Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
3D-Printed COVID-19 immunosensors with electronic readout

Jose Muñoz \(^a\), Martin Pumera \(^a,b,c,d,e\)

\(^a\) Future Energy and Innovation Laboratory, Central European Institute of Technology, Brno University of Technology (CEITEC-BUT), Brno 61600, Czech Republic
\(^b\) Department of Medical Research, China Medical University Hospital, China Medical University, No. 91 Haach-Shih Road, Taichung, Taiwan
\(^c\) 3D Printing & Innovation Hub, Department of Food Technology, Mendel University in Brno, Zemedelska 1, Brno CZ-613 00, Czech Republic
\(^d\) Department of Chemical and Biomolecular Engineering, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea

**ABSTRACT**

3D printing technology has brought light in the fight against the COVID-19 global pandemic event through the decentralized and on-demand manufacture of different personal protective equipment and medical devices. Nonetheless, since this technology is still in an early stage, the use of 3D-printed electronic devices for antigen test developments is almost an unexplored field. Herein, a robust and general bottom-up biofunctionalization approach via surface engineering is reported aiming at providing the bases for the fabrication of the first 3D-printed COVID-19 immunosensor prototype with electronic readout. The 3D-printed COVID-19 immunosensor was constructed by covalently anchoring the COVID-19 recombinant protein on a 3D-printed graphene-based nanocomposite electrode surface. The electrical readout relies on impedimetrically monitoring changes at the electrode/electrolyte interface after interacting with the monoclonal COVID-19 antibody, fact that hinders the redox conversion of a benchmark redox marker. Overall, the developed 3D-printed system demonstrates the advantage of light-of-speed distribution of 3D printing datafiles with localized point-of-care low-cost printing and bioelectronic devices to help contain the spread of emerging infectious diseases such as COVID-19. This technology is applicable to any post-COVID-19 SARS diseases.

1. Introduction

Since the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) was identified in December 2019, this virus has been spread over multiple countries worldwide. SARS-CoV-2 is a highly virulent pathogen that has promoted the Corona Virus Disease 2019 (COVID-19) pandemic event, as was declared by World Health Organization (WHO) on 31 March 2020 \(^1\). SARS-CoV-2 virus is characterized by a rapid human-to-human transmission and even worse, high mortality. Accordingly, the resulting COVID-19 outbreak has become one of the major public health crises of the 21st century \(^2\). While the scientific community has started a race against time for the vaccine or antiviral therapy development \(^3,4\), another pivotal challenge remains opened: the fast widespread testing of the vast majority of the citizens in order to identify suspected and/or asymptomatic cases \(^5\). Although this fact would help curb the spread of the COVID-19 global pandemic, it supposes a huge economic cost for the governments \(^6\).

Following the recommendation of WHO, the standard workhorse assay in the detection of SARS-CoV-2 is being the well-known real time reverse transcription polymerase chain reaction (PCR) technology \(^7\). Besides PCR allows to accurately identify and target the virus based on its genomic sequences, this technology is time-consuming (more than 3 h), requires of expensive bench-top instrumentation and experienced personnel, hindering its use for in-field analysis. In this regard, antigen-based rapid lateral-flow tests are simpler and can be in situ performed routinely, providing the results in a couple of minutes \(^8,9\). Although antigen-based tests are called to be used for the first-line diagnosis of COVID-19 owing to their high specificity, their sensitivity is generally worse than the PCR tests, fact that may lead to false-negative responses \(^10\). In general, antigen-based lateral-flow tests involve a qualitative (positive or negative) optical detection employing labelled antibodies, being not able to quantify the virus load. This drawback can be overcome by employing electrochemical methods, which are particularly appealing for the development of easy-to-automate analytical devices since the transduction method is electronic \(^11,12\). To date, several electrochemical immunosensing strategies (i.e., label-free, sandwich,
direct/competitive or lateral-flow assays) have been devised for the rapid determination and quantification of different viruses [13,14,15,16], also including the SARS-CoV-2 [17,18,19,20,21].

