Electrochemical immunosensors using electrodeposited gold nanostructures for detecting the S proteins from SARS-CoV and SARS-CoV-2

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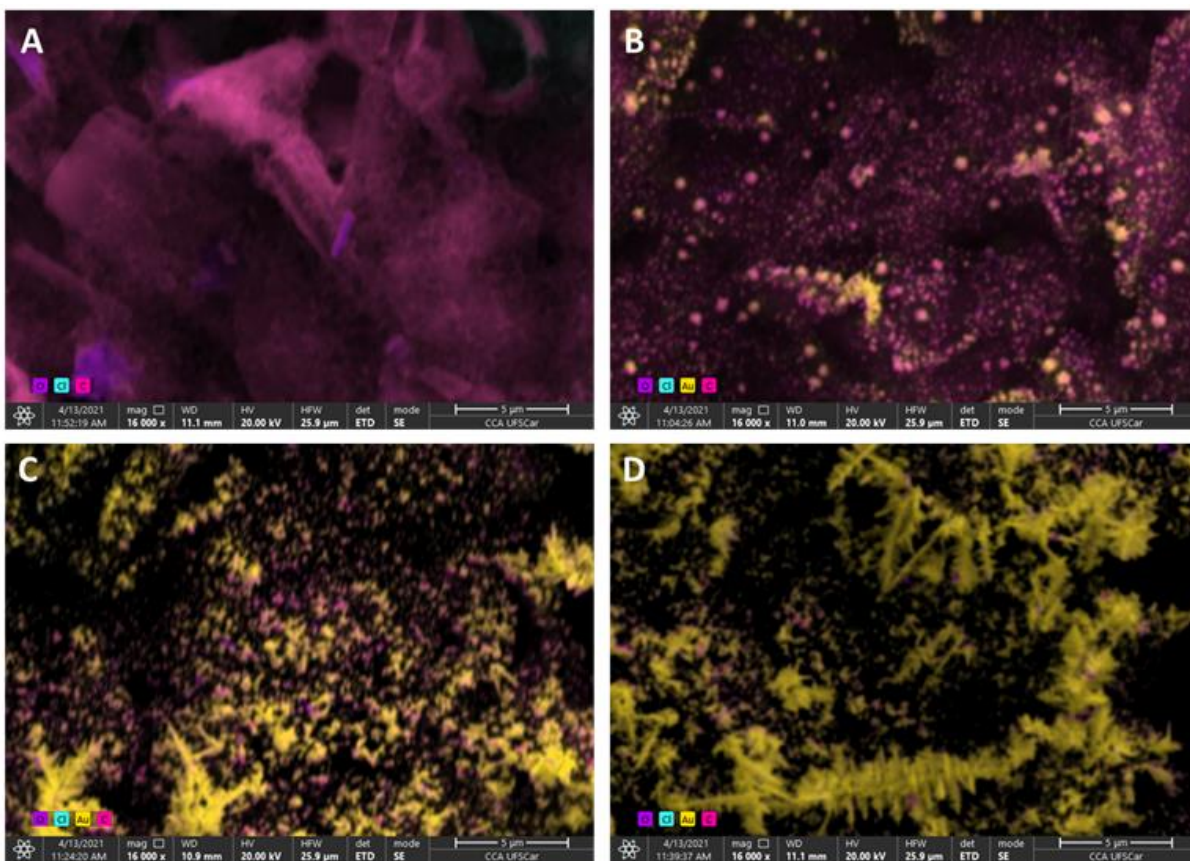
