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Detecting and inactivating severe acute respiratory syndrome coronavirus-2 under the auspices of electrochemistry

Ghazala Ashraf [a], Ayesha Aziz [a,b], Rubina Naz Qaisrani [c], Wei Chen [a,**], Muhammad Asif [b,*]

[a] Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology (HUST), Wuhan, 430074, PR China
[b] Hubei Key Laboratory of Plasma Chemistry and Advanced Materials, School of Materials Science and Engineering, Wuhan Institute of Technology, Wuhan, 430205, China
[c] Institute of Chemical Sciences, Gomal University, Dera Ismail Khan, 29050, Pakistan

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ABSTRACT

The recent epidemic of novel coronavirus (COVID-19) has turned out to be a huge public health concern owing to its fast transmission. Rapid and cost-effective detection of SARS-CoV-2 is crucial to classify diseased individuals. Serological examination based on antibody chromatography as a substitute to RT-PCR provides inadequate help owing to sophisticated personnel, false-positive results, special equipment and high cost. Biosensing techniques provide sensitive and specific detection, recognition and quantification of pathogens. Herein, after an introduction, we review potential electrochemical (EC) biosensors for COVID-19 diagnosis, emphasizing plasmonic, optical, colorimetric and aptamer-based sensors with a special focus on EC biosensors and point-of-care (POC) diagnostic methods. We have conferred the working principle of these biosensors, EC performance in terms of particular analytical figures of merit and their real-time applications in biological matrices. Lastly, we have described briefly the inactivation of SARS-CoV-2 by EC oxidation. In the end, we have concluded this review by clearing up the strengths and weaknesses of EC sensors and future directions. Advancement in research and technology would be our unsurpassed weapons in the fight against COVID-19 and preventing imminent pandemics.

1. Introduction

The COVID-19 is a contagious ailment instigated by SARS-CoV-2 (Miripour et al., 2020). World Health Organization has announced the COVID-19 disease as a pandemic in March 2020 (Udugama et al., 2020). The SARS-CoV-2 with virion size of 50–200 nm comprises 28 proteins (Wu et al., 2020) including nucleocapsid (N), membrane (M), envelope (E) and spike (S) structural glycoproteins, can be practically employed as an antigen for detection of COVID-19 (Fig. 1A). It attaches to the host individual via binding its spike protein to the ACE2 receptor. The genetic population investigation of SARS-CoV-2 concluded that it has two advanced genotypes (L and S type) (Tang et al., 2020). SARS-CoV-2 with L type of genotype is more virulent than S type with 70% of total cases. The health risks of COVID-19 are long-lasting and continuous which not only harmful to public health but also risky for worldwide economic stability. As the vaccine improvement is still on the way that could be a possible solution to overcome this catastrophe now and to circumvent resurfacing again (Ayesha et al., 2020). Initially, a combination of computed tomography (CT) scans, next-generation sequencing, Polymerase Chain Reactions (RT-qPCR), ELISA and CRISPR based diagnostic approaches were used to scan and detect SARS-CoV-2 (Udugama et al., 2020; Asif et al., 2020). These molecular methods (RT-PCR, CRISPR) are more appropriate than syndromic testing and CT scans for correct identifications of virus (Xi et al., 2020). Though, CT scanners are restricted to bigger hospitals, but cannot differentiate a particular virion from different viruses.

Besides, (RT-PCR) based detection of epidemiological RNA is considered the benchmark method for diagnosing SARS-CoV-2. On contrary, it is a laborious assay that may take 4 h to perform a single test and has a great possibility of inaccurate results. The affected individual with preliminary false-negative results can spread the disease through foiling the appropriate control of contamination. Meanwhile, antibody response becomes evident around the 10th day afterward the start of signs thus all the assays that can detect antibodies cannot be consistent

* Corresponding author.
** Corresponding author.
E-mail addresses: chen1980wei@hust.edu.cn (W. Chen), asif83chemist@gmail.com (M. Asif).

