

Freestanding Nanofiber-Assembled Aptasensor for Precisely and Ultrafast Electrochemical Detection of Alzheimer's Disease Biomarkers

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Amyloid beta-protein ($A\beta$) is a main hallmark of Alzheimer's disease (AD), and a low amount of $A\beta$ protein accumulation appears to be a potential marker for AD. Here, an electrochemical DNA biosensor based on polyamide/polyaniline carbon nanotubes (PA/PANI-CNTs) is developed with the aim of diagnosing AD early using a simple, low-cost, and accessible method to rapidly detect $A\beta$ 42 in human blood. Electrospun PA nanofibers served as the skeleton for the successive in situ deposition of PANI and CNTs, which contribute both high conductivity and abundant binding sites for the $A\beta$ 42 aptamers. After the aptamers are immobilized, this aptasensor exhibits precise and specific detection of $A\beta$ 42 in human blood within only 4 min with an extremely fast response rate, lower detection limit, and excellent linear detection range. These findings make a significant contribution to advancing the development of serum-based detection techniques for $A\beta$ 42, thereby paving the way for improved diagnostic capabilities in the field of AD.

pivotal role in causing the progressing cognitive decline in AD.^[2] $A\beta$ plaques consist of intracellular neurofibrillary tangles^[3] that encompass hyperphosphorylated tau protein and diverse $A\beta$ peptides, including $A\beta$ 42 and $A\beta$ 40, derived from the cleavage of the amyloid precursor protein.^[4] Recent studies suggest that targeted removal of $A\beta$ plaques from the brain can yield notable clinical benefits, potentially slowing cognitive decline in affected individuals.^[5] Traditionally, two well-established techniques are used to detect the formation of $A\beta$ plaques: one utilizes a positron emission tomography scan to show the regional distribution in the brain,^[6] and the other collects cerebral spinal fluid (CSF) through lumbar punctures.^[7] However, both of these methods suffer from high costs and invasive

1. Introduction

Amyloid beta-protein ($A\beta$) aggregation in the brain is a main hallmark of Alzheimer's disease (AD),^[1] and is considered to play a

operations, particularly the extraction of CSF, which carries high surgical risks, making early and onsite screening of potential Alzheimer's patients difficult.^[8] To address this above issue, several blood assay methods have been developed to determine the $A\beta$ level for AD diagnosis, such as enzyme-linked immunosorbent assay,^[9] immunoprecipitation mass spectrometry,^[10] and immunohistochemistry (IHC).^[11] These routes often have drawbacks such as time consumption (normally 2–5 h), high costs, bulky instruments, and unsatisfactory detection specificity.^[12] As a result, the innovation of a novel blood assay technique that delivers fast, low-cost, and sensitive determination of $A\beta$ without the need for bulky instruments is desirable.

Electrochemical biosensors facilitate the precise and discriminating detection of minute biomarkers in bodily fluids, showcasing an exceptional level of sensitivity and selectivity.^[13] Moreover, the device is easy to miniaturize into portable or handheld configurations for point-of-care or non-hospital assays.^[14] Among the various electrochemical biosensors, the aptasensors, which employ oligonucleotide probes with binding properties toward specific proteins or small non-protein molecules, have an ultralow detection limit and fast response time.^[15] Generally, the performance of an aptasensor is determined by the recognition strategy and the electrode material.^[16] For the $A\beta$ test, labeled and label-free aptamers as the probes are the two main strategies adopted for target binding and signal generation.^[17] For the labeled aptasensors, fluorescent dyes, enzymes, biotin, and aminated compounds are often used to supply an electrochemical signal source to DNA or RNA probes.^[18] This method enables

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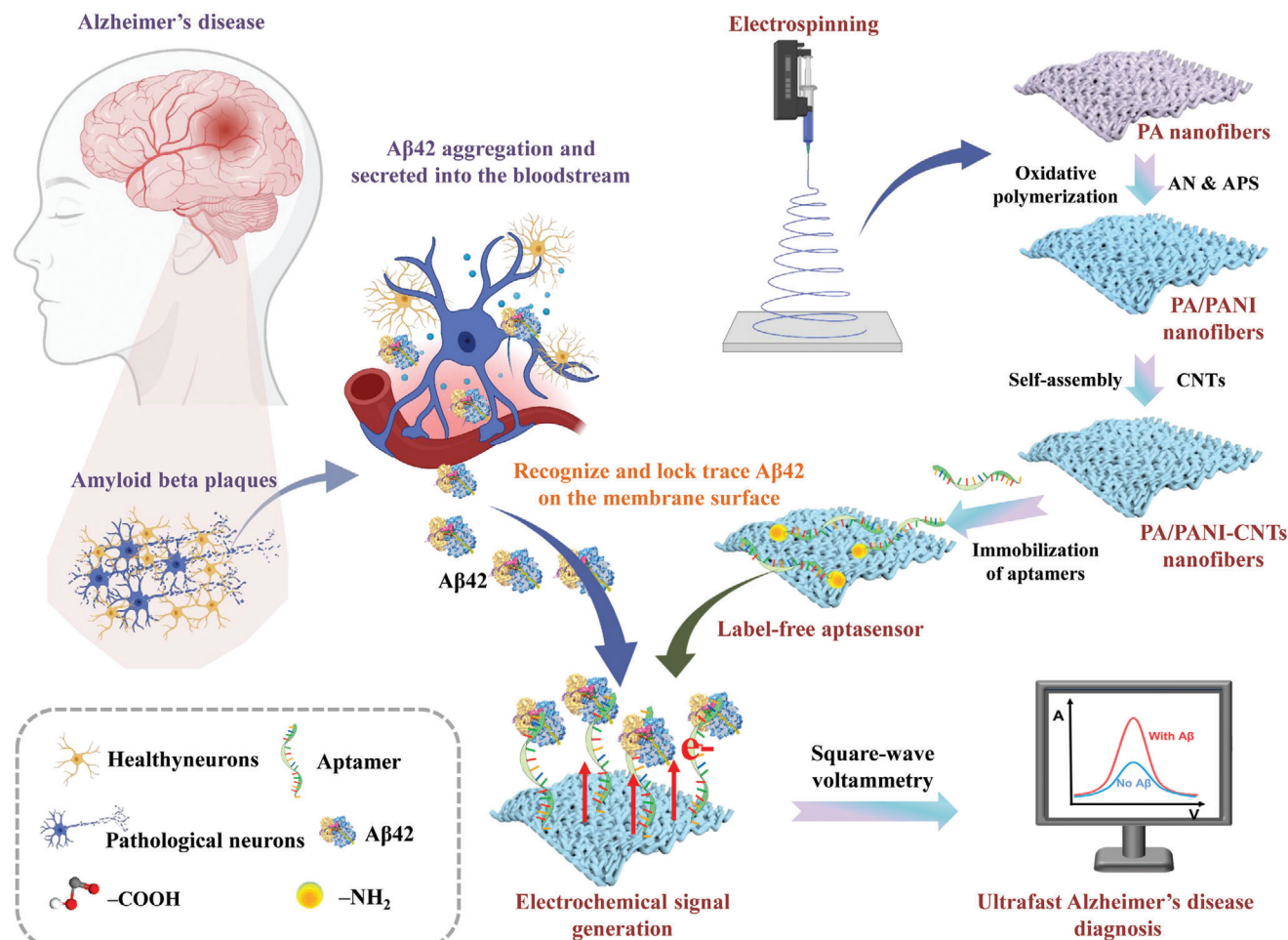
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DOI: 10.1002/adhm.202304355