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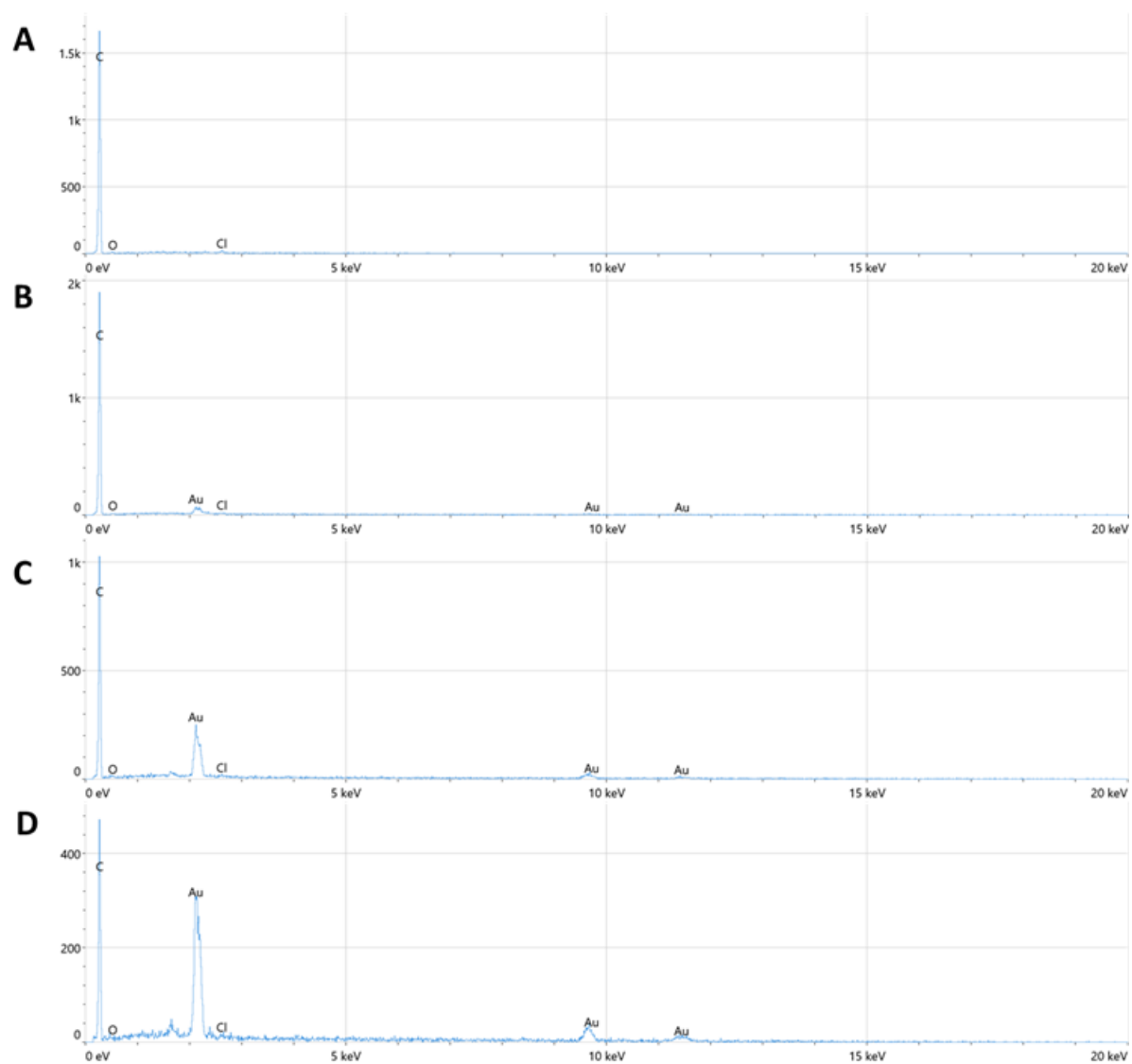**Figure S1.** Elemental mapping of a) bare SPCE; SPCE with b) 9s, c) 30s and d) 90s gold deposition time.

