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An electrochemical membrane-based aptasensor for detection of severe acute respiratory syndrome coronavirus-2 receptor-binding domain

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A R T I C L E   I N F O

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- Nanoporous anodic aluminium oxide membrane decorated with gold nanoparticles
- Laser-engraved graphene electrode
- 3-D printed flow-cell
- Electrochemical aptasensor
- SARS-CoV-2-RBD

ABSTRACT

Herein, we report an electrochemical membrane-based aptasensor for the determination of the SARS-CoV-2 receptor-binding domain (SARS-CoV-2-RBD). For this purpose, the nanoporous anodic aluminium oxide membrane (NPAOM) was first fabricated electrochemically. The NPAOM was then functionalized with 3-mercapto- propyl trimethoxysilane (NPAOM-Si-SH). After that, the NPAOM-Si-SH was decorated with gold nanoparticles by using gold ion and sodium borohydride. The NPAOM-Si-S-Au nano was then attached to the surface of the working electrode of a laser-engraved graphene electrode (LEGE). Subsequently, the LEGE/NPAOM-Si-S-Au nano was fixed inside a flow cell that was made by using a three-dimensional (3D) printer, and then thiolated aptamer was transferred into the flow cell using a pump. The electrochemical behavior of the LEGE/NPAOM-Si-S-Au nano Aptamer was studied using square wave voltammetry (SWV) in the presence of potassium ferrocyanide as a redox probe. The response of the LEGE/NPAOM-Si-S-Au nano Aptamer to the different concentrations of the SARS-CoV-2-RBD in human saliva sample was investigated in the concentration range of 2.5-40.0 ng/mL. The limit of the detection was found to be 0.8 ng/mL. The LEGE/NPAOM-Si-S-Au nano Aptamer showed good selectivity to 5.0 ng/mL of SARS-CoV-2-RBD in the presence of five times of the interfering agents like hemagglutinin and neuraminidase as the influenza A virus major surface glycoproteins.

1. Introduction

The International monetary fund estimates the coronavirus disease 2019 (COVID-19) pandemic will cost the global economy $12.5 trillion by 2024 [1]. For this reason, it is essential to decrease the global economic losses and solve the global health issue caused by COVID-19. Beside the fabrication of the vaccines, the fabrication of a cheap, high sensitive, and selective device that can detect the syndrome coronavirus-2 (SARS-CoV-2) before appearing the symptom of COVID-19 is also important. Hence, several research groups have been working to fabricate the biosensors to detect the SARS-CoV-2 in the early stage of COVID-19. The spike protein [2–5], nucleocapsid protein [6–8], a short fragment of its gene [9–11], and receptor-binding domain (RBD) [12–14] of the SARS-CoV-2 are the common biomarkers that were detected in the real samples to diagnose the COVID-19.

Nowadays, several methods have been reported for the detection of the biomarkers related to the SARS-CoV-2 like optical [15–18] and electrochemical methods [19–21]. Among them, the electrochemical method has several advantages for the diagnose viruses such as low cost, high sensitivity, ease to use and being portable [22–24].

The electrochemical aptamer-based sensors (aptasensors) and immune-based sensors (immunosensors) are widely used biosensors for the detection of the biomarkers related to SARS-CoV-2 [25,26]. The electrochemical aptasensors [27–29] have several advantages over the immunosensors [30–32] including low price, high stability, ease of fabrication, and in some cases better specificity and affinity.

Nowadays, nanomaterials have been used to increase the analytical performance of electrochemical sensors [33,34]. Among them, the nanoporous anodic aluminium oxide (NPAOM) has drawn tremendous interest in developing electrochemical biosensors due to its three-dimensional (3D) structure, high surface area, low-cost, easy fabrication, biocompatibility, and high mechanical stability [35–37]. Hence it is one of the favorite nanomaterials to fabricate the electrochemical biosensor [38,39].

Hence, we decided to take the advantage of the electrochemical method, aptamer-based recognizer, and NPAOM to fabricate a biosensor for diagnosing the COVID-19.