Scheme 1. Schematic of the detection mechanism of the PA/PANI-CNTs nanofiber-based A β 42 aptasensor for ultrafast AD diagnosis. The electrochemical biosensor based on PA/PANI-CNTs enables the early diagnosis of AD through a simple, low-cost method that rapidly detects A β 42 in human blood.

the production of a strong electrochemical reaction, which improves the sensitivity. However, owing to the further modification of reporter molecules, the high cost, long binding time, and multiple processes on the oligonucleotide always block the large-scale applications of the labeled aptasensor.^[19] To overcome these deficiencies, the label-free aptasensors have attracted increasing attention. Nevertheless, owing to the lack of a signal reporter, this biosensor exhibits a relatively weak signal intensity derived from the binding reaction between the oligonucleotide and target protein. Therefore, the conductivity, surface area, and active sites of the aptamer assembly of the electrode material are essential for determining the detection performance.^[20]

In this work, we proposed an ultrafast label-free aptasensor by building a free-standing nanofiber membrane for loading DNA probes, which achieved the precise detection of A β 42 within only 4 min (Scheme 1). This membrane was prepared using an electrospinning technique to weave the polyamide/polyaniline-carbon nanotubes (PA/PANI-CNTs) nanofibers as a free-standing skeleton for the aptamer binding. Based on the reaction between the -NH_2 group of the DNA and the -COOH group of the CNTs, the designed single-strand

DNA matching the A β 42 protein was immobilized on the membrane as both capturer and reporter. This nanofiber membrane enabled the recognition and locking of trace A β 42 on the membrane surface, resulting in an obvious resistance decrease for the signal change. Using human serum as a sample, the as-prepared aptasensor exhibited excellent selectivity, detection limit, linear range, usage repeatability, and storage stability.

2. Results and Discussion

2.1. Structures and Properties of the Electrospun PA/PANI-CNTs Nanofibers

The main challenge in electrochemical aptasensors is to capture and amplify the response signal generated upon the binding of the aptamer to its target. Therefore, the construction of a conductive interface is crucial for providing abundant active sites to improve the loading capacity of DNA strands, thus playing a dominant role in the detection performance. PANI, a commonly used conductive polymer, was adopted as the main aptamer platform owing to its excellent extensibility and biocompatibility.^[21]