Figure S2. EDX of a) bare SPCE; SPCE with b) 9s, c) 30s and d) 90s gold deposition time.
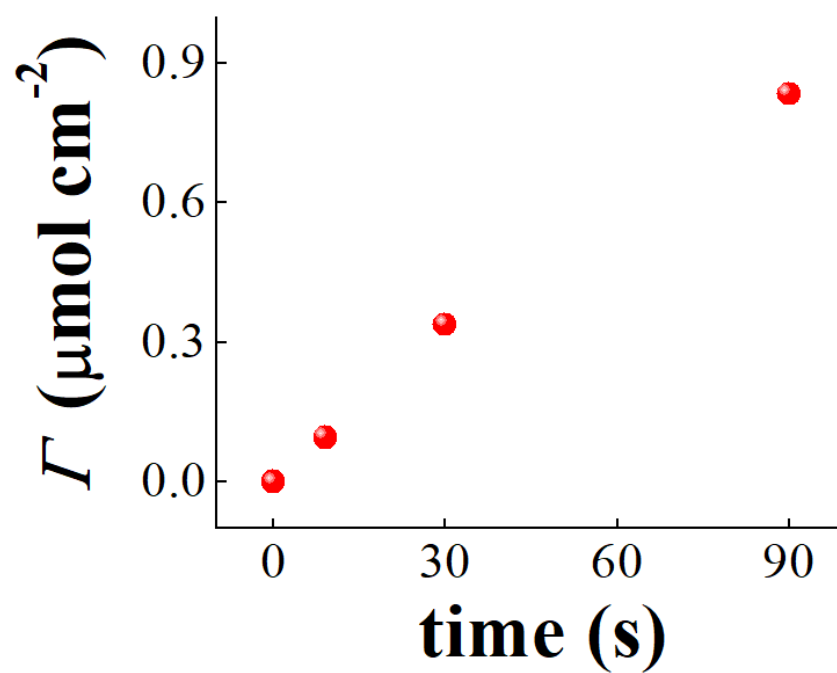
Figure S3. Effect of AuNP deposition time on the number of electroactive species (Γ) on the sensor surface.
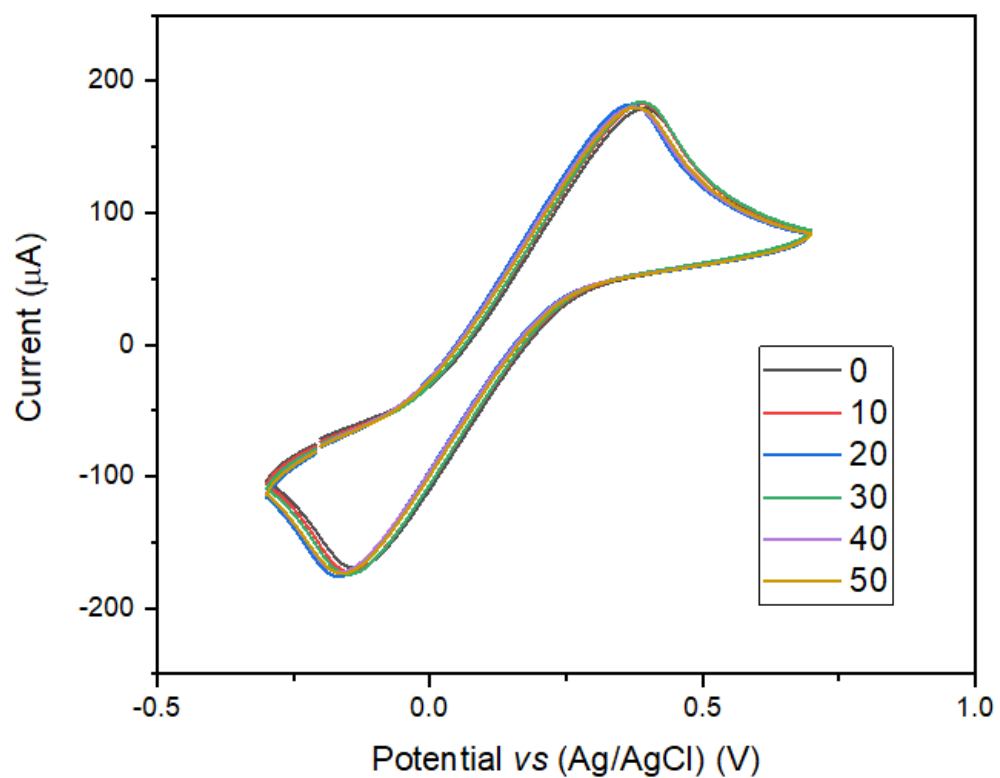
Figure S4. Cyclic voltammograms after 0, 10, 20, 30, 40 and 50 mechanical flexions. CVs were performed in 0.1 mol L^{-1} PBS containing 4.0 mmol L^{-1} [Fe(CN)$_6$]$^{4-/3-}$ at a scan rate 100 mV s^{-1}.

