

Label-Free Electrochemical Detection of Transthyretin for Targeted Therapeutic Monitoring in ATTR-CA Patients

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Abstract—This paper presents an electrochemical biosensor for transthyretin (TTR), a cardiovascular disease biomarker, using antibody-functionalized Au screen-printed electrodes. The point-of-care sensor demonstrated a concentration-dependent increase in charge transfer resistance (R_{ct}) for residual native TTR. The limit of detection in buffer solution was estimated at 18 ng/mL, demonstrating sufficient sensitivity for detecting TTR levels well below the clinical threshold of 100 µg/mL in critical patients. To the authors' knowledge, this is the first study to electrochemically monitor TTR levels in patient fluids, with a specific focus on detecting residual TTR tetramers.

Index Terms—Transthyretin (TTR), electrical impedance spectroscopy, cardiac amyloidosis, screen printed electrodes

I. INTRODUCTION

Transthyretin cardiac amyloidosis (ATTR-CA) accounts for approximately 10-15% of global heart failure cases [1]. The condition arises from misfolding of the transthyretin (TTR) protein, which leads to the deposition of amyloid fibrils in cardiac tissue. TTR, also known as prealbumin, is a liver- and choroid plexus-derived homotetrameric protein that transports thyroxine and retinol via retinol-binding protein 4 (RBP4) [2]. Fig. 1 illustrates the pathogenic process, wherein TTR tetramers misfold into a continuum of non-native species that exist in dynamic equilibrium and are challenging to isolate, detect, or quantify [6].

Several disease-modifying therapies have been approved by the FDA for this amyloidosis condition, suggesting advances in targeted therapies [3]. These include protein stabilizers such as tafamidis, which preserves the integrity of TTR tetramer, and gene-silencing therapies like vutrisiran that reduce hepatic TTR production [4]. Despite these therapeutic advances, current practices of monitoring treatment efficacy are indirect and rely on surrogate indicators such as NT-proBNP, troponins, cardiac imaging, and functional assessments including the 6-minute walk test and NYHA classification [5]. This underscores the need for diagnostic platforms capable of directly measuring circulating TTR levels and assessing tetramer stability in real time during therapy.

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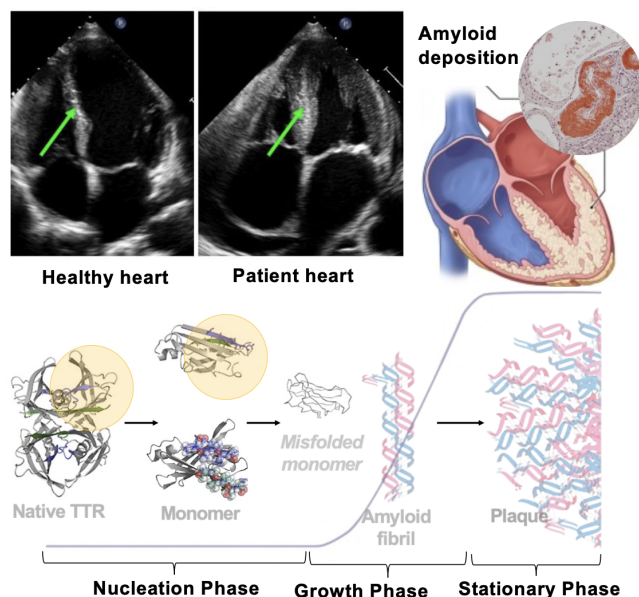


Fig. 1: Schematic representation of transthyretin cardiac amyloidosis (ATTR-CA) pathogenesis. Native TTR tetramers undergo dissociation into monomers, which subsequently misfold and aggregate into oligomers and insoluble amyloid fibrils, leading to progressive myocardial deposition and structural remodeling [6].

Given these monitoring limitations, label-free detection using impedimetric biosensors has emerged as a promising diagnostic strategy, particularly in easily accessible biological fluids [7]. These sensors operate on the principle of electrochemical impedance spectroscopy (EIS), a non-destructive technique that enables the detection of specific molecular interactions by measuring changes in capacitance or charge-transfer resistance at the sensor surface [8]. EIS offers distinct advantages in high-ionic-strength biofluids, as it is unaffected by Debye screening and supports multiplexed detection [9]. The present work focuses on developing an impedimetric immunosensor using antibody-functionalized gold screen-printed electrodes (SPEs), establishing a scalable, reproducible, and

clinically viable platform for TTR detection.

II. METHODS AND MATERIALS

A. Antibody Modification of Au Screen Printed Electrodes

Fig 2 describes the systematic protocol employed for antibody immobilization on Au SPEs. Electrodes were cleaned with isopropanol (IPA), rinsed with Milli-Q water, and dried under a nitrogen (N_2) stream.