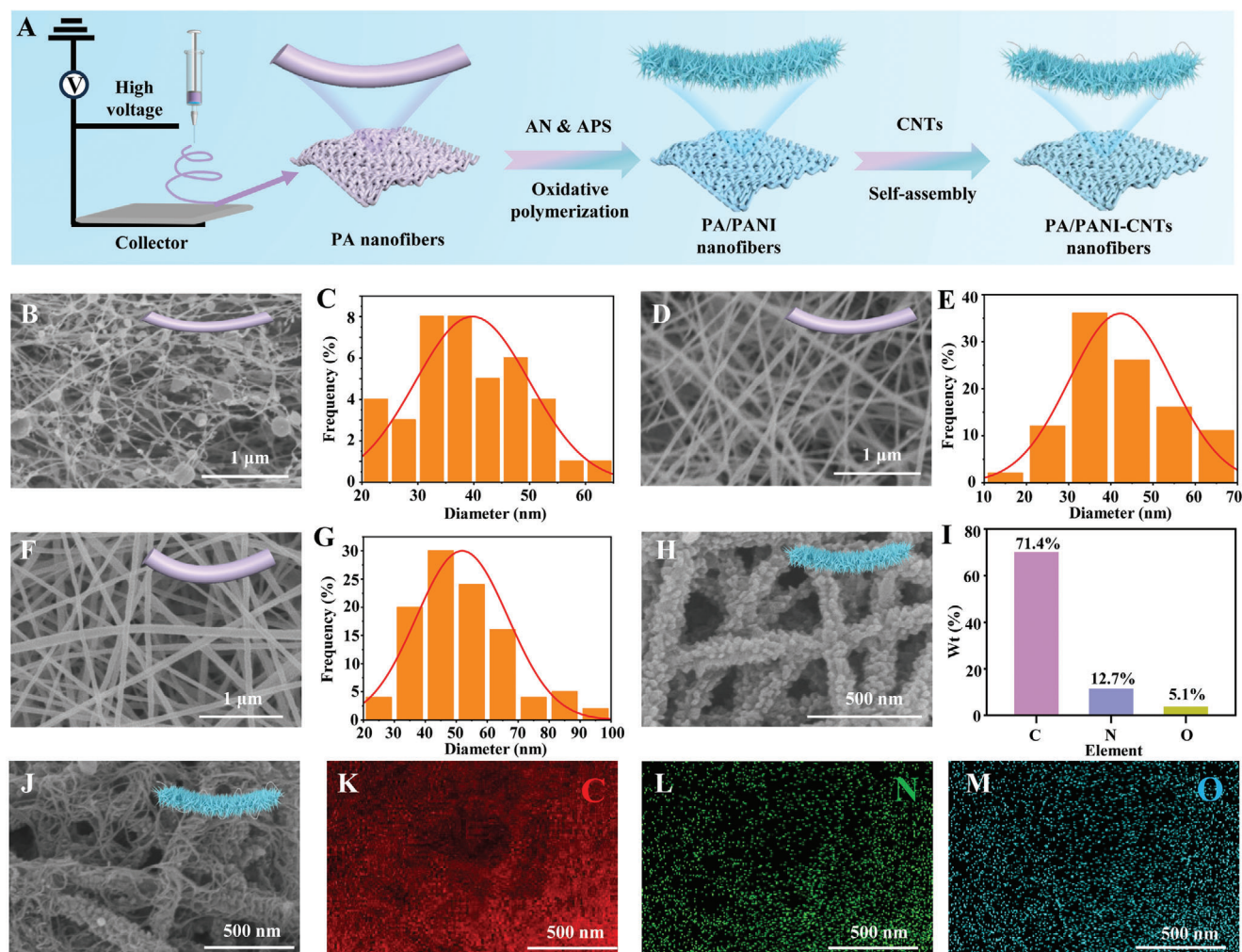


Figure 1. Characterization of nanofiber membranes. A) Schematic of the synthesis process of PA/PANI-CNTs nanofiber membranes. FESEM images and nanofiber diameter quantitative analysis of different electrospun nanofibers prepared at B,C) 10 wt.%, D,E) 16 wt.%, and F,G) 20 wt.% PA concentrations; FESEM images of H) PA/PANI nanofiber membranes. I) Elemental content analysis of EDX mapping on PA/PANI-CNTs. J) FESEM images of PA/PANI-CNTs. K–M) EDX mapping images of PA/PANI-CNTs nanofiber membranes showing the composition of C, N, and O.

To provide a soft growth skeleton for PANI, PA nanofibers were prepared to investigate the effects of the PA concentration in the spinning solution on the membrane nanostructure (Figure 1A). At the mass concentrations of 10 or 16 wt.% PA, the treated nanofibers showed a cracked surface with many beads (Figure 1B–E), resulting in lower catalytic activity and effective electrochemical specific surface area (Figures S1,S2, Supporting Information). If the mass concentration is increased to 20 wt.% PA, nanofibers with smooth surfaces and uniformly distributed diameters of ≈ 50 nm can be obtained, which is more conducive to the in situ deposition of PANI (Figure 1F,G). Moreover, the prepared nanofibers containing 20 wt.% PA presented a higher catalytic activity and effective electrochemical specific surface area of 0.25 cm^2 , which is approximately double those of the nanofibers at 10 wt.% PA (Table S1, Supporting Information). Upon continuous enhancement to 24 wt.% PA, the attained nanofibers' diameters were increased to ≈ 120 nm, with a smaller specific surface area (Figure S3, Supporting Information). However, if we further

increased the PA concentration to 28 wt.%, the spinning solution became too sticky to block the production of complete nanofiber structures (Figure S4, Supporting Information). Therefore, the prepared nanofibers membrane (Figure 1F) served as the optimum growth skeleton. For the PANI synthesis, the obtained PA nanofibers were immersed into aniline monomers and ammonium persulfate was added for polymerization. After the formation of PANI (Figures S5,S6, Supporting Information), many nanospikes were created in situ in the radial direction, significantly increasing the roughness of the nanofibers (Figure 1H).

As reported, PANI possesses good conductivity owing to its π - π conjugation.^[22] In this work, we designed $-\text{NH}_2$ modified aptamers matching A β 42. However, PANI cannot provide the corresponding functional groups to firmly immobilize aptamers through chemical bonds. Hence, we introduced CNTs as anchors to contribute abundant $-\text{COOH}$ groups to aptamer loading. Carboxylic carbon nanotubes (COOH-CNTs) were in situ on the PANI surface via layer-by-layer self-assembly. According to the

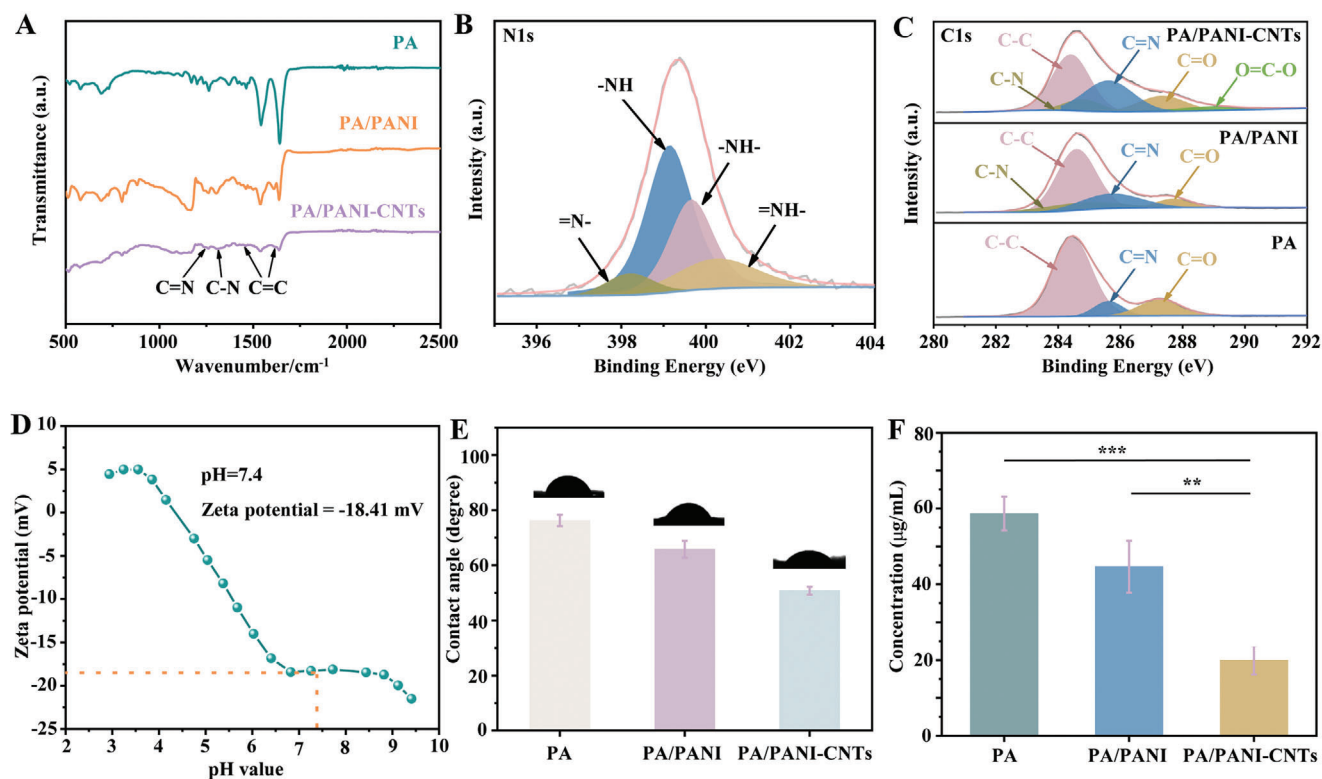


Figure 2. Performance characterization of nanofiber membranes. A) FTIR spectra of the PA, PA/PANI and PA/PANI-CNTs. B) N1s XPS spectrum of the PA/PANI-CNTs membrane and C) comparison of the C1s spectra of the PA, PA/PANI, and PA/PANI-CNTs. D) Zeta potential of the PA/PANI-CNTs in environments of varying pH environments. E) Contact angle and F) anti-protein adsorption performance of the PA, PA/PANI, and PA/PANI-CNTs.

field-emission scanning electron microscopy (FESEM) image in Figure 1J, the tubes dominate the surface of the nanofibers. This architecture enabled a further increase in the surface area derived from the cavities produced by the twinning of the nanotubes. Moreover, the energy-dispersive X-ray spectroscopy (EDS) results showed a uniform distribution of C, N, and O (Figure 1I) at concentrations of 71.4% (C), 12.7% (N), and 5.1% (O), indicating the existence of oxygen functional groups (Figure 1K–M).

