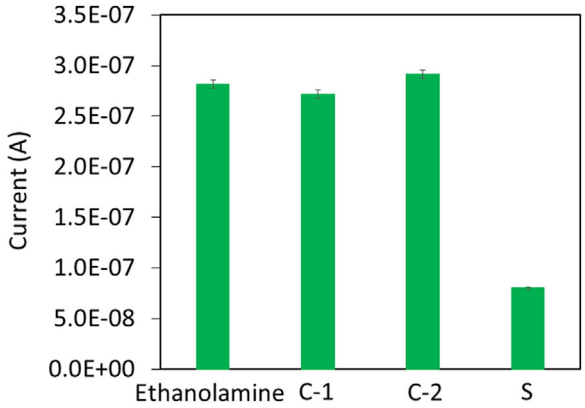

FIG. 5 *Control experiment for the specific NT-proBNP detection. Control experiment was carried out with complementary aptamer sequence (C-1) and the surface with no aptamer (C-2) and compared with the specific interaction of NT-proBNP (S). Control experiments did not show significant current changes, indicating the specific detection of NT-proBNP. Data were averaged by the number of replicates (*n = *3).*

NT-proBNP concentrations, the current flows were gradually decreased (Fig. 4A). The changes in current were plotted and the limit of NT-proBNP detection was found as 1 pg/mL (Fig. 4B).

3.4. Specific detection of NT-proBNP

Specific detection of NT-proBNP was analyzed with control experiments such as with complementary aptamer sequence and the surface with no aptamer attachment. As shown in Fig. 5, NT-proBNP failed to bind at the conditions with controls; at the same time, the current flow with only anti-NT-proBNP aptamer is clearly evident, indicating the specific detection of NT-proBNP.

4. Conclusions

In this study, identification of a vascular disease, AAA, was carried out with the biomarker NT-proBNP. Anti-NT-proBNP aptamer was used as the probe and immobilized on IDE surface by complexing the GNR. Aptamer-conjugated GNR was attached on IDE through the amine linker and detected NT-proBNP. Limit of detection of NT-proBNP was found as 1 pg/mL on linear dose-dependent concentrations and the control experiments with complementary aptamer sequence confirmed the specific NT-proBNP detection. This detection method with a finely tuned strategy helps to diagnose AAA and its follow-up treatment.

5. Conflict of Interest

The authors declare no conflict of interest.

5.1. Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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