This research work contains three major fabrication steps: (1) The Fabrication of the nanoporous anodic aluminium membrane decorated with gold nanoparticles; (2) The immobilization of the aptamer inside...
the membrane; and (3) The fabrication of the disposable electrode.

The anodic aluminium oxide membrane was first electrochemically fabricated and then modified with the thiol-silane coupling agent by using 3-mercaptopropyl trimethoxysilane. According to the previous report, anodic aluminium oxide functionalized with thiol groups can interact with gold ions [40]. After that, gold ions were reduced to gold nanoparticles by using sodium borohydride solution. Consequently, the thiol-terminal aptamer probes were then immobilized on an anodic aluminium oxide membrane decorated with gold nanoparticles due to the covalent bond between thiol groups and gold [41]. The mechanism of the proposed membrane-based aptasensor relies on the change in the diffusivity of the membrane to a redox probe like ferrocyanide (Fe(CN)_6^3-)..

According to the literature [39,42,43], as a huge molecule like a protein incubates with the immobilized bio-recognized inside the nanochannel of the membrane, the diffusivity of the membrane toward a redox probe would decrease because of the mass transfer limiting of a redox probe to the surface of the electrode. Consequently, the recorded faradaic current caused by a redox probe decreases. The amount of the change in the faradaic current depends on the concentration of the target molecule.

In this research work, we measured the RBD of the SARS-CoV-2 which is made of 70 amino acids [44], and its size is small (~6 nm) [45]. Since the size of the SARS-CoV-2 (100 nm) [44] is bigger than the diameter of the nanochannels of the NPAOM (55 nm), the proposed membrane-based aptasensor cannot be used for the detection of the SARS-CoV-2.

Finally, we have fabricated a disposable electrode using the laser-engraved graphene method [46–48]. To do that, a Kapton tape (poly polyimide) was used as a substrate to carbonize it to graphene by using a diode laser. During the process of the carbonization of Kapton tape to graphene, the pulsed laser irradiation converts the sp^3-carbon atoms of polyimide into sp^2-carbon atoms [49,50]. This method has several advantages in comparison to the wet-chemistry method such as one step, easy, eco-friendly, and safe method [51].

The obtained results exhibited that the proposed membrane-based aptasensor showed a high analytical performance to the SARS-CoV-2-RBD.

2. Experimental section

2.1. Reagents and chemicals

All chemicals were of analytical reagent grade and used without further purification. Double deionized (DI) water (18.6 MΩ) was used throughout the research work. Aluminium (Al) discs of 15 mm diameter were obtained from Good fellow. The thiol terminal aptamer was purchased from the Nyttech company and its sequence was: SH-(CH_2)_6-5’-CAG CAC CCA CCT TGT GCT TTG GGA GTG CTG GTC CAA GGG CGT TAA TGG TCA A-3’. Gold (III) acetate Au (III) acetate, sodium borohydride (NaBH_4), phosphoric acid (H_3PO_4), potassium hydroxide (KOH), 3-mercaptopropyl trimethoxysilane (3-MPS), oxalic acid (Ox), perchloric acid (HClO_4), hydrogen peroxide (H_2O_2), and chromium (VI) oxide (H_2CrO_4), potassium ferrocyanide (Fe(CN)_6^3-), and magnesium chloride (MgCl_2) were obtained from Alfa Aesar. 3-mercaptop-1-propanol (3-MCP), Tris (2-carboxyethyl) phosphate hydrochloride (TCEP), syndrome coronavirus-2 receptor-binding domain (SARS-CoV-2), hemaglutinin (HA) and neuraminidase (NA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Kapton tape (Polyimide, 100 mm wide, 33 mm long) was purchased from Sakitik (Tarragona, Spain).

2.2. Apparatus

Scanning electron microscopy (SEM) and atomic force microscopy (AFM) were performed using a Field Electron and Ion (FEI, Hillsboro, OR, USA) and a DME DualScope Scanner DS 95–200 (Herlev, Denmark). The elemental analysis was performed using an Energy-dispersive X-ray (EDX) spectroscopy (EDAX, Mahwah, NJ, USA). The attenuated total reflectance spectrum (ATR) analysis and Raman scattering were carried out using a Nicolet iS50 Fourier transform infrared spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) and Renishaw’s in Via Raman spectrometer (laser wavelength: 514 nm), respectively. The square wave voltammetry (SWV) studies were performed using a potentiostat–galvanostat from Metrohm-DropSens Model µStat 300.