The Fourier transform infrared spectroscopy (FTIR) spectra revealed the characteristic absorption peaks at 1252, 1306, 1468, and 1564 cm^{-1} , corresponding to C=N, C–N, and C=C functional groups (Figure 2A).^[23] Compared to the bare PA nanofibers, the presence of C–N and C=N functional groups indicates the successful deposition of PANI. Furthermore, in the PA/PANI-CNTs composite, the characteristic peaks of C=C peaks were observed owing to the graphitic structure of the CNTs.^[24] During the synthesis of PA/PANI-CNTs, the presence of carboxyl groups on the CNTs surface enhanced its solution dispersibility and adhesion, allowing the formation of amide bonds (–CONH–) through the reactions with amines (–NH₂).^[25] The bonding involving C, N, and O in the prepared PA, PA/PANI, and PA/PANI-CNTs was studied using X-ray photoelectron spectroscopy (XPS; Figure S7, Supporting Information). In Figure 2B, four absorption peaks can be distinguished to represent the =N–, –NH–, –NH–, and =NH– bonds in the PA/PANI-CNTs, confirming the typical molecular structure of PANI.^[26] Compared with the C1s spectra of the above three nanofibers (Figure 2C),

the appearance of C–N and C=N peaks is derived from PANI deposition, while the O=C–O peak is attributed to the CNTs loading,^[27] which produces an amidation reaction between the carboxyl groups on acidic CNTs and amine groups on PANI.

The surface properties of the PA/PANI-CNTs were further investigated to evaluate their biocompatibility with real serum samples. According to the zeta potential curve result (Figure 2D), in a serum environment of pH 7.4, the PA/PANI-CNTs were electronegative. These results indicated that the PA/PANI-CNTs nanofibers network could effectively inhibit the adhesion of a large number of negatively charged proteins during a real blood assay, thereby reducing the detection interferences. Furthermore, the water contact angle experiment confirmed that after the successive modification with PANI and CNTs, the hydrophilicity of the membrane was obviously increased (Figure 2E). This characteristic is beneficial for the adsorption of the target proteins in the serum onto the membrane surface, enhancing recognition and signal generation. After the deposition of both PANI and CNTs, the membrane exhibited the best anti-protein adsorption ability, which can effectively avoid the pollution of the nanofibers by non-target proteins in the blood.^[28] In electrochemical biosensor applications, non-specific protein adsorption frequently poses challenges that can interfere with sensor performance. As evidenced by the anti-protein adsorption assay (Figure 2F), the PA/PANI-CNTs demonstrated the lowest protein adsorption levels compared to PA and PA/PANI, indicating their superior resistance to protein adsorption.