In the heat of the COVID-19 pandemic, 3D printing technology has been placed at the forefront in the fight against this health crisis, enabling a decentralized and tailored fabrication of on-demand substrates—from personal protection equipment to medical devices and isolation wards—, which can be readily available in a matter of minutes [22,23,24,25]. However, to the best of our knowledge, no references about the use of 3D printing technology for the development of COVID-19 sensing systems have been reported yet. In this regard, 3D printing technology is currently revolutionizing the way to manufacture electronic devices since provides a fast-large-scale eco-friendly production of customized-shape objects with minimised waste [26,27,28,29,30]. Huge advantage of 3D printing is that 3D-printed devices containing files can be send electronically around the world—for example, to the International Space Station—at the speed of light and printed locally [31]. Further, 3D-printed conductive structures can be simply electronically updated/upgraded and distributed electronically at the same time, making software updates to precipitate in real-world applications with detection limits at part per million (ppm, μg·mL⁻¹) levels; however, this is only the tip of the iceberg and there is plenty of room for improvement. Thus, this work aims at building up the bases towards the almost unexplored field of 3D-printed electrochemical immunosensors, a challenge in the bioelectronics field that responds to the current needs of our Society.

2. Materials and methods

2.1. Chemicals and materials

COVID-19 recombinant protein (COVID-19 Spike Protein RBD Domain Coronavirus) and monoclonal COVID-19 antibody (Humanized COVID-19 Spike S1 Protein Coronavirus Monoclonal Antibody) were purchased from MyBioSoruce (San Diego, USA). HAuCl₄ and NaBH₄ (Au-NPs precursors), KCl, K₂Fe(CN)₆, K₄Fe(CN)₆, glutaraldehyde (25%), cysteamine hydrochloride, bovine serum albumin (BSA), human serum from human male AB plasma (hemoglobin ≤ 20 mg·dL⁻¹), phosphate buffer saline (PBS, pH 7.4) and tris-buffer saline (TBS) were supplied by Sigma-Aldrich (St. Louis, USA). DMF (99.5%) was acquired from Lach-Ner (Neratovice, Czech Republic). All the solutions were prepared in PBS pH 7.4 with the exception of BSA solution, which was prepared in TBS 1x.

Concretely, 3D-printed G/PLA electrodes are very appealing transducers since they combine i) electrochemical performances comparable and even better than those displayed by other conventional high-cost commercially available carbon-based electrodes (i.e., glassy carbon electrode or screen-printed electrodes) [34,35,36] with ii) the custom and large-scale benefits of 3D printing technology [28,37]. As a proof case study, the COVID-19 global pandemic event has been considered. The electroanalytical approach (see Scheme 1b for illustration) relies on an indirect electrochemical immunoassay based on the competition of a fixed concentration of monoclonal COVID-19 antibody to interact with either the free COVID-19 recombinant protein (antigen) in the sample or the one immobilized on the electrode surface (biomarker). The electronic outputs derived from different concentrations of antigen were impedimetrically monitored by means of charge transfer resistance (R_C_T) changes at the electrode/electrolyte interface, using [Fe(CN)₆]³⁻/⁴⁻ as the redox probe [38]. Overall, the first 3D-printed COVID-19 immunosensor prototype exhibits promising electroanalytical capabilities and large-scale benefits of 3D printing technology [28,37] as the redox probe [38].