2.3. The fabrication of LEGE

A 3-electrode system (working, auxiliary, and reference) was designed using a diode laser (Power: 1.5 W, Intensity: 30%) in the following steps. First, two layers of Kapton tapes were attached to a photo paper. After that, the surface of the Kapton-photo paper was cleaned with ethanol to remove any figure prints and wax. Consequently, the designed pattern of the electrode was engraved on the surface of the Kapton tape (Fig. S1A). The fabricated LEGE was finally washed and used for the fabrication of the membrane-based aptasensor. A Microsoft PowerPoint was used for the design of the 3-electrode system. The SEM image of the LEGE is shown in Fig.S1B. As can be seen, the fabricated LEGE has a uniform sheet structure. The Raman spectrum of the LEGE was also recorded (Fig.S1C). As can be seen, the G band at 1575 cm^-1 is assigned to the E2g phonon of C sp^2, and the D band at 1355 cm^-1 is assigned to the breathing mode of K-point phonons of A1g symmetry [53].

2.4. The fabrication of the flow cell

Sketch Up software was used to design the 3-D image and converted it to STL format (Fig S2A-D). Then, the STL format of the design was converted to g-code using Ultimaker Cura 4.12.1 software. The electrochemical flow cell setup was then designed using a 3D printer and a transparent filament (thermoplastic polyurethane) (Fig S3A-B). After the fabrication, the flow cell was dangled in an acetone bath for 5 min to smooth the surface of the flow cell. The final size of the flow cell was 30 mm, 30 mm, and 7 mm in length, width, and height respectively.

2.5. Fabrication of nanoporous anodic aluminium oxide-gold nanoparticles-aptamer (LEGE/NPAOM-Si-S-Au nanoparticles-Aptamer) and the measurement process of NPAOM

NPAOM has been fabricated by 2-step anodization electrochemical anodization of a high purity Al in 0.3 M Ox, according to the previous method [54]. The fabrication process is mentioned in the electronic supporting material. To convert the nanoporous anodic aluminium oxide (NPAO) to nanoporous anodic aluminium oxide membrane (NPAOM), the remaining Al should be removed at the backside of NPAO. For that purpose, the NPAO was dipped in CuCl_2/HCl solution. The sample was then washed with DI water and subsequently put in 5% H_3PO_4 for 90 min at room temperature to widen the pore diameters of the NPAOM. Finally, the NPAO membrane was rinsed with DI water and put in an oven (70°C) for 2 h to dry. Fig.S4 shows the photo image of an NPAOM.

To silanize the NPAOM, 5 mL of dry toluene and then 0.2 mL of 3-MPS were mixed, and then the NPAOM was immersed in it for 1 h under stirring conditions. The silanized NPAOM (NPAOM-Si-SH) was then removed from the solution and rinsed with dry toluene thoroughly. NPAOM-Si-SH was dried under a nitrogen stream slowly and finally put in an oven (70°C) for 6 h.

To decorate the membrane with gold nanoparticles, the NPAOM-Si-SH was immersed in a solution of 1 mM Au (III) acetate for 12 h under the stirring condition to assemble gold ions (Au^{3+} ions) into the pores of NPAOM-Si-SH [40]. Then, the extra Au^{3+} ions were washed away by rinsing the membrane with DI water. After that, 1 mL of NaBH_4 (0.1 M)
was dropped on the solution and stirred for 2 h. During this time, the self-assembled Au$^{3+}$ ions were reduced to Au nanoparticles.