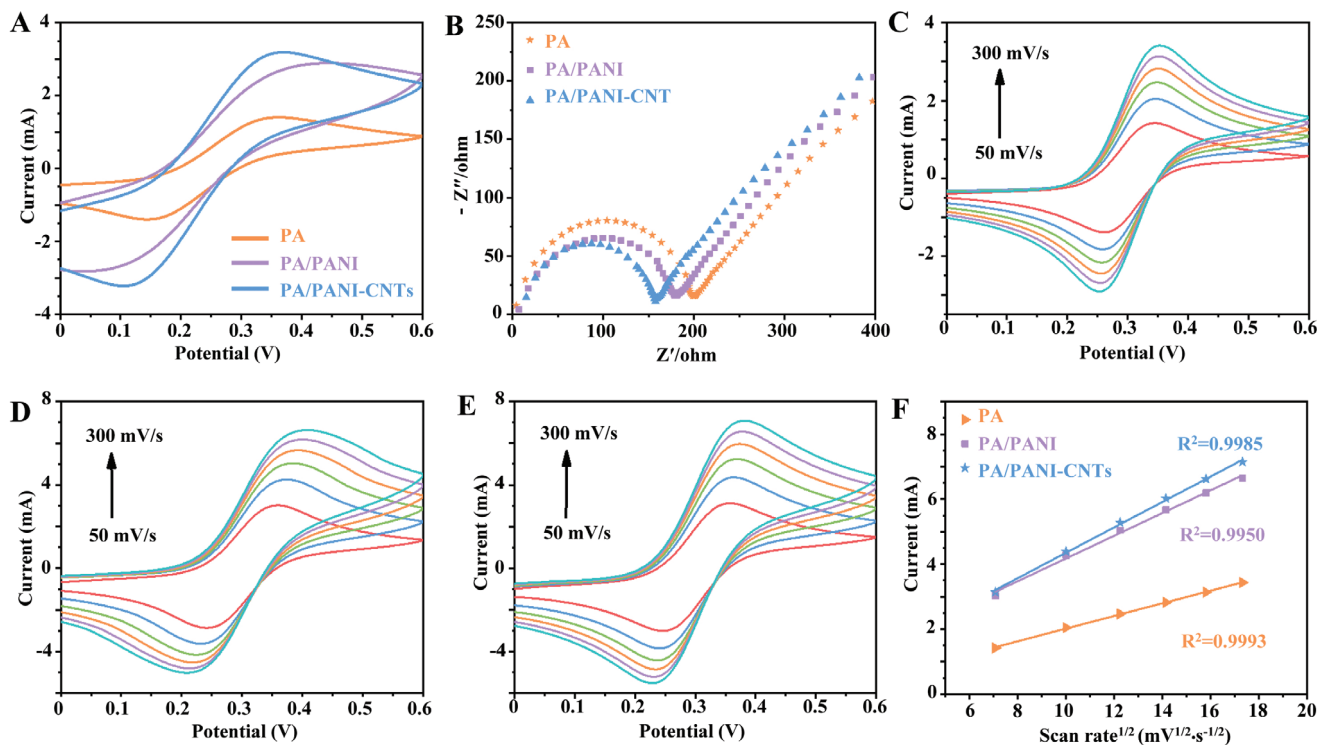


Figure 3. Electrochemical behaviors of nanofiber membranes. A) CV curves of the PA, PA/PANI and PA/PANI-CNTs in a solution containing 10 mM Fe (CN)₆^{3-/4-} and 0.1 M KCl. B) EIS diagram of the above three electrodes in the frequency range of 0.1 Hz to 1 MHz characterized in a solution containing 5 mM Fe (CN)₆^{3-/4-} and 0.1 M KCl. CV diagrams of C) PA, D) PA/PANI, and E) PA/PANI-CNTs-modified electrodes in a solution containing 10 mM Fe (CN)₆^{3-/4-} and 3 M KCl scanned at rates of 50, 100, 150, 200, 250 and 300 mV s⁻¹; F) Effective electrochemical surface area characterized in a solution containing 10 mM Fe (CN)₆^{3-/4-} and 3 M KCl.

2.2. Electrochemical Performance of the PA/PANI-CNTs-Modified Electrodes

As mentioned previously, the performance of the electrochemical biosensors is always determined by the electrocatalytic activity and conductivity of the electrode material. Hence, the electrocatalytic and conductive behaviors of the different nanofiber-modified electrodes were systematically evaluated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). **Figure 3A** shows the catalytic activities of the bare PA, PA/PANI, and PA/PANI-CNTs toward the redox reaction of Fe(CN)₆^{3-/4-}. After CNTs loading, the nanofibers exhibited the highest reduction and oxidation current peaks, confirming that the best electrocatalyst was derived from the excellent conductivity of the CNTs. Additionally, **Figure 3B** shows that the above PA/PANI-CNTs nanofibers provided a smaller Nyquist semicircle, suggesting a lower electron transfer impedance for enhancing the conductive capability, which is consistent with the CV results. Furthermore, the electrochemically effective surface areas of the bare PA, PA/PANI, and PA/PANI-CNTs were investigated using the Randles–Sevcik equation:

$$\frac{I_p}{v^{1/2}} = (2.69 \times 10^5) n^{3/2} D o^{1/2} C o \times A \quad (1)$$

where I_p is the redox peak current; v is the scan rate; n is the total number of electrons transferred during the redox process;

and D_o , C_o , and A referred to the molecular diffusion coefficient, probe molecule concentration, and electrode area, respectively. Obviously, the effective catalytic area was proportional to $I_p/v^{1/2}$. **Figure 3C–E** presents the CV curves of these three electrodes in a solution of 10 mM Fe (CN)₆^{3-/4-} containing 3 M KCl. By fitting Equation (1) (**Figure 3F**), the effective areas of the PA, PA/PANI, and PA/PANI-CNTs modified electrodes were calculated as 0.25, 0.451, and 0.504 cm², respectively (Table S2, Supporting Information). As a result, the PANI and CNTs deposited on the PA nanofibers can evidently increase the surface area of the electrode, which can also contribute to more catalytic sites to generate more electrons that participate in the reaction and attract more DNA strands for the signal magnification.

2.3. Biosensing Performance of the as-Prepared Aβ42 Aptasensor

Specific sequences of DNA probes can form stable spatial configurations with protein molecules through coordination or affinity interactions, enabling specific recognition of protein molecules. Compared to protein detection through immune reactions, the reaction time between DNA sequences and protein molecules is typically shorter, allowing for higher detection efficiency. To further enhance the specificity of our aptasensor for Aβ42 protein detection, we assembled Aβ42 aptamers onto the PA/PANI-CNTs nanofibers to obtain an electrochemical biosensor capable of recognizing AD biomarkers (**Figure 4A**). The incubation time is a critical parameter because both excessively long and short