Scheme 1. a) Illustration of the 3D-printed electrochemical COVID-19 immunosensor fabrication steps. b) Indirect competitive assay carried out for detecting the COVID-19 recombinant protein (antigen), the one against the SARS-CoV-2 virus.
Pendulum-like 3D-printed electrodes were fabricated by fused deposition modeling (FDM) using a Prusa i3 MK3 printer (Prusa Research, Czech Republic). A commercially available Black Magic 3D filament (New York, USA) was used as the raw material, which is made of a conducting graphene-based nanocomposite filler dispersed throughout an insulating PLA polymeric matrix (G/PLA). For the printing, the raw G/PLA filament was extruded down through the nozzle (Olsson Ruby-tipped 0.4 mm, 3DVerkstan, Sweden). The nozzle and the bed temperatures were 215 °C and 60 °C, respectively. For electrochemical measurements, the geometric area employed was 0.28 cm², which corresponds to the one-face spherical part of the pendulum-like 3D-printed G/PLA electrodes exposed in the electrochemical cell, while the linear part was utilized for the electrical contact (see Scheme 1a for illustration). Since the as-printed G/PLA electrodes possess poor conductivity, they were activated by following the standard procedure [39]. In brief, as-printed G/PLA electrodes were immersed in DMF for 3 h—in order to partially remove the insulating PLA polymer—and then electrochemically (EC) activated (bias potential: 2.5 V vs. Ag/AgCl; activation time: 300 s; electrolyte: PBS pH 7.2) to promote the formation of oxygenated groups on the carbon walls exposed on the 3D-printed G/PLA electrode surface.

2.3. Apparatus and procedures

The morphological characterization of the 3D-printed G/PLA electrode surfaces was carried out by using a scanning electron microscopy (SEM, TESCAN LYRA 3) operated at 15 kV. The atomic composition at the surface level was analyzed by X-ray photoelectron spectroscopy (XPS, AXIS Supra instrument). Electrochemical experiments by means of cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were run using a PGSTAT 204 potentiostat/galvanostat (MetrohmAutolab, The Netherlands) operated by a NOVA 2.1 software. Electrochemical measurements were carried out in a three-electrode configuration cell filled with 10 mM [Fe(CN)₆]³⁻/⁴⁻ in 0.1 M PBS (pH 7.2), which was used as the redox marker. A single junction Ag/AgCl, a platinum wire and the 3D-printed immunosensors were used as the reference, counter and working electrodes, respectively. Immunosassays employing serological fluids were run by incubating the 3D-printed COVID-19 immunosensors in a 100x diluted human serum sample.

3. Results and discussion

3.1. Fabrication of 3D-printed electrochemical immunosensors

The surface engineering related to the fabrication of 3D-printed electrochemical immunosensors implies the biofunctionalization of 3D-printed G/PLA electrodes following four consecutive stages, as illustrated in Scheme 1a. As a first prototype, the experimental conditions were adapted from the optimized ones reported by Layqah et al. along the fabrication of a conventional Au-based volumetric immunoassay for the detection of the MERS-Cov virus [40]. All the functionalization steps were carried out at room temperature (20 °C).

1) *In situ* incorporation of Au–NPs: The activated 3D-printed G/PLA electrodes were in *situ* functionalized with Au–NPs via an eco-friendly Intermatrix Synthesis approach [41]. For this, the activated electrodes were firstly immersed in an Eppendorf vial containing a 2.5 mM HAuCl₄ aqueous solution (precursor for Au–NPs synthesis) for 1 h. The acting force for such formation relies on the electrostatic interactions between the oxygenated groups contained on the G/PLA electrode surface and the Au³⁺ metal ion precursor. Subsequently, the Au³⁺-loaded electrodes were immersed in another Eppendorf vial containing a 10 mM NaBH₄ aqueous solution in order to induce the zero-valent form of the Au metal, resulting in the *in situ* formation of Au–NPs upon the electrode surface.

2) Anchoring of a thiolated moiety: Au–NPs were used as nanotemplates to bottom-up the 3D-printed electrode surface. Thus, the Au-functionalized electrodes were immersed in a 10 mM cysteamine solution—a thiolated molecule carrying a terminal amine group—for 2.5 h in order to promote the well-known gold-thiol interactions.

3) Anchoring of the cross-linker: The resulting electrodes were incubated in a 2.5% v/v glutaraldehyde solution—a homobifunctional cross-linker acting as a coupling reagent for covalently linking antigens to the amine groups of cysteamine—for 1 h.