To immobilize the aptamer inside the NPAOM-Si-S-Au$_{nano}$, a LEGE was first planted inside of a flow cell (bottom part) and then the NPAOM-Si-S-Au$_{nano}$ was attached to the surface of a LEGE. After that, the top part of a flow cell was put on the bottom part of the flow cell and fixed with screws (Fig. S5). Consequently, the thiolated aptamer probe solution (100 nmol aptamer, 1 mM TCEP, 1 mM Mg$^{2+}$, pH 7.4) was pumped to the flow cell set up for 10 h to link the thiolated aptamer probe to the gold nanoparticles through the self-assemble of the gold–sulfur (Au–S) bonds. After that, the LEGE/NPAOM-Si-S-Au$_{nano}$-Aptamer was thoroughly washed by pumping 0.1 M of PBS to the flow cell for 10 min to wash away any loosely bounded aptamer. After that, 1 μM of 3-MCP solution was pumped into a cell for 1.0 h at room temperature to block the non-specific sites on the surface of the gold nanoparticles. Finally, the LEGE/NPAOM-Si-S-Au$_{nano}$-Aptamer was thoroughly washed with 0.1 M PBS to wash away any loosely bounded 3-MCP. The flow cell containing aptasensor was stored at 4 °C in a refrigerator when not in use.

During the measurement process, 100.0 μL of saliva sample was mixed with 400.0 μL of PBS (0.125 M, pH 7.4) containing a fixed concentration of the SARS-CoV-2-RBD with a mini rotator for 5 min. The saliva sample was mixed with PBS to increase the ionic conductivity (ionic conductance) of a saliva sample for the electrochemical measurement and adjust its pH level to 7.4 which is a biological pH.

The mixture was then transferred to the flow cell with a pump for 20

Fig. 1. Schematic illustration for the fabrication of the membrane-based aptasensor.
min. After that, the electrode was rinsed thoroughly with 0.1 M PBS. Finally, 100 μL of Fe(CN)₆⁴⁻ solution (0.1 M PBS, pH 7.4) was then pumped into the flow cell for 30 sec. After that, the pump was stopped and the signal of the LEGE/NPAOM-Si-S-Auₙano-Aptamer to the different concentrations of SARS-CoV-2-RBD was recorded by using the SWV method.

Fig. 1 shows the schematic illustration of the LEGE/NPAOM-Si-S-Auₙano-Aptamer fabrication and the sensing mechanism.

3. Results and discussion
3.1. The characterization of the prepared nanochannel membrane

The SEM images of NPAOM (A-B) and NPAOM-Si-S-Auₙano (C-E) are shown in Fig. 2. As can be seen in (Fig. 2A and B), the NPAOM has a 3-D nonporous structure. The average pore diameter and length are 55 ± 2 nm and 50 ± 5 μm, respectively. Also, (Fig. 2C) (top view) and D, E (cross-section views) show the SEM images of the NPAOM-Si-S-Auₙano. As shown, the gold nanoparticles decorated the outside and inside of the membrane uniformly. The average size of the gold nanoparticles is 25.7 nm.

Fig. 2. SEM images of the NPAOM (A-B) and NPAOM-Auₙano (C-E).
nm. To take the cross-section SEM image, the NPAOM-Si-S-Au\textsubscript{nano} was broken in half. Therefore, some of the gold nanoparticles must be on the other side of the membrane.

The surface morphology of the NPAOM-Si-S-Au\textsubscript{nano} was also investigated with AFM (Fig. S6). As can be seen, the AFM image was consistent with the SEM image.

The elemental analyses of the NPAOM (A), NPAOM-Si-S-Au\textsubscript{nano} (B), and NPAOM-Si-S-Au\textsubscript{nano}-Aptamer (C) were also carried out by EDX (Fig. S7). After the decoration of the NPAOM with gold nanoparticles, a gold element appeared in the EDX spectrum (B), proving the membrane was decorated with a gold element. As the thiolated aptamer was immobilized in the NPAOM-Si-S-Au\textsubscript{nano}, the peaks related to sulfur (related to the thiol function group of aptamer) and phosphorus elements (related to the backbone of the aptamer) appeared. It indicated that aptamer immobilized inside membrane via the Au-S bond.