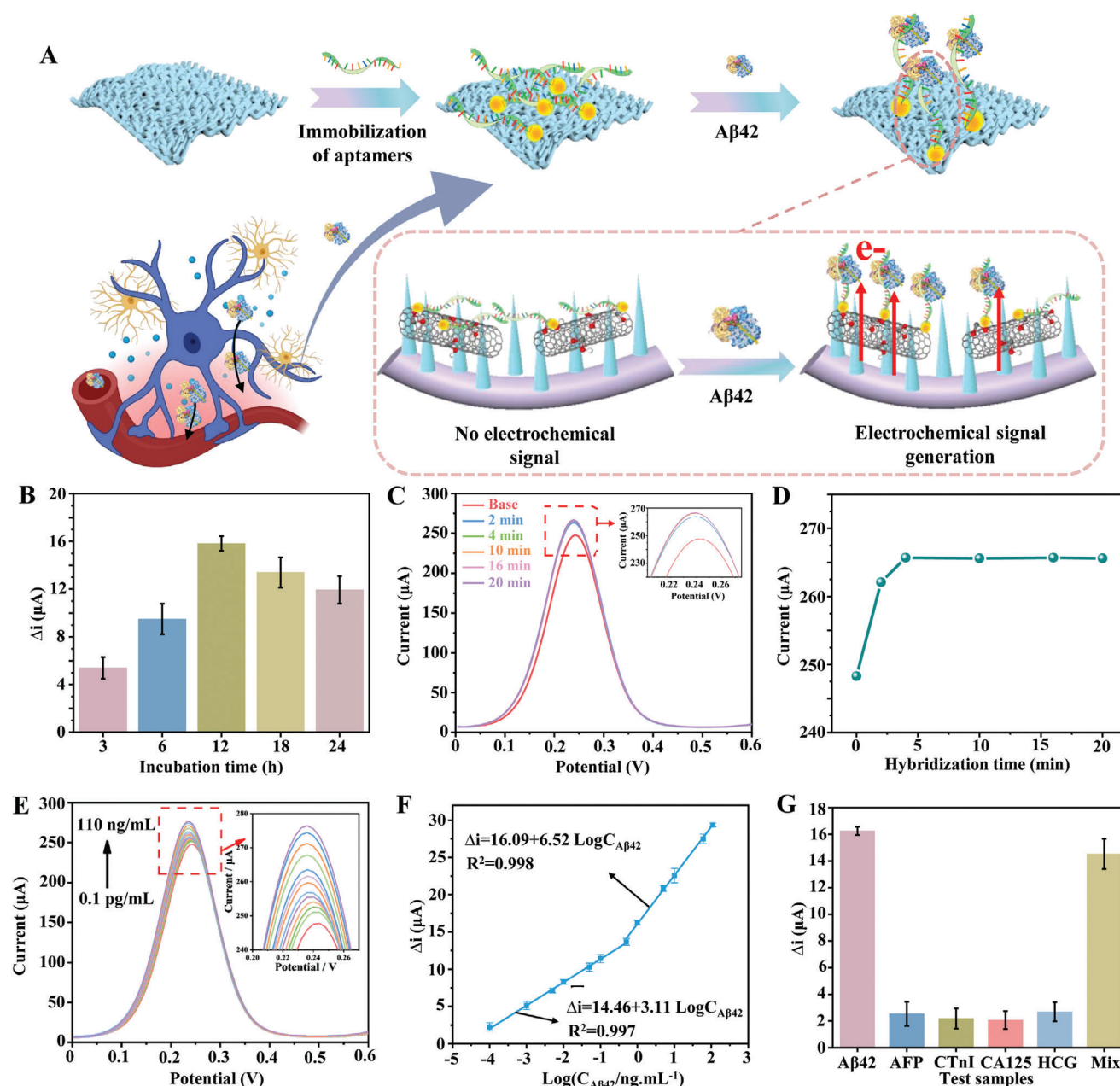


Figure 4. Biosensing performance of PA/PANI-CNTs-based aptasensor for detected $A\beta 42$. A) Schematic of the $A\beta 42$ detection process and electrochemical signal generation mechanism of the PA/PANI-CNTs-based aptasensor. B) Response signals of the aptasensor with varying DNA incubation times. C, D) Response signals of the aptasensor with different hybridization times of DNA and $A\beta 42$. E) Response signals of the as-prepared aptasensor for the detection of $A\beta 42$ with concentrations from 0.1 $pg\ mL^{-1}$ to 110 $ng\ mL^{-1}$. F) Linear calibration of the response current versus the $A\beta 42$ concentration. G) Selectivity test of the aptasensor in 1 $ng\ mL^{-1}$ $A\beta 42$ with 100 $ng\ mL^{-1}$ of various biomarkers as the interfering substances.

incubation times may negatively affect functionalization. We observed that when the incubation time between the aptamer and PA/PANI-CNTs nanofibers was 12 hours (Figure 4B), the newly developed aptasensor exhibited the strongest current signal, indicating saturation of the $A\beta 42$ aptamer binding on the surface of the PA/PANI-CNTs nanofibers. When the DNA probe was successfully loaded on the surface of the PA/PANI-CNTs nanofibers, the fabricated aptasensor was capable of $A\beta 42$ detection. Furthermore, it is necessary to determine the reaction time required

to reach the reaction equilibrium between the DNA probe and $A\beta 42$, which is also related to the rapid recognition and response of the biosensor to $A\beta 42$. When the developed aptasensor was used to assay $A\beta 42$ in the detection system of $A\beta 42$, as shown in Figure 4C,D, it can be determined that the current signal increases by 72.6% in 2 min and stabilizes within 4 min. The results indicated that the as-prepared aptasensor for the detection of $A\beta 42$ only needs 4 min to generate a high-intensity, stable current signal, which can effectively achieve the early warning

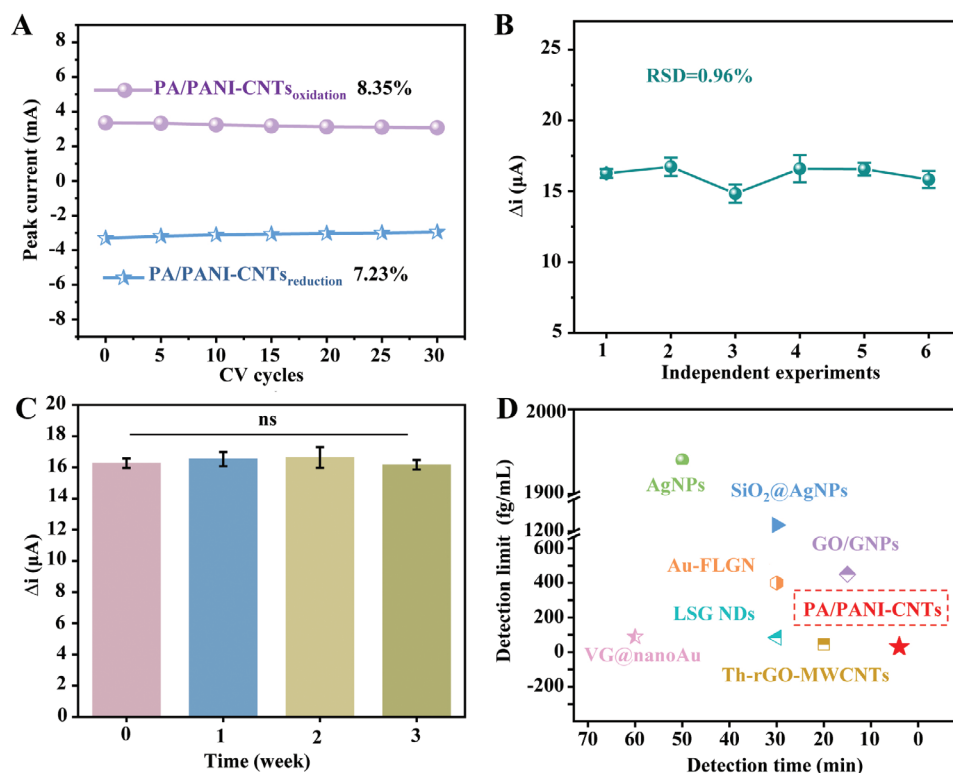


Figure 5. Electrochemical stability and performance advantages of PA/PANI-CNTs. A) Electrochemical stability of the prepared PA/PANI-CNTs investigated by 30 repetitive CV scans; B) Reproducibility of the aptasensor in 1 ng mL⁻¹ Aβ42; C) Long-term stability over 30 days; D) Comparison of the detection performance of the as-prepared aptasensor with those reported in the literature. The corresponding data and references are listed in Table S3 (Supporting Information).