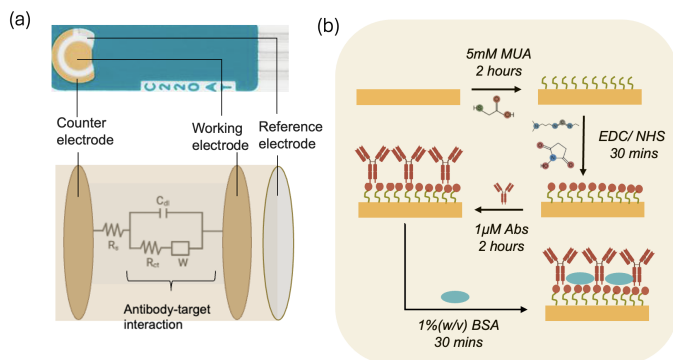


Fig. 2: (a) Schematic of a three-electrode electrochemical sensor setup with corresponding Randles circuit for impedance analysis. (b) Stepwise functionalization of Au SPEs using EDC/NHS activation for directed antibody orientation.

A self-assembled monolayer (SAM) was formed by incubating the Au working electrode with 10 μL of 5mM 11-mercaptoundecanoic acid (MUA) in a 90:10 ethanol–water solution for 2 hours. Carboxyl groups on the SAM were activated by a 30-minute incubation with 20 μL of a solution containing 10 mM N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and 20 mM N-Hydroxysuccinimide (NHS) in MES buffer (pH 6.0). Anti-TTR antibodies (DAKO; 1 $\mu g/mL$) were immobilized by 2-hour incubation, followed by blocking with 1% (w/v) bovine serum albumin (BSA) in PBS. Electrodes were rinsed with PBS and stored until further use. A similar approach was reported by Stefano *et al.* and Brazaca *et al.* [10], [11]. All incubations were performed at room temperature (22 $^{\circ}C$) in a humidified chamber to prevent droplet evaporation.

B. Cyclic Voltammetry for Confirming Chemical Modification of Au Electrodes

Cyclic voltammetry and EIS were conducted using an Autolab electrochemical station (Metrohm, Switzerland). Electrodes were scanned across a potential range of -0.4 to 0.6 V in 5 mM $[Fe(CN)_6]^{3-/4-}$ at a scan rate of $100 mVs^{-1}$. The progressive changes in peak position and current intensity were evaluated after each modification step.

C. Faradaic electrical impedance spectroscopy for electrochemical detection

Following immobilisation of antibodies, the electrodes were incubated with 1x PBS spiked with varied TTR concentrations,

and measurements were performed in a redox solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$, in open-circuit conditions (0.1 to 100 kHz at an amplitude of 10 mV). Sensor interface was evaluated using the Randles circuit, accounting for solution resistance, charge transfer (R_{ct}), double-layer capacitance, and diffusion effects. Detection sensitivity was quantified based on the absolute change in R_{ct} before and after TTR exposure.

III. RESULTS AND DISCUSSION

A. Validating Antibody Immobilisation on Au Electrodes

Cyclic voltammetry (CV) was employed to monitor changes in charge transfer properties during Au SPE functionalization. Overlapping scans confirmed a contaminant-free surface. As shown in Fig. 3, well-defined redox peaks were observed for bare Au, indicative of a clean, conductive surface with fast electron transfer. Following MUA modification, a decrease in anodic peak current was observed, consistent with SAM formation.

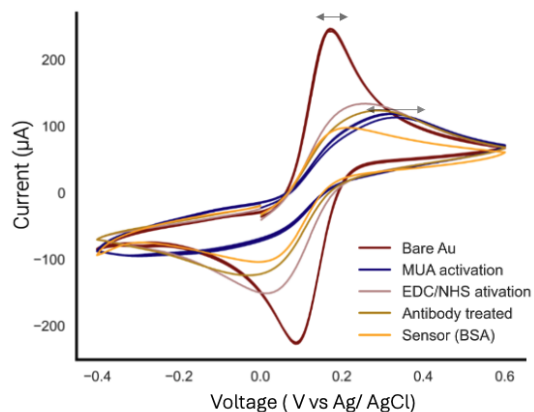


Fig. 3: Cyclic voltammogram characterizing chemical modification of Au SPEs, arrows denote the progressive increase in peak-to-peak separation, reflecting reduced electron transfer rates ($N=3$).

The decline in anodic peak current after MUA treatment results from its insulating nature, which partially hinders charge transfer due to the presence of terminal carboxyl ($-COOH$) groups. Subsequent EDC activation further reduces current, confirming carboxyl activation through formation of amine-reactive intermediates that increase steric hindrance. Antibody immobilization leads to an additional drop in current density, consistent with protein-layer insulation. Final BSA blocking causes a slight decrease, indicating successful passivation of unreacted sites. The observed increases in peak-to-peak separation (ΔE_p) and reduced anodic current quantitatively indicate progressive surface modification and hindered electron transfer.