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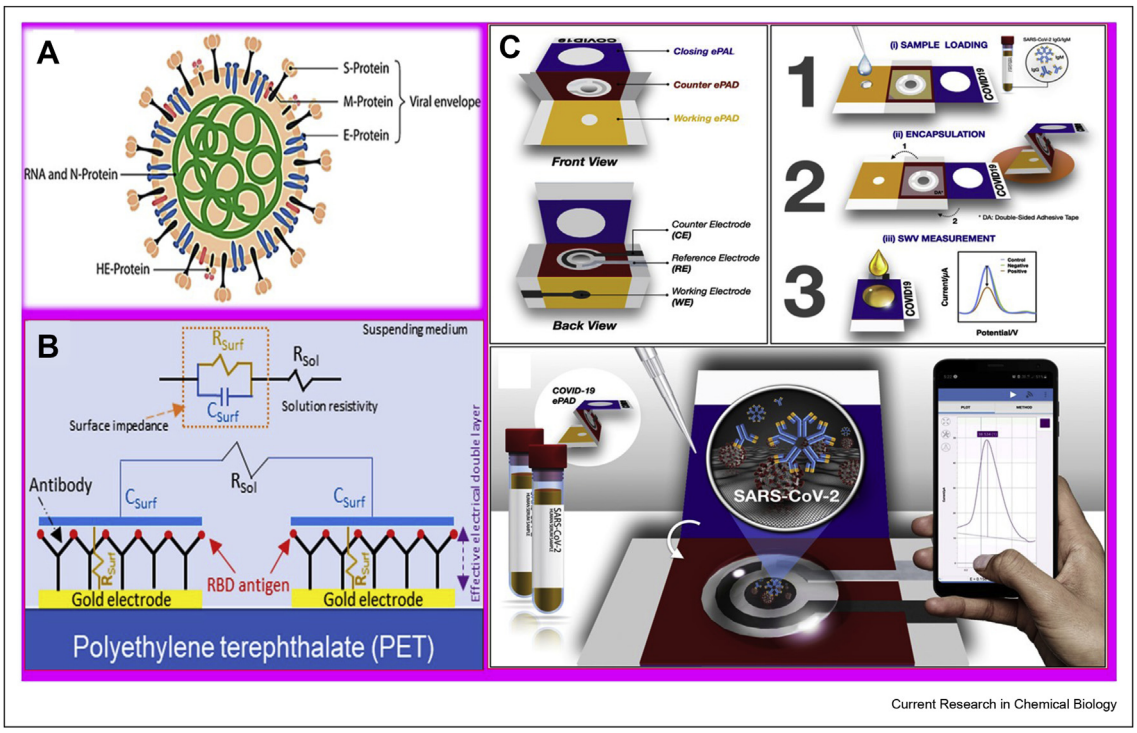

Fig. 1. (A) Structural illustration of SARS-CoV-2 Virion and specific proteins, (B) A 16-well plate EC sensor based on EIS analysis of SARS-CoV-2, (C) schematic of a low-cost, paper-based EC sensor for detection of SARS-CoV-2 antibodies employing SWV technique. Reprinted with permission (Rashed et al., 2021; Yakoh et al., 2020).

for early disease diagnosis of asymptomatic persons. The false positives are most possibly due to the interferences caused by other proteins in serum samples. Moreover, the RT-PCR system may not be on time available or affordable in several countries (Morales and Dincer, 2020). Above mentioned methods are sophisticated and reliable but they have particular practical restrictions. Yet, EC sensing systems have attained ample research attention owed to the compactness, low cost, swift response, and easy handling. EC sensors translate the reactions of biological mediums with analyte into EC indicators that can be observed and documented by different techniques (*i-e.,* impedance spectrometry, amperometry and voltammetry). The EC sensors can differentiate various targets together with proteins, nucleic acids, chemicals, biomolecules, microorganisms, antigens, and antibodies (Ashraf et al., 2021). To ensure extremely accurate and flexible detection, recognition and quantification of viruses, EC sensors strive to grow more advanced nanomaterials and functional developments (Khan et al., 2020; Ashraf et al., 2020; Ashraf et al., 2019). These sensors include optical, EC, colorimetric and surface plasmon resonance (SPR), etc (Asif et al., 2018b; Aziz et al., 2019a; Aziz et al., 2019b). Among these, EC label-free sensors provide point of care (POC) diagnosis and mass production (Kaya et al., 2020; Guo et al., 2020).

In this review, we outline the recent developments in EC biosensing platforms with main focus on their working principle and core analytical figures of merit for early diagnosis of SARS-CoV-2. Biosensors have the desired aptitude to detect infectious agents directly with high sensitivity and specificity without sample pretreatment (Asif et al., 2018a; Asif et al., 2019). Besides, we have added a section on the inactivation of SARS-CoV-2 by EC oxidation method and then we have concluded this review by clearing up the strengths and weaknesses of EC sensors and future directions. The review paper demonstrating the topical diagnostic discoveries solely on biosensors may play a significant part in guiding practices to improve initial diagnostics along with a remedial window for the COVID-19 pandemic.