of AD. The prepared electrochemical aptasensor was utilized to detect varying concentrations of Aβ42 to examine the relationship between its response signal and Aβ42 concentration. Based on the reaction between the -NH₂ group of the DNA and the -COOH group of the CNTs, the designed single-strand DNA matching the Aβ42 protein was immobilized on the membrane as both capturer and reporter. This nanofiber membrane enabled the recognition and locking of trace Aβ42 owing to the change in DNA configuration, which resulted in an obvious resistance decrease for the signal change. Therefore, the signal peak intensity gradually increased as the Aβ42 concentration increased from 0.1 pg mL⁻¹ to 110 ng mL⁻¹ (Figure 4E). Furthermore, the fitting results between the signal change (Δi) and the logarithm of Aβ42 concentration demonstrate a good linear relationship. When the Aβ42 concentration increases from 0.1 pg mL⁻¹ to 1 ng mL⁻¹, the signal changes caused by the combination of DNA and target is weak, and the regression equation is $\Delta i = 14.46 + 3.11 \log(C_{A\beta42})$. When the Aβ42 concentration was further increased from 1 to 110 ng mL⁻¹, more DNA strands bind to Aβ42, and the change of DNA configuration leads to a significant decrease in resistance. Therefore, in this case, the aptasensor demonstrated a higher sensitivity. (Figure 4F). Additionally, based on this signal amplification strategy, the detection limit of the Aβ42 aptasensor reaches as low as 30 fg mL⁻¹, meeting the requirements for trace-level Aβ42 detection. To investigate the effect of free CNTs, we have designed another PA/PANI-based aptasensor to assay Aβ42. As shown in Figure S8 (Supporting Information), the PA/PANI-

based aptasensor possessed a high detection limit, narrow detection range, and low sensitivity because PANI cannot provide the corresponding functional groups to firmly immobilize aptamers through chemical bonds and can physically adsorb only a few aptamers. Therefore, in our work, we designed PA/PANI-CNTs with a 3D network architecture to provide abundant binding sites for loading the aptamer recognizing Aβ42, so that achieving the rapid signal response and high sensitivity during the detection of trace Aβ42.

In addition, the anti-interference performance of the aptasensor is essential for the practical analysis of Aβ42 in real serum. The Aβ42 selectivity of the fabricated PA/PANI-CNTs-based aptasensor was investigated by comparing the current response values in Aβ42 and other antibiotics, including alpha-fetoprotein, cardiac troponin I (CTnI), cancer antigen 125 (CA125), and human chorionic gonadotropin. As shown in Figure 4G, when the sensor detected 1 ng mL⁻¹ Aβ42 alone, it generated a signal response of ≈16 μA. However, when a single interfering protein at a concentration of 100 ng mL⁻¹ was added to the detection system, the signal response was only ≈2 μA. Furthermore, when a mixture of proteins at a concentration of 100 and 1 ng mL⁻¹ Aβ42 was added, the current response signal was similar to that generated when 1 ng mL⁻¹ Aβ42 alone was detected. These results indicate that the PA/PANI-CNTs-based aptasensor in this study exhibits excellent anti-interference capability and high stability.

The electrochemical stability of the PA/PANI-CNTs-based aptasensor was examined, and the results are shown in Figure 5A.

Table 1. Detection results of A β 42 concentration in real serum samples by using the as-prepared aptasensor.

Sample	Added [pg mL ⁻¹]		Detected [pg mL ⁻¹]		Recovery [%]	RSD [%]
1	10	13.05	11.48	8.91	111.50	2.09
2	50	49.50	53.33	43.65	97.65	4.87
3	100	97.72	103.77	111.69	104.39	7.00
4	1000	970	1040	1150	105.33	0.09
5	60000	61650	60340	56230	99.01	2.82

After 30 CV scans, the oxidation–reduction peaks of this aptasensor exhibited only slight variations, with decreases of 8.35% and 7.23% respectively. This suggests that the biosensor prepared using these nanofibers possesses excellent electrochemical stability. Furthermore, six independent aptasensors were prepared using the same method and applied to detect 1 ng mL⁻¹ A β 42 (Figure 5B). The results show minimal differences in the response signals among these sensors (RSD = 0.96%), confirming the excellent reproducibility of the aptasensor. To further investigate the long-term stability of the aptasensor, we prepared multiple sensors in batches for a one-month testing period, as shown in Figure 5C. Following a 30-day long-term testing period, the aptamer-based sensor displayed an exceptional capability to maintain its initial performance with minimal degradation, indicating a minimal impact on its performance from long-term low-temperature storage and fatigue testing and demonstrating good stability. As shown in Figure 5D and Table S3 (Supporting Information), the aptasensor exhibited an ultralow detection limit, a wide linear range, and, compared to previously reported A β 42 sensors, a significantly better detection time of only 4 min.

2.4. Practical Analysis of Human Serum Samples Constructed Aptasensor

To evaluate the performance of the sensor in practical applications, the PA/PANI-CNTs aptasensor was applied for the detection of A β 42 in human serum. Serum is a component of blood that does not contain red blood cells, white blood cells, or clotting factors. The serum utilized in this investigation was randomly sampled from patients and subsequently subjected to protein-removal procedures, assuming the absence of the A β 42 protein within the serum. The results (Table 1) showed that when different concentrations of A β 42 were added to the serum for detection, the sensor exhibited recovery rates close to 100% and low RSD values. This observation underscores the substantial potential of the PA/PANI-CNTs aptasensor for detecting minute quantities of A β 42.

3. Conclusion

In this study, we innovatively designed an aptasensor based on PA/PANI-CNTs for the ultrasensitive detection of A β 42. The PA/PANI-CNTs aptasensor exhibited high sensitivity, strong specificity, and a rapid response—surpassing similar sensors—with excellent accuracy and stability in real serum sample detection. The design of this ultrasensitive label-free aptasensor provides robust support for the early diagnosis, research, and treatment of AD, offering broad prospects for clinical application.

4. Experimental Section

Reagents: Polyamide (PA) (Mw $\approx$ 20 000) was purchased from Shanghai Macklin Biochemical Co., Ltd. Formic acid (HCOOH) was acquired from Shanghai Ling Feng Chemical Reagent Co., Ltd. Aniline (AN, 99.5%), and carboxylic carbon nanotubes (COOH-CNTs) were provided by Sinopharm Chemical Reagent Co., Ltd., China. Ammonium persulfate (APS, 98.0%) was obtained from Nanjing Xianfeng Nanomaterial Technology Co., Ltd. The A β 42-targeting-aptamer applied in this work was synthesized, purified, and modified by Sangon Biotech (Shanghai) Co., Ltd., China and its base sequence was as follows: A β 42-Apt: 5′-NH₂-(CH₂)₆-CCGG TGGG GGAC CAGT ACAA AAGT GGGT AGGG CGGG TTGG AAAA-3′. Tris(2-carboxyethyl) phosphine hydrochloride (TCEP, 98%), 6-mercapto-1-hexanol (MCH), tris(hydroxymethyl)aminomethane (Tris-HCl, 99.0%), and ethylenediaminetetraacetic acid (EDTA, 99.5%) were purchased from Aladdin, China. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC-HCl, 95%) and N-hydroxysuccinimide (NHS, 98%) were obtained from Macklin Biochemical Co., Ltd. DNA immobilization buffer (I-buffer, pH = 7.4): 10 mM Tris-HCl + 1 mM EDTA + 0.1 M NaCl. Washing buffer (W-buffer, pH = 7.4): 10 mM Tris-HCl. All the chemical reagents were of analytical grade, and deionized water was used in the whole experiment.