Figure S5. 2D score plot obtained by principal component analysis (PCA) of measurements of BSA, and the S protein of SARS-CoV-1 and SARS-CoV-2 (10^{-9} mol L^{-1}, PBS).

Table S1. k_{app} values for each of the Au modified SPCEs. R_{ct} values were obtained from measurements in a potassium hexacyanoferrate (II)/(III) solution (4 mmol L^{-1} each, PBS 0.1 mol L^{-1}, pH 7.0).

Electrode	R_{ct}/Ω	k_{app} /cm s^{-1}
Bare electrode	1335 ± 74	3.968 x 10^{-10}
9s	88 ± 24	6.016 x 10^{-9}
30s	14 ± 5	3.78 x 10^{-8}
90s	0.02 ± 0.04	2.65 x 10^{-5}

S1. Literature review on SARS-CoV-2 diagnosis using biosensors

An overview of biosensors for SARS-CoV-2 detection using S protein as a target analyte is shown in Table S2. Several techniques were reported for biosensing platforms, with different matrices. Seo et al. [1] have developed a graphene-based field-effect transistor (FET) device for SARS-CoV-2 spike protein detection. The antibody biocomponent was immobilized on the fabricated device using PBAS as a probe linker, which was tested in nasopharyngeal swab clinical samples. The assay presented a limit of detection of 242 copies mL^{-1}. Lee et al. [2] have designed a lateral flow immunoassay (LFIA) comprising a sample pad, a conjugation pad, an absorbent pad and a nitrocellulose membrane. A SARS-CoV-2 receptor (ACE2) was immobilized on the nitrocellulose membrane as a capture probe and the S1-mAb as a detection antibody, forming a matched pair. The assay was sensitive for S1 protein (5 ng reaction^{-1}) in diluted samples of SARS-CoV-2 specific antigen, and the limit of detection was 1.86 x10^5 copies mL^{-1} in the clinical specimen. An electrochemical immunoassay was developed by Fabiani and coworkers [3] with carbon black-based screen-printed electrodes (SPEs) combined with PALM SENS portable potentiostat as a reader. Magnetic beads (MBs) were used for the immobilization of antibodies for SARS-CoV-2 S and N proteins. The detection limit was 19 ng mL^{-1} and 8 ng mL^{-1} for S and N proteins, respectively, in standard solutions. Electrochemical detection of SARS-CoV-2 spike protein was made with a paper-based platform in which the working electrode was modified with graphene oxide (GO) and its carboxylic group (-COOH) was activated with 20mM EDC/40 mM NHS [4]. The SARS-CoV-2 IgM antibody was immobilized, and the electrochemical response was monitored using the

square-wave voltammetry (SWV) technique. The detection limit of the sensor for the virus spike protein was 0.11 nb mL^{-1}, which was not sufficient to detect the antigen in human nasal swabs.

A cell-based biosensor platform was produced with mammalian Vero cells engineered by electroinserting specific antibodies for SARS-CoV-2 S protein [5]. When those specific antibodies bind the target molecules, the electric properties of the biorecognition elements change, as measured in Bioelectric Recognition Assays (BERA). The device had a detection limit of 1 fg mL^{-1} in standard solutions of SARS-CoV-2 Spike protein [5]. In a different approach, Ahmadivand et al. [6] have fabricated a miniaturized assay with a plasmonic immunosensor based on the toroidal electrodynamics concept. Functionalized colloidal gold nanoparticles were introduced with the antibody to capture the spike proteins in the sample (standard solution of SARS-CoV-2 spike protein). The assessment of these biomolecules around the toroidal-resonant unit cell was executed by defining the difference between the transmission tensors in the absence and presence of spike protein (concentrations, from 4 to 12 fM). The sensor LOD was 4.2 fM.