Fig. 3A shows the FTIR spectrum of NPAOM (a) and NPAOM-Si-S-Au\textsubscript{nano}-Aptamer (b). As shown, an absorption band at 3192 cm\textsuperscript{-1} due to the \textendash OH stretching, and two absorption bands at 1150 cm\textsuperscript{-1}, 926 cm\textsuperscript{-1} due to the \textendash Al-O stretching (a) were recorded. After the immobilization of aptamer (b), a band at 3390 cm\textsuperscript{-1} due to \textendash NH\textsubscript{2}, a band at 2918 cm\textsuperscript{-1}, a band at 1119 cm\textsuperscript{-1} due to \textendash PO\textsubscript{2}, two bands at 1150 cm\textsuperscript{-1} and 926 cm\textsuperscript{-1} due to the \textendash Al-O-Si stretching, a band at 1538 cm\textsuperscript{-1} due to the \textendash C = O stretching, a band at 1313 cm\textsuperscript{-1} due to the \textendash C-N stretching of amide III, stretching a band at 1277 cm\textsuperscript{-1} due to the \textendash PO\textsubscript{2} stretching, a band at 1047 cm\textsuperscript{-1} due to the \textendash C-O-P bending, an absorption band at 792 cm\textsuperscript{-1} due to the = C-H bending appeared, indicating the aptamer probe was immobilized inside the membrane [55].

The NPAOM-Si-S-Au\textsubscript{nano}-Aptamer was also analyzed using a Raman spectroscopy (Fig. 3B). As can be seen, a sharp peak related to the carbon–carbon (C-C) stretching of adenine (A), guanine (G), and thymine (T) of aptamer at 1390 cm\textsuperscript{-1}, a band related to the C-C stretching of cytosine (C), G, and T at 1180 cm\textsuperscript{-1}, a band related to the C-C stretching of deoxyribose the aptamer at 890 cm\textsuperscript{-1}, a sharp peak related to the O-P-O group, the C-C stretching of C and T of aptamer at 780 cm\textsuperscript{-1} [56,57], a sharp peak related to -Al-O-Si bending at 500 cm\textsuperscript{-1}, and a sharp peak related to -Si-O-Si bending at 447 cm\textsuperscript{-1} [58].

Fig. 3. Fourier transform infrared spectrum of NPAOM (a) NPAOM-Si-S-Au\textsubscript{nano}-Aptamer (b) (A). Raman spectrum of NPAOM-Si-S-Au\textsubscript{nano}-Aptamer (B).
The FTIR and Raman results indicated that the aptamer probes were immobilized inside the membrane.

### 3.2. Electrochemical characterization of the membrane-based aptasensor

The stepwise electrochemical characterization of the fabricated nanochannel-based aptasensor was studied. Fig. 4 shows the SWV of the LEGE/NPAOM-Au nano as a substrate (a), the LEGE/NPAOM-Au nano–Aptamer (b), and the LEGE/NPAOM-Au nano–Aptamer/SARS-CoV-2-RBD (c) in a solution containing 1 mM Fe(CN)₆³⁻ as the redox probe and 0.1 M PBS (pH 7.4). As can be seen, before the immobilization of aptamer, the intensity of the recorded signal for the LEGE/NPAOM-Au nano is high (a), indicating that the redox probe could go inside the nanochannels of the membrane without being limited. After the immobilization of aptamer probes on the nanochannels of the membrane (b), the intensity of the signal decreased. The reasonable explanation is that the immobilized aptamer probes have a negative charge repelled the negatively charged redox probe (Fe(CN)₆³⁻), hindering the access of the redox probe toward the electrode surface [38]. As the SARS-CoV-2-RBD (5.0 ng/mL) incubated with the immobilized aptamer probes inside the nanochannels of the membrane (c), the intensity of the signal of the redox probe decreased more. The reasonable explanation is that the SARS-CoV-2-RBD which is a huge molecule (35 kDa), blocked the nanochannels of the membrane, hindering the redox probe to diffuse over the working electrode of LEGE.

### 3.3. Optimization of effective parameters on membrane-based aptasensor response

The effect of the amount of the aptamer probe and incubation time on the response of the LEGE/NPAOM-Si-S-Au nano–Aptamer to 40.0 ng/mL of SARS-CoV-2-RBD (0.1 M PBS, pH 7.4) was investigated (Fig. 5). During the process of investigation, a saliva sample containing 40.0 ng/mL of SARS-CoV-2-RBD (0.1 M PBS, pH 7.4) was pumped into the flow cell where the LEGE/NPAOM-Si-S-Au nano–Aptamer was planted. After a while, the pump was turned off and then, the signal of the aptasensor was recorded using the SWV method.