2. Potential EC biosensors for SARS-CoV-2 detection

Biosensors are excellent platforms for continuous monitoring and real-time diagnosis of clinical analytes. A twin fold plasmonic LSPR sensor based on Au nanoisland chip was developed to detect ORF1ab, RdRp virus sequences and E gene from SARS-CoV-2 in clinical samples. Photothermal plasmonic heat effect was employed on 2D Au NIs functionalized with cDNA which increased the *in-situ* annealing temperature and enabled the precise differentiation of RdRp gene sequence from SARS-CoV-2, SARS-CoV genes with the lowest (0.22 pM) detection limit (Qiu et al., 2020). The practical application of the biosensor was confirmed for COVID-19 diagnosis in respiratory samples with high selectivity. Of late, this SPR constructed biosensor was developed for specific anti-SARS-CoV-2 nucleocapsid antibodies sensing in pure human serum samples (Djaileb et al., 2020). The sensor was coated with a single layer peptide monolayer and functional moieties of recombinant SARS-CoV-2 nucleocapsid protein. The sensor performance was evaluated by anti-SARS-CoV-2 antibodies detection in the nM concentration range within 15 min. Furthermore, a graphene-based FET biosensing platform was reported for COVID-19 diagnosis in different cultured and clinical samples (Seo et al., 2020). The sensor was fabricated by coating a particular spike protein antibody on a graphene sheet of FET platform. The sensor exhibited (1 fg mL^{-1}, 100 fg mL^{-1}) LOD for S protein of SARS-CoV-2 in PBS and therapeutic transport medium respectively. The biosensor displayed excellent selectivity towards SARS-CoV-2 in presence of MERS-CoV. The real-time detection of COVID-19 disease was evaluated by employing biosensor in clinical samples and nasopharyngeal swabs. The sensor showed 1.6×10^1 PFU mL LOD in cultured media. An EC immunosensor was reported recently for the detection of (S) protein of SARS-CoV-2 and anti-SARS-CoV-2 in serum samples (Rashed et al., 2021). A 16-well plate from ACEA Biosciences (see Fig. 1B) was projected for non-invasive exposure of structural changes and cell proliferation. The plate comprised of sensing electrodes pre-treated

receptor-binding domain (RBD) of (S) protein followed by washing with PBS-Tween-20 solution, filled with a blocking solution and tested for negative CR3022 antibody with (0.1, 1.0 and 10 μg mL^{-1}) concentration. The serum samples from infected and healthy individuals were tested by EIS measurements within 5 min. The designed sensor was compared with the ELISA test that showed consistent results with ($R^2 = 0.9$).

Similarly, a label-free EC paper-based sensor was designed to detect immunoglobulins (IgG and IgM) of SARS-CoV-2 (Yakoh et al., 2020). The working mode of the designed EC sensor consisted of 3 (working, counter and closing) electrodes/pads. The test pad of the sensor was coated with RBD of (S) protein to capture SARS-CoV-2 antibodies. The counter pad was folded up on working pad and fixed with a closing pad (Fig. 1C). This arrangement minimized the direct interaction with biofluid and prevented contact with the surrounding. The EC performance was evaluated by using SWV that exhibited decreased current response immunocomplex development. The sensor detected SARS-CoV-2 immunoglobulins in clinical serum samples with 1 ng mL^{-1} LOD that was 3 times higher than traditional colorimetric assay. The colorimetric bioassays based on nanomaterials are suitable in biosensor fabrication owing to their ease of handling, visual result, and simple instrumentation. A colorimetric-SPR bioassay based on (Au NPs) capped with carefully designed thiol treated antisense oligonucleotides (ASO) was reported for COVID-19 detection from RNA samples within 10 min (Moitra et al., 2020). In presence of a specific RNA sequence of SARS-CoV-2, the ASO/AuNPs aggregate selectively and demonstrate a variation in its SPR signal (Fig. 2A). The injection of ribonuclease H splits the RNA components from its hybrid that leads to the formation of visible precipitates in the solution facilitated by the supplementary accumulation of (AuNPs). The fabricated biosensors demonstrated remarkable selectivity towards SARS-CoV-2 RNA with 0.18 ng μL^{-1} LOD. A serological testing approach based on biolayer interferometry system was developed by employing immunosorbent assay for early detection of SARS-CoV-2 antigen-specific-antibodies (Fig. 2B). The designed fiber optics-based assay measured binding between biosensor surface and biomolecules that produced a real time output because of wavelength shift in refracted light (Dzimianski et al., 2020). The developed biosensor provided feasible dip and read set up for real-time detection of antigen loading, specific antibodies/plasma binding and total antibodies in a single reaction mixture free from any fluidics. Also, (S1) protein was used as biomarker for early diagnosis of SARS-CoV-2 by a cell-based biosensor (Mavrikou et al., 2020). The sensor was designed by S1 protein attachment of membrane engineered antibodies that resulted in a specific and significant variation in bioelectric properties of the cell where it is measured by a smartphone-based readout device. The novel portable biosensor provided test results within 3 min with no cross-reaction against (N) protein of SARS-CoV-2 with lowest LOD of 1 fg mL^{-1} and 10 fg mL^{1-} to 1 μg mL^{1-} linear range.

Recently, a molecularly imprinted polymer (MIP) sensor was reported for nucleocapsid protein detection of SARS-CoV-2 antigen (Raziq et al., 2021). A portable potentiostat attached to the sensing platform was controlled by a software in mobile phone (Fig. 2C). The main component of the sensor comprised of Au-thin film electrode modified with nucleocapsid protein-MIP produced from poly-m-phenylenediamine in an EC cell *vs* Ag/AgCl/KCl in adjusted charge density. The sensor chip was