Table S2. Comparison of different biosensors using S protein as a detection target for SARS-CoV-2

Biosensor	Method	Technique	Detection target	Sample	Analytical features	References
Electrochemical immunosensor	Magnetic bead-based immunosensor combined with carbon black-modified screen-printed electrode	PALM SENS portable potentiostat	SARS-CoV-2 Spike and Nucleocapsid protein	Untreated saliva	**LOD:** 19 ng mL^{-1} and 8 ng mL^{-1} for S and N protein, respectively **Analytical Sensitivity:** 6.5 PFU mL^{-1} and 6.5 x 10^3 PFU mL^{-1} for antibodies directed against S and N protein, respectively	[3]
Field-Effect transistor (FET)-based biosensor	Label-free, real-time electrical detection with graphene-based FET functionalized with antibody	Electrical performance on a semiconductor analyzer and probe station	SARS-CoV-2 Spike Protein	Nasopharyngeal Swabs	**LOD:** 1 fg mL^{-1} (spike protein in PBS) **LOD:** 100 fg mL^{-1} (Nasopharyngeal Swabs containing spike protein) **LOD:** 242 copies mL^{-1} (Nasopharyngeal Swabs - clinical samples)	[1]

Electrochemical diagnostic kit	Fixed/Screen printed electrodes modified with a layer of graphene oxide (GO) along with sensitive chemical compounds and gold nanostars (AuNS)	DPV (differential pulse voltammetry)	Viral Spike glycoproteins	Aquatic biological media	**LOD:** 1.68×10^{-22} µg mL^{-1} **Analytical Sensitivity:** 0.0048 µAµg mL^{-1} cm^{-2} **Sensitivity:** 95% (compared with RT-PCR)	[7]
Paper-based electrochemical immunosensor	Label-free paper-based electrochemical platform; working ePAD modified through embedded graphene oxide (GO)-EDC/NHS chemistry	SWW (square wave voltammetry)	SARS-CoV-2 Spike Protein and antibody	Human serum	**LOD:** 0.96 ng mL^{-1} for IgG; 0.14 ng mL^{-1} for IgM; 0.11 ng mL^{-1} for spike protein sensing **Sensitivity:** 100% (compared with RT-PCR) **Specificity:** 90%	[4]

Cell-based biosensor	Portable cell-based biosensor customized with a multichannel potentiometer containing eight gold screen-printed electrodes on a disposable sensor strip.	Bioelectric Recognition Assay (BERA)	SARS-CoV-2 Spike Protein	Standard solution of the SARS-CoV-2 Spike protein	**LOD**:1 fg mL^{-1}	[5]
THz plasmonic biosensor	Immunosensor based on THz plasmonic toroidal metasurface; The binding properties of the metasurface were improved by introducing functionalized colloidal AuNPs	Transmission spectra of the THz metasensor device	SARS-CoV-2 Spike Protein	Standard solution of the SARS-CoV-2 Spike protein	**LOD:** 4.2 fM	[6]

Lateral flow immunoassay (LFIA)	LFIA sensor strip using the ACE2 (SARS-CoV-2 receptor); ACE2 and S1-mAb were paired with each other for capture and detection in a lateral flow-based immunoassay	Image scanner and analyzer	SARS-CoV-2 spike protein	Clinical Specimen	**LOD**: 1.86×10^5 copies mL^{-1} **Analytical sensitive:** 5 ng reaction^{-1}	[2]
Gr-FET immunosensor	Sensitive graphene field-effect transistor (Gr-FET) with highly selective antibody-antigen interaction	Conductance/ resistance via field effect	SARS-CoV-2 Spike Protein	Antigen buffer solution	**LOD**: 0.2 pM	[8]