4) Immobilization of biomarker and BSA blocking: The terminal aldehyde groups of the linker contained on the electrode surface were utilized for anchoring the biomarker (concentration: 10 µg mL⁻¹; incubation time: 1 h) with the amino groups of the protein via imide bond formation. Herein, the COVID-19 recombinant protein was used as the recognition biomarker for the competitive antigen-antibody assay. Finally, the biofunctionalized electrodes were immersed in a 1% w/v BSA solution (TBS 1x) for 30 min in order to block the unreacted aldehyde groups, as well as to avoid nonspecific interactions, resulting in the first 3D-printed COVID-19 immunosensor prototype with electrical readout. While the cost of manufacturing a 3D-printed G/PLA electrode is ~ 0.25 €, the total cost to produce a single 3D-printed COVID-19 immunosensor is around 9 € due to the current prize of the commercially available antigen/antibody kits for COVID-19 determination.

3.2. Characterization of the 3D-printed COVID-19 immunosensor

The stepwise functionalization process for the fabrication of 3D-printed electrochemical immunosensors was interrogated electrochemically by means of CV and EIS (Fig. 1), using [Fe(CN)₆]³⁻/⁴⁻ as the benchmark redox couple. The voltammograms from Fig. 1A indicates the accessibility of the redox marker to be oxidized/reduced at the electrode surface by analyzing the intensity peak currents (Iₚ). While (a) as-printed G/PLA electrodes exhibited an almost flat voltammetric behavior due to their poor conductivity, the presence of a pair of redox peaks was observed (b) after activation, indicating that the chemical/electrochemical treated electrodes are electrically active [39]. In addition, a significant Iₚ enhancement was achieved (c) after functionalization with Au–NPs. This fact can be ascribed to the increase in the surface area as well as the intrinsic catalytic activity of Au–NPs [42]. After anchoring both (d) cysteamine and (e) glutaraldehyde molecules, an improvement in the electrochemical capabilities was yielded. This can be attributed to two different factors: i) the electrostatic attraction between the positive amine terminal groups of cysteamine and the negative anions of the redox marker, and ii) the incorporation of more oxygenated groups on the electrode surface after anchoring the glutaraldehyde molecule, increasing the hydrophilicity of the electrode surface. Finally, (f) the last functionalization step—which consisted of immobilizing the biomarker and the blocking agent (BSA) on the transducer surface to achieve the 3D-printed electrochemical immunosensor—also provided a remarkable enhancement on the electrochemical performance of the electrode. Interestingly, this behavior is contrary to that exhibited by conventional pure electrodes, where the hindrance effect provided by proteins immobilization leads to a current decrease [40]. Hence, this unconventional performance can be associated with an increased surface wettability (see Figure S1, water contact angles), facilitating the interfacial electron transfer rate between the redox marker and the nanocomposite electrode surface, which resulted in a notable Iₚ increase [43,44].

Further electrochemical characterization at each functionalization stage was carried out by EIS (see Fig. 1B). EIS is known to be a powerful technique for studying surface phenomena and electronic transfer capabilities along the frequency domain when the system is subjected to an AC bias potential [38]. Among the different pivotal parameters that can
be obtained from EIS measurements, the $R_{CT}$ value provides information about the facility of an electrode reaction. Interestingly, the histogram depicted in Fig. 1B shows a clear downward trend in the $R_{CT}$ value after each functionalization stage, indicating that the electrode surface became more and more conductive after each post-treatment. These results are also consistent with the ones obtained by CV ($I_p$ increases with each functionalization step). Therefore, the devised bottom-up functionalization procedure allows the achievement of biofunctionalized 3D-printed electrodes exhibiting excellent electrochemical performances.

Additionally, a morphological and elemental characterization of the developed 3D-printed immunosensors was also conducted by SEM and XPS, respectively. Both as-printed, activated and Au–functionalized electrodes were used for comparison. SEM images from Fig. 2 provide an insight of the surface changes achieved at the 3D-printed G/PLA electrode surfaces along different functionalization stages. Fig. 2A shows the electrode surface of the as-printed electrode with the carbon fiber embedded within the insulating PLA polymeric matrix. Fig. 2B clearly present a morphology that resembles a network of wires due to the activation post-treatments, fact that partially removes the insulating PLA polymer. Importantly, the presence of spherical nanoparticles on the graphene walls was evidenced after Au–NPs incorporation (Fig. 2C), suggesting the successful formation of metallic Au–NPs with an estimated average diameter around 35 nm (see Figure S2). Herein, Au–NPs act as nanotemplates for further functionalization via gold–thiol interactions [45]. Thus, after the last immobilization stage, the surface roughness of the resulting 3D-printed electrochemical immunosensor (Fig. 2D) dramatically increased, indicating the proper biofunctionalization of the 3D-printed G/PLA electrode surface with the protein-based biomarker.