Nyquist plots (Fig. 4a) were fitted using a Randles equivalent circuit, with charge transfer resistance (R_{ct}) serving as the primary metric for interfacial changes. The linear response observed for bare Au indicated a diffusion-controlled electron transfer process with minimal R_{ct} . A substantial increase in

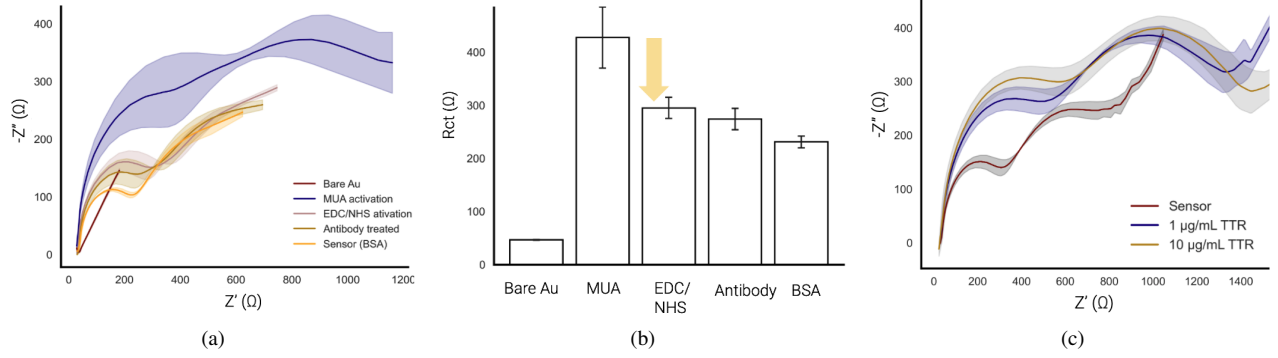


Fig. 4: (a) Nyquist plot depicting charge transfer resistance (R_{ct}) at different functionalization stages, with increasing impedance upon antibody immobilization (b) R_{ct} variation after each modification step, highlighting formation of insulating organic and biomolecular layers (c) Nyquist plot showing an increase in (R_{ct}) with TTR concentrations, indicating protein adsorption at the sensor surface ($N=3$)

R_{ct} ($> 400 \Omega$) was observed after MUA modification, consistent with the formation of a resistive self-assembled monolayer (Fig. 4b). A subsequent decrease following EDC/NHS activation reflected enhanced surface hydrophilicity due to conversion of carboxyl groups to amide bonds, facilitating antibody attachment. Stepwise increases in R_{ct} throughout the remaining functionalization stages further confirmed successful and progressive interface modification.

TABLE I: Nyquist parameters for antibody functionalization: R_{ct} and C_{dl} variation across electrode-electrolyte interface for $N=3$

Functionalization process	R_{ct} (Ω)	C_{dl} (μF)
Bare Au SPE	46.41	63.0
MUA (SAM)	427.55	11.2
EDC/NHS (carboxyl group activation)	294.88	12.1
Anti-TTR Antibodies	273.90	13.4
1% BSA (for non-specific interactions)	230.71	14.2

B. Residual TTR detection in 1x PBS via Modified Sensors

Upon incubation with $1 \mu g/mL$ and $10 \mu g/mL$ TTR, the nyquist plots showed a clear increase in semicircle diameter, corresponding to R_{ct} values of 750Ω and 840Ω , respectively (Fig. 4c). This increase reflects the insulating nature of the adsorbed TTR layer, which impedes charge transfer at the interface. The trend was consistent across replicates, confirming the reliability of the sensor response.

Quantitative analysis of charge transfer resistance (R_{ct}) across increasing TTR concentrations (Fig. 5) revealed a non-linear response profile. Modest changes were observed at lower concentrations ($1-10 \mu g/mL$), followed by a pronounced increase at $100 \mu g/mL$. This trend suggests a biphasic adsorption mechanism, wherein initial binding is limited by the availability of antibody sites, and higher concentrations lead to surface saturation and protein crowding. The baseline R_{ct} of the antibody-functionalized sensor was approximately 470Ω ,

which increased to 950Ω at the highest TTR concentration tested.

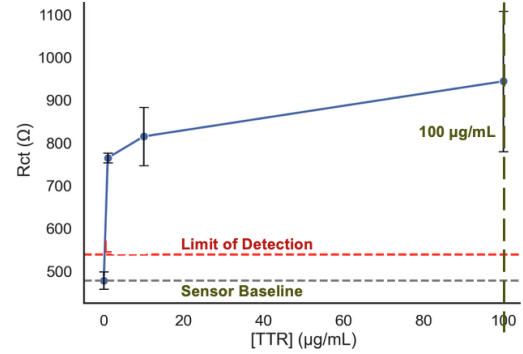


Fig. 5: Residual TTR detection via modified sensors showing a concentration-dependent increase in R_{ct} ($N=3$).

The sensor demonstrated a limit of detection (LoD) of 18 ng/mL , calculated over its full dynamic range. While this level of sensitivity is promising, further improvements are needed in resolution and specificity, particularly at low concentrations where small impedance changes must be reliably distinguished from background fluctuations. The platform however shows strong potential for sensitive TTR detection but requires refinement to improve analytical resolution and standardize interfacial binding kinetics.

CONCLUSION

This study demonstrates the viability of a label-free electrochemical immunosensor for detecting residual TTR at clinically relevant concentrations for personalised therapy. Sensor's low detection limit highlights its analytical sensitivity in buffer solutions. Future work will focus on enhancing detection fidelity and specificity in complex biological matrices and expanding the sensor's capacity for multiplexed cardiac biomarker profiling, enabling broader applicability in pre-symptomatic cardiovascular diagnostics.

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