Apparatus: The JNS-SBS-01 microfluidic spinning device (Nanjing Janus New Materials Co., Ltd., Nanjing, China) was used for the microfluidic spinning process. FESEM (Hitachi, Model S-4800II, Japan) was used to characterize micromorphology. An X-ray diffractometer (D/MAX 2500 V/PC) with a Cu-K α line (0.15 419 nm), X-ray photoelectron spectrometer (Thermo, ESCALAB 250XI), and Raman spectrometer (Thermo Scientific DXR) were used to analyze the quality of the prepared materials. In addition, electrochemical measurements, including CV chronoamperometry, EIS, and square-wave voltammetry (SWV) were performed at electrochemical workstations (CHI660E or CHI 920D, Shanghai Chenhua Instrument Co., Ltd., China).

Fabrication of Electrospun PA Nanofibers: Electrospun PA nanofibers were synthesized through an electrospinning process. First, 10 g of PA solid was dissolved in 40 g of HCOOH solvent and stirred at room temperature for 12 h to acquire a 20 wt.% spinning solution. Subsequently, the solution was electrospun under a positive voltage of 18 kV and a negative voltage of 6 kV, with a flow rate of 0.6 mL h⁻¹ and a rotation speed of 1000 rpm. The electrospinning conditions were constantly monitored at an ambient temperature of 25 °C and a relative humidity of 40 $\pm$ 2%. Finally, the collected nanofiber membrane was transferred to a vacuum oven and dried at 60 °C for 10 h to remove the residual HCOOH.

Synthesis of PA/PANI-CNTs Nanofibers: Conductive polymers PANI were synthesized in situ on the electrospun PA nanofibers through a low-temperature chemical oxidative polymerization process. First, 0.2 M AN in 1 M H₂SO₄ was prepared to form an AN/H₂SO₄ uniform solution. APS (0.2 M) in 1 M H₂SO₄ was prepared to form the APS/H₂SO₄ uniform solution. The PA nanofibers were immersed into the AN/H₂SO₄ mixed solution at 2 °C for 2 h. Next, the APS/H₂SO₄ solution was added to the AN/H₂SO₄ mixed solution and stirred uniformly. After that, PA nanofibers were incubated in the uniform solution to react for 2 h at 2 °C. Finally, the PA/PANI nanofibers were washed three times with 1 M H₂SO₄ and deionized water and dried at 60 °C for 8 h. Thus, the PA/PANI nanofibers were obtained. To provide grafting sites for DNA probes and further amplify

sensing signals, carboxylic carbon nanotubes (COOH-CNTs) were loaded onto PA/PANI nanofibers by layer-by-layer self-assembly. Then, the developed PA/PANI-CNTs nanofibers were washed with deionized water and dried at 60 °C for 8 h.

Fabrication of PA/PANI-CNTs-Based A β 42 Aptasensors: The PA/PANI-CNTs nanofibers constructed from the novel self-supporting electrode were immersed in a mixed solution of EDC (100 μ L, 0.3 M) and NHS (100 μ L, 0.1 M) at 2 °C for 2 h to activate the carboxyl groups in the PA/PANI-CNTs. Then, the electrode was incubated with A β 42-Apt (100 μ L, 2 μ M) in an I-buffer for 12 h at 2 °C. After the reaction was completed, the physically adsorbed DNA probe was washed and removed with deionized water. Then, the obtained electrode was treated with 5 mg mL⁻¹ BSA solution for 2 h to block nonspecific sites, washed with W-buffer for three times to reduce physical adsorption, and used for the performance tests.

Anti-Protein Adsorption Experiment: Using bovine serum albumin (BSA, Thermofisher, USA) as the model protein, the anti-adsorption performance of the membranes (PA, PA/PANI, and PA/PANI-CNTs) through static protein adsorption assays was detected. Initially, membrane samples were trimmed to an effective area of 2 cm² and then rehydrated by immersing them in 2 mL of deionized water for 30 minutes. Subsequently, the samples were exposed to a phosphate-buffered saline solution (PBS, Corning, USA) for another 30 minutes followed by immersion in the BSA solution (750 μ g mL⁻¹) at room temperature for 12 h. The concentration of BSA solution before (C_b = 750 μ g mL⁻¹) and after (C_a) sample immersion was measured using a BCA protein assay kit (Thermofisher, USA).

The protein adsorption (C) was determined using the following Equation:

$$C = C_b - C_a \quad (2)$$

Detection of A β 42 in the Real Serum Samples Using the Constructed Aptasensor: Human serum was obtained from Nanjing Drum Tower Hospital (Nanjing, China). The Institutional Review Board for Clinical Investigations at Affiliated Drum Tower Hospital of Medical School of Nanjing University approved this study on November 27, 2018 (No. 2018-252-01). First, the obtained serum samples were centrifuged and treated for 15 min at a rotating speed of 10 000 rpm to further remove the residual macromolecular substances in the serum samples. Subsequently, the serum samples containing A β 42 were diluted with PBS (pH = 7.4) at different concentrations as the solution to be tested. The aptasensor was used to determine the content of A β 42. The electrochemical experiments SWV (the modified PA/PANI-CNTs electrode, a Pt wire, and an Ag/AgCl (saturated KCl) electrode were utilized as the working electrode, counter electrode, and reference electrode, respectively) were employed to explore its signal response in the real serum sample.

Statistical Analysis: This data was statistically analyzed with one-way ANOVA, depending on the data set, and visualized using GraphPad Prism 9 software. All statistical results were represented as mean $\pm$ standard deviation with a significance of $P < 0.05$ unless indicated differently (ns: not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

H.L. and X.Y. contributed equally to this work. This work was financially supported by the National Key R&D Program of China, China (2021YFC2103300), the National Natural Science Foundation of China (22108120, 22078148 and 82301104), the Natural Science Foundation of Jiangsu Province (BK20220002, BK20230160), Development Project of Nanjing University of Technology (No. KL20-01) and the "3456" Cultivation Program for Junior Talents of Nanjing Stomatological Hospital, Medical School of Nanjing University (0222R212). The authors also thank the BioRender for providing drawing elements.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

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

Keywords

Alzheimer's, electrochemical aptasensors, electrospun nanofibers, real serum detection, ultrasensitive trace A β 42 assay

Received: December 7, 2023

Revised: February 7, 2024

Published online: February 28, 2024

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