This was also confirmed by XPS. Fig. 3A presents the wide-range survey XPS spectra of the 3D-printed G/PLA electrodes before (as-printed) and after being functionalized with Au–NPs and the biomarker, indicating that C 1 s and O 1 s are the two main elements present in all three substrates. Importantly, the presence of Au 4f peaks in the Au–functionalized electrode evidenced the effective in situ incorporation of Au–NPs. Moreover, an additional peak (N 1 s) was observed after anchoring the recognition biomarker owing to the nitrogen atoms from cysteamine and the immobilized protein. The absence of the Au 4f peaks in this substrate also indicates that the electrode surface was perfectly blocked by ABS. Regarding to the two different activation post-treatments — chemical (DMF) and electrochemical (EC) activation— carried out to achieve electrically active 3D-printed G/PLA electrodes, XPS was also run in order to quantify the %O. As shown in Fig. 3B, the atomic percentages obtained by the XPS spectra presented a significant %O increase from 27.6% to 30.2% after the EC treatment, indicating an optimum activation with oxygenated functional groups [39]. It is important to highlight that no metallic impurities (e.g., Ti or Fe) were observed in the raw carbon filament employed here after activation treatments, which might be found in the filament depending on the batch utilized for 3D-printed G/PLA electrodes fabrication [46,47].

Therefore, these electrochemical and physical characterization results clearly demonstrated the success of the surface engineering devised to achieve an unprecedented 3D-printed G/PLA platform for immunosensing achievements, which implies an in situ functionalization of 3D-printed G/PLA electrode surfaces with Au–NPs followed by a covalently immobilization of the biomarker via fundamental chemistry.

### 3.3. Immunosensing assay with electronic readout

Since the specific recognition biomarker (COVID-19 recombinant protein) is the one against the COVID-19 antibody, the developed 3D-printed electrochemical immunosensor suppose the first 3D-printed electronic prototype against the COVID-19 outbreak.

Firstly, an indirect competitive immunoassay was run for electrochemically monitoring the EIS changes achieved at the electrode/electrolyte interface when the 3D-printed electrochemical COVID-19 immunosensor was exposed to different free COVID-19 recombinant protein (antigen) concentrations in the presence of a fix concentration (10 µg mL$^{-1}$) of monoclonal COVID-19 antibody (incubation time: 20 min). Figure S3 shows the Nyquist plots from the EIS measurements. EIS signals were collected by means of charge transfer resistance ($R_{CT}$) values (a parameter inversely related to the electron transfer rate). All measurements were acquired per triplicate ($n = 3$) and represented with their corresponding standard deviation (SD) for reproducibility. A normalization procedure was carried out by means of $\Delta_{ratio} = (R_{CT,R0}/R_{CT})/R_{0}$, where $R_0$ and $R_{CT}$ are the $R_{CT}$ values before and after incubating with an antigen/antibody mixture, respectively) in order to obtain independent and reproducible results while comparing the different electrodes used here [48].

As shown in Figure S3, the binding between the biomarker and the accessible antibody (molecular weight greater than 100 kDa) promotes the formation of an insulating layer on the electrode surface that hinders the Fe(CN)$_6^{3−/4−}$ redox reaction, leading to a $R_{CT}$ increase ($\Delta_{ratio} = 1.03$). Since the analytical method relies in a competitive immunoassay, the concentration of free COVID-19 antibody available to interact with the recognition biomarker exposed on the electrode surface decreases with increasing the concentration of antigen in the media. Thus, a clear $\Delta_{ratio}$ decrease with increasing the concentration of COVID-19 protein from 1.0 to 50 µg mL$^{-1}$ was reached (see Fig. 4A). The electroanalytical
achievements of the 3D-printed electrochemical COVID-19 immuno-
sensor are shown in Fig. 4B. Promising electroanalytical results from the 
calibration curve were extracted, which includes an excellent linear 
regression ($r^2 = 0.995$) that displayed good sensitivity (0.076 ppm$^{-1}$) 
with a lineal range from 1.0 to 10 μg·mL$^{-1}$ and a limit of detection (LOD) 
of 0.5 ± 0.1 μg·mL$^{-1}$ for the COVID-19 antigen protein. This supposes 
that the developed 3D-printed COVID-19 immunosensor detects target 
COVID-19 antigen protein in physiological buffered pH at trace (ppm) 
levels. In addition, Table S1 shows a comparison between the 
electroanalytical parameters achieved by the 3D-printed COVID-19 
immunosensor with other antigen-based electronic devices developed 
for the determination of different coronaviruses (including the SARS-
CoV-2). The LOD obtained here is as good as that obtained by a con-
ventional colorimetric assay employing a paper-based device (see 
Ref. [3] from Supporting Information). Importantly, some pivotal elec-
tronoanalytical parameters achieved by this 3D-printed G/PLA electrode, 
such as LOD (0.5 μg·mL$^{-1}$), lineal range (1.0 to 10 μg·mL$^{-1}$) and 
reproducibility (error bars), were significantly improved with respect to

Fig. 2. Morphological SEM characterization of different 3D-printed G/PLA electrode surfaces: A) as-printed, B) activated, C) Au-functionalized and D) 3D-printed 
COVID-19 immunosensor.

Fig. 3. Wide XPS spectra of 3D-printed G/PLA electrodes at different functionalization stages. A) XPS spectra of as-printed, Au-functionalized and 3D-printed COVID-
19 immunosensor. B) XPS spectra of the chemical (DMF) activated electrode before and after the electrochemical (EC) treatment with their corresponding elemental 
percentage of C and O.

J. Muñoz and M. Pumera
those obtained by the only alternative 3D-printed immunosensing device —made of carbon black/PLA— reported to date (LOD of 22 μg mL⁻¹ and lineal range from 30 to 240 μg mL⁻¹ for the determination of the Hantavirus Araucaria nucleoprotein) [32]. However, since 3D-printing technology is still in early stages, further research is highly encouraged in light of this promising cross-disciplinary field.

Secondly, the selectivity of the monoclonal COVID-19 antibody employed towards the biomarker/antigen (COVID-19 recombinant protein) was explored against other unrelated proteins, using the BSA as the proof. This control experiment was run in order to elucidate the key role of the monoclonal COVID-19 antibody for recognizing the biomarker. For this aim, a 3D-printed biosensor was fabricated by immobilizing the non-specific protein as the biomarker and used for the determination of a fixed concentration of monoclonal COVID-19 antibody (see inset from Fig. 5 for illustration). As shown in Fig. 5, the EIS capabilities of the developed 3D-printed COVID-19 immunosensor notably changed after interacting with a 10 μg mL⁻¹ concentration of antibody, yielding to a Δ_ratio value of 1.03. Remarkably, no significant EIS changes were noticed after interacting the resulting control biosensor with such a fixed concentration of COVID-19 antibody (Fig. 5B), resulting in a Δ_ratio value as low as 0.03. Comparing both Δ_ratio values (Fig. 5C), the interfering signal achieved at the control 3D-printed bioelectrode was lower than 3%. This demonstrates i) the selectivity of the antigen-antibody interactions at the 3D-printed COVID-19 immunosensor. EIS measurements were obtained before and after being incubated with 10 μg mL⁻¹ COVID-19 antibody for 20 min using a 10 mM [Fe(CN)₆]³⁻⁻/⁴⁻ PBS (pH 7.2) as the electrolyte.
immunosensor—it taking into account that the monoclonal COVID-19 antibody did not interact with the proof non-specific (BSA)—as well as ii) the specificity of the 3D-printed COVID-19 immunosensor since no non-specific adsorption was observed without the immobilization of the specific biomarker (COVID-19 recombinant protein). Importantly, these anti-interfering results are in agreement with the monoclonal nature of the COVID-19 antibody employed. Monoclonal antibodies provide higher specificity than polyclonal counterparts because they recognize a single epitope, fact that avoids cross reactions with non-specific proteins [49]. In addition, monoclonal antibodies also favor reproducibility since they do not change batch to batch and therefore, they are widely used for clinic diagnosis [50].