Fig. 5A shows the effect of the immobilized aptamer probe on the response of the proposed membrane-based-aptasensor. As shown, the change in the signal of the aptasensor to 40.0 ng/mL of SARS-CoV-2-RBD increased as the concentration of the immobilized aptamer probe increased from 0 nmol to 100 nmol and then decreased in the high concentration of the aptamer. The reasonable explanation is that the interaction between the thiolated aptamer probe and gold nanoparticles inside the channels reached the highest level as 100 nmol of aptamer was used. When the concentration of aptamer increased to 125 nmol, the electrostatic repulsion interaction between the aptamers that have the negative charge hindered thiolated aptamers to interact with gold nanoparticles inside the nanochannel of the NPAOM-Si-S-Au nano. For that reason, the change in the signal of the aptasensor to 40.0 ng/mL of SARS-CoV-2-RBD is low as 125 nmol of aptamer probe was used for the fabrication of the aptasensor compare to that aptasensor that was made by using 100 nmol of the aptamer.

The effect of the incubation time between the aptamer probe and 40.0 ng/mL of SARS-CoV-2-RBD was also studied (Fig. 5B). As it can be seen, the change in the response of the aptasensor increased by increasing the incubation time from 5 min to 20 min and then remained unchanged at a longer incubation time due to reaching the interaction of aptamer-target to the steady-state equilibrium.

### 3.4. Analytical performance

Fig. 6A and B show the SWV (A) and the corresponding calibration plot of SWV response (B) of the LEGE/NPAOM-Si-S-Au nano–Aptamer to the different concentrations of SARS-CoV-2-RBD in saliva samples, respectively. As shown in (Fig. 6A), the SWV peak of the aptasensor decreased with increasing the concentration of SARS-CoV-2-RBD. The corresponding calibration plot (Fig. 6B) shows that the response of the proposed membrane-based aptasensor had a linear relation with the concentration of SARS-CoV-2-RBD in the range of 2.5–40.0 ng/mL. The limit of detection (LOD) of the LEGE/NPAOM-Si-S-Au nano–Aptamer was found to be 0.8 ng/mL (based on 3 σ/S, where σ is the standard deviation of the blank measurements, and S is the slope). According to the literature [59], there are between 25 and 40 RBD on each SARS-CoV-2.
Therefore, the LOD of the LEGE/NPAOM-Si-S-Au nano-Aptamer would be $5.5 \times 10^8 - 3.4 \times 10^8$ copies/mL using the equation below:

$$\text{Copies/mL} = \frac{\text{Molarity of SARS-cov$_2$ RBD}}{\text{Number of RBD in a SARS-cov$_2$}} \times \text{Avagadro number}$$

Since the SARS-CoV-2 level reaches $6.6 \times 10^8$ copies/mL after 4 days in a COVID-19 patient [60], the fabricated aptasensor can be used for the early-stage detection of COVID-19. The selectivity of the LEGE/NPAOM-Si-S-Au nano-Aptamer was studied in the absence and presence of HA and NA (Fig. 6 C). No sensible interference was also observed for 10$^2$-fold quantities of HA (red curve) and NA (green curve) in the determination of 5.0 ng/mL of SARS-CoV-2-RBD due to the high affinity of the aptamer to SARS-CoV-2-RBD. The stability of the LEGE/NPAOM-Si-S-Au nano-Aptamer was also evaluated (Fig. 6 D). No obvious change in the signal of the aptasensor to 5.0 ng/mL of SARS-CoV-2-RBD was observed after 3 weeks. It can be related to the preparation of a bio-friendly micro-environment for the aptamer probes by NPAOM.

The responses of aptasensor to two different concentrations of the SARS-CoV-2RBD in saliva samples were compared with the enzyme-linked immunosorbent assay (ELISA) method (Table S1). No
statistically significant difference between the responses of the LEGE/NPAOM-Si-S-Au nano-Aptamer and the ELISA, indicating that the proposed aptasensor is a reliable sensor for the SARS-CoV-2RBD determination in saliva serum samples.