Electrochemical immunosensor	Label-free electrochemical immunoassay; screen printed electrodes modified with a layer of graphene oxide (GO) functionalized with anti-spike antibodies	Square Wave Voltammetry (SWV)	SARS-CoV-2 Spike Protein	Dilutions of spike protein	**Lowest concentration of spike protein detected:** 20 µg mL^{-1}	[9]
Opto-microfluidic sensing platform	label-free microfluidic platform; a platform consisting of a gold nanospike covered glass substrate, fabricated by gold electrodeposition, integrated with a microfluidic chip coupled with a reflection probe	Surface plasmon resonance (LSPR)	Antibodies specific to the SARS-CoV-2 spike protein	Human plasma diluted in buffer solution	**LOD:** 0.08 ng mL^{-1}	[10]

Plasmon Enhance Biosensor Platform	Multiplexed grating-coupled fluorescent plasmonic (GC-FP) biosensor platform	Fluorescent plasmonic imaging instrument.	Antibodies against three spike protein antigens (RBD; spike S1 fragment; spike S1S2 extracellular domain)	Human blood serum and dried blood spot (DBS)	**For DBS Sensitivity:** 86,7% **Selectivity:** 100% (compared with RT-PCR) **For blood serum: Sensitivity:** 100% **Selectivity:** 100% (compared with RT-PCR)	[11]
Aptamer-based electrochemical sensor	High-surface wrinkled gold electrodes functionalized with aptamer tethered with redox probe	Difference Pulse Voltammetry (DPV)	SARS-CoV-2 Spike Protein	Pooled saliva diluted 10%	**Lowest concentration of spike protein detected:** 1 ag mL^{-1}	[12]
Electrochemical immunosensor	Screen-printed carbon electrodes (SPCEs)	Electrochemical impedance spectroscopy (EIS)	SARS-CoV Spike Protein	Dilutions of spike protein	**Low limit of detection (LOD):** $(3.39 \text{ pmol L} - 1)$	Current Paper

Table S3. Comparison of different biosensors to detect SARS-CoV virus

Biosensor	Method	Technique	Detection target	Sample	Analytical features	Reference
Surface plasmon resonance (SPR)-based biosensor	GBP-E-SCVme-coated SPR sensor chip for detection of anti-SCVm	Surface plasmon resonance	SARS-CoV antigen (SCVme)	Anti-SCVme in PBS	**Detection response:** 906 RU (Resonance Units) **LOD**: 200 ng L^{-1} **Time**: 10 min	[13]
Surface plasmon resonance (SPR) biosensor	Single biotinylated DNA/streptavidin coated sensor chip	Surface plasmon resonance	SARS-CoV RNA	RNA extracted from cells infected with SARS-CoV strain WHU	**Binding affinity between SARS-CoV N protein and the target RNA:** 4.60 nM	[14]
Surface plasmon resonance (SPR) biosensor	Home-developed Reference SPR Instrument; Monoclonal antibodies to SARS-CoV were immobilized	Surface plasmon resonance	SARS-CoV antigens	Sterilized SARS-CoV solution	**Binding rate:** 1.4 unit min^{-1}	[15]

	on the reaction spots via selective chemical modification					
Piezoelectric (PZ) immunosensor	Label-free immunosensor; PZ crystal with an antibody immobilized on its surface	Frequency counter	SARS-CoV antigen	Sputum in gas phase (1 µL)	**Time**: 2 min **Reproducibility:** 100 times **Concentrations of Antigen**: 1-4 µg µL^{-1} **Correlation coefficient:** 0.958 **Detection limit:** 0.60 mg mL^{-1}	[16]
Metal oxide (In$_2$O$_3$) nanowire based biosensors	Label-free, Nanowire biosensors utilizing antibody mimics as capture probes	Field-effect transistor	SARS-CoV Nucleocapsid (N) protein	N-protein in PBS	**High binding affinity to the N protein**: (KD = 3.3 nM) **Response time:** 10 min	[17]
Fiber-optic biosensor that	Label-based sandwich immunosensor; Localized Surface plasmon coupled fluorescence (LSPCF) combined with the	Localized Surface Plasmon (LSP)	SARS-CoV Nucleocapsid (N) protein	Human Serum	**Coefficients:** 0.9624 **Linear range:** 0.6–4 µg mL^{-1} **Sensitivity:** 1 pg mL^{-1}	[18]

	sandwich immunoassay					

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