Additionally, the EIS behavior of the 3D-printed COVID-19 immunosensor in human fluids was interrogated by using a 100x diluted human serum sample solution. Histograms from Fig. 5C also compare the \( \Delta_{\text{ratio}} \) values achieved after incubating the 3D-printed COVID-19 immunosensor in either PBS (pH 7.2) or human serum samples with a fixed concentration (10 \( \mu \text{g.mL}^{-1} \)) of COVID-19 antibody for 20 min. While a \( \Delta_{\text{ratio}} \) value of 1.03 was obtained in the buffered solution, a close \( \Delta_{\text{ratio}} \) value of 1.01 was reached using the serological sample. Thus, the electrochemical performance of the electronic device was not significantly affected by potential interferences from proteins/biomolecules present in human serum through non-specific binding. Further, the applicability of the developed 3D-printed immunosensing system was evaluated in a spiked serological sample containing 3.5 \( \mu \text{g.mL}^{-1} \) of COVID-19 antigen, where an average \( \Delta_{\text{ratio}} \) of 0.77 was obtained (Fig. 5C). After interpolating this value on the calibration curve (see Figure S4), a concentration as good as 3.6 \( \pm 0.1 \mu \text{g.mL}^{-1} \) was yielded, which corresponds to a recovery of 103%. Accordingly, these results clearly suggest the potential of the developed 3D-printed COVID-19 immunosensor towards rapid antigen-based COVID-19 tests in real samples.

Finally, the feasibility of a direct impedimetric immunosassay was also interrogated by immobilizing the COVID-19 antibody on the 3D-printed electrode surface as the biomarker (see Figure S4). No significant EIS changes at the electrode/electrolyte interface were observed after incubating the resulting antibody-based 3D-printed COVID-19 immunosensor with different concentrations of antigen for 20 min, demonstrating that an indirect competitive immunosassay is a must for a fruitful EIS recognition of this explored antigen–antibody system.

4. Conclusions

Along this COVID-19 global pandemic crisis, 3D printing technology has been at the forefront in the fight against the SARS-CoV-2 virus, enabling the fast and on-demand fabrication of personal protection equipment or isolation wards. However, a clear gap was observed regarding to the development of low-cost antigen-based 3D-printed electronic devices. Accordingly, this work reports the bases for the fabrication of the first 3D-printed electrochemical COVID-19 immunosensor prototype for point-of-care detection. For this aim, surface engineering has been devised to exploit the carbon reactivity of 3D-printed G/PLA electrodes via a simple bottom-up approach. The feasibility of the 3D-printed electrochemical COVID-19 immunosensor towards the impedimetric screening of the antigen against the SARS-CoV-2 virus has been demonstrated in both buffered and diluted serological samples, achieving a detection limit at trace levels in just 20 min. As the first prototype, this 3D-printed COVID-19 immunosensor just represents the tip of the iceberg, and there is enough room for the improvement. Thus, the slump from the prototype to the massive screening in real samples would require the exploration of additional interfering proteins (i.e., other coronaviruses) in human fluids and/or their integration for a point-of-use analyses.

In the light of the state-of-the-art, this work combines i) the use of 3D printing technology for the large-scale and eco-friendly production of customized-shape electronic devices on-demand, anywhere at any time with ii) the electrical readout of antigen–antibody systems. Owing to electronic devices allow rapid turnaround time from sample to results, such a combination could bring light to this emerging health crisis by massively manufacturing at-point-of-use antigen tests with file being distributed by speed-of-light internet. Finally, this approach is general and could be easily customized to assess alternative antigen–antibody systems at 3D-printed electrodes.

CRediT authorship contribution statement

Jose Muñoz: Conceptualization, Investigation, Formal analysis, Validation, Writing – original draft, Drafting - review & editing. Martin Pumera: Conceptualization, Writing - review & editing, Supervision, 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.

Acknowledgements

M. P. acknowledges the financial support of Grant Agency of the Czech Republic (EXPRO: 19-26896X). J.M. acknowledges CzechNanoLab Research Infrastructure supported by LM2018110 MEYS CR 2020–2022.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcej.2021.131433.

References

[1] D. Cucinotta, M. Vanelli, WHO declares COVID-19 a pandemic, Acta Biomed. 91 (2020) 157–160.
[2] G. Chauhan, M.J. Madou, S. Kalra, V. Chopra, D. Ghosh, S.O. Martinez-Chapa, Nanotechnology for COVID-19: therapeutics and vaccine research, ACS Nano. 14 (2020) 7760–7782.
[3] Y.H. Chung, V. Beits, S.N. Fiering, N.F. Steinmetz, Covid-19 vaccine frontrunners and their nanotechnology design, ACS Nano. 14 (10) (2020) 12522–12537.
[4] X. Cao, COVID-19: immunopathology and its implications for therapy, Nature. Rev. Immunol. 20 (5) (2020) 269–270.
[5] W.C. Chu, Nano research for COVID-19, ACS Nano. 14 (4) (2020) 3719–3720.
[6] J.E. Corral, S.A. Hoogenboom, P.T. Kroner, M.I. Vazquez-Roque, M.F. Picco, F. A. Farrarye, M.B. Wallace, COVID-19 polymerase chain reaction testing before endoscopy: an economic analysis, Gastrointest. Endosc. 92 (3) (2020) 524–534.e6.
[7] B. Udagama, F. Kudhiresan, H.N. Koslowski, A. Malekjahani, M. Osborne, V.Y. C. Li, H. Chen, S. Muberska, J.B. Gubbay, W.C.W. Chan, Diagnosing COVID-19: the disease and tools for detection, ACS Nano. 14 (2020) 3822–3835.
[8] Y. Hirotsu, M. Maejima, M. Shibawada, Y. Nagakubo, K. Hosaka, K. Amemiya, H. Sueki, M. Hayakawa, H. Mochizuki, T. Tsutui, Y. Kihikazi, Y. Miyashita, S. Yagi, S. Kojima, M. Omata, Comparison of automated SARS-CoV-2 antigen test for COVID-19 infection with quantitative RT-PCR using 313 nasopharyngeal swabs, including from seven serially followed patients, Int. J. Infect. Dis. 99 (2020) 397–402.
[9] S. Griffin, Covid-19: lateral flow tests are better at identifying people with symptoms, finds Cochrane review, BMJ 372 (2021).
[10] T. Ogawa, T. Fukumori, Y. Nishihara, T. Sekine, N. Okuda, T. Nishimura, H. Fujikura, N. Hirai, N. Imakita, K. Kasahara, Another false-positive problem for a COVID-19 antigen test in Japan. J. Clin. Virol. 131 (2020) 104612.
[11] T. Xiao, F. Wu, J. Hao, M. Zhang, P. Yu, L. Mao, In vivo analysis with electrochemical sensors and biosensors, Anal. Chem. 89 (1) (2017) 300–313.
[12] R. Li, H. Qi, Y. Ma, Y. Deng, S. Liu, Y. Jie, J. Jing, J. He, X. Zhang, L. Whearey, C. Huang, X. Sheng, M. Zhang, L. Yin, A flexible and physically transient electrochemical sensor for real-time wireless nitric oxide monitoring, Nat. Commun. 11 (2020) 3207.
[13] S.D. Bakkigar, N.P. Shetti, T.M. Aminabhavi, Electrochemical investigations for COVID-19 detection–a comparison with other viral detection methods, Chem. Eng. J. 420 (2021) 127575.
[14] R.R.X. Lim, A. Bonanni, The potential of electrochemistry for the detection of coronavirus-induced infections, TiAC - Trends Anal. Chem. 133 (2021), 116081.