The analytical performances of the LEGE/NPAOM-Si-S-Au nano-Aptamer were also compared with the other aptasensors that were applied for the detection of the biomarkers related to the SARS-CoV-2 (Table 1). As can be seen, All in all, the proposed aptasensor has good

| Aptsensor | Biomarkers | Technique | Linear range | Limit of detection | Incubation time | Ref. |
|-----------|------------|-----------|--------------|--------------------|-----------------|-----|
| SPCE/Aunano/MPA/ biotinylated aptamer | SARS-CoV-2-RBD | DPV | 10–50 ng/mL | 2.63 ng/mL | 40 min | [61] |
| CB/CSPE combined with Magnetic bead-based immunoassors | SARS-CoV-2 nucleocapsid | DPV/DPV | 0.01–0.6 µg/mL | 8 ng/mL/19 ng/mL | 30 min/30 min | [62] |
| Pd/Aptamer | SARS-CoV-2 nucleocapsid protein | SPR | 0.5–16 ng/mL | 1 ng/mL | 110 min | [63] |
| Gold electrode/Aptamer-Methylene blue | SARS-CoV-2-RBD | SWV | 10^1–10^3 | – | 5 min | [2] |
| GCE/Yb-TCP-4/ Au NPs/ Atpamer | Spike protein | PEC | 0.5–8 µg/mL | 72 ng/mL | 70 min | [64] |
| ITO electrode/GdS QDs- Chitosan/ gC3N4/Aptamer | SARS-CoV-2-RBD | PEC | 0.5–32.0 nM | 0.12 nM | 40 | [13] |
| LEGE/NPAOM-Si-S-Au nano-Aptamer | SARS-CoV-2-RBD | SWV | 2.5–40.0 ng/mL (71.5 pM–1.14 nM) | 0.8 ng/mL (23 pM) | 20 min | This work |

DPV: Differential pulse voltammetry; SPR: Surface plasmon resonance; PEC: Photoelectrochemistry; MPA: 3-Mercaptopropionic acid; Cu(OH)2 NRs: Copper hydroxide nanorods; GCE: Glassy carbon electrode; Au NPs: Gold nanoparticles; Yb-TCP-4: Two-dimensional metal–organic framework
performance in comparison with other aptasensors.

4. Conclusion

In conclusion, we used a nanoporous anodic aluminium oxide-gold nanoparticles membrane to design an electrochemical aptasensor for the SARS-CoV-2-RBD detection. The highly ordered nanoporous aluminium oxide membrane was fabricated first. Subsequently, the membrane was functionalized with mercapto-silane to decorate the nanochannel of the membrane with gold nanoparticles. The thiolated nanoparticles membrane to design an electrochemical aptasensor for the detection of SARS-CoV-2-RBD was recorded using the SWV method in the presence of Fe(CN)₅⁻₃⁻ as a redox probe. The aptasensor was able to detect SARS-CoV-2-RBD in the range 2.5–40.0 ng/mL with a LOD of 0.8 ng/mL. Furthermore, no obvious change in response was observed after 21 days, indicating the high stability of the aptasensor. Moreover, the aptasensor was able to detect SARS-CoV-2-RBD in the human serum sample. Although the fabricated aptasensor could detect COVID-19 before the symptom of the disease appears, however, it has some disadvantages to be a point of care device and it can only be used in clinics and hospitals. First, in the proposed method, a pump should be used to transfer the solution to the measurement cell. Second, the RBD of the SARS-CoV-2-RBD should be extracted and separate from the virus. Because the diameter of the channels is smaller than the diameter of the SARS-CoV-2.

CRediT authorship contribution statement

Mahmoud Amouzadeh Tabrizi: Supervision, Project administration, Funding acquisition, Conceptualization, Methodology, Data curation, Formal analysis, Validation, Investigation, Visualization, Writing – original draft. Pablo Acedo: Supervision, Writing – review & editing, Resources, Project administration, Funding acquisition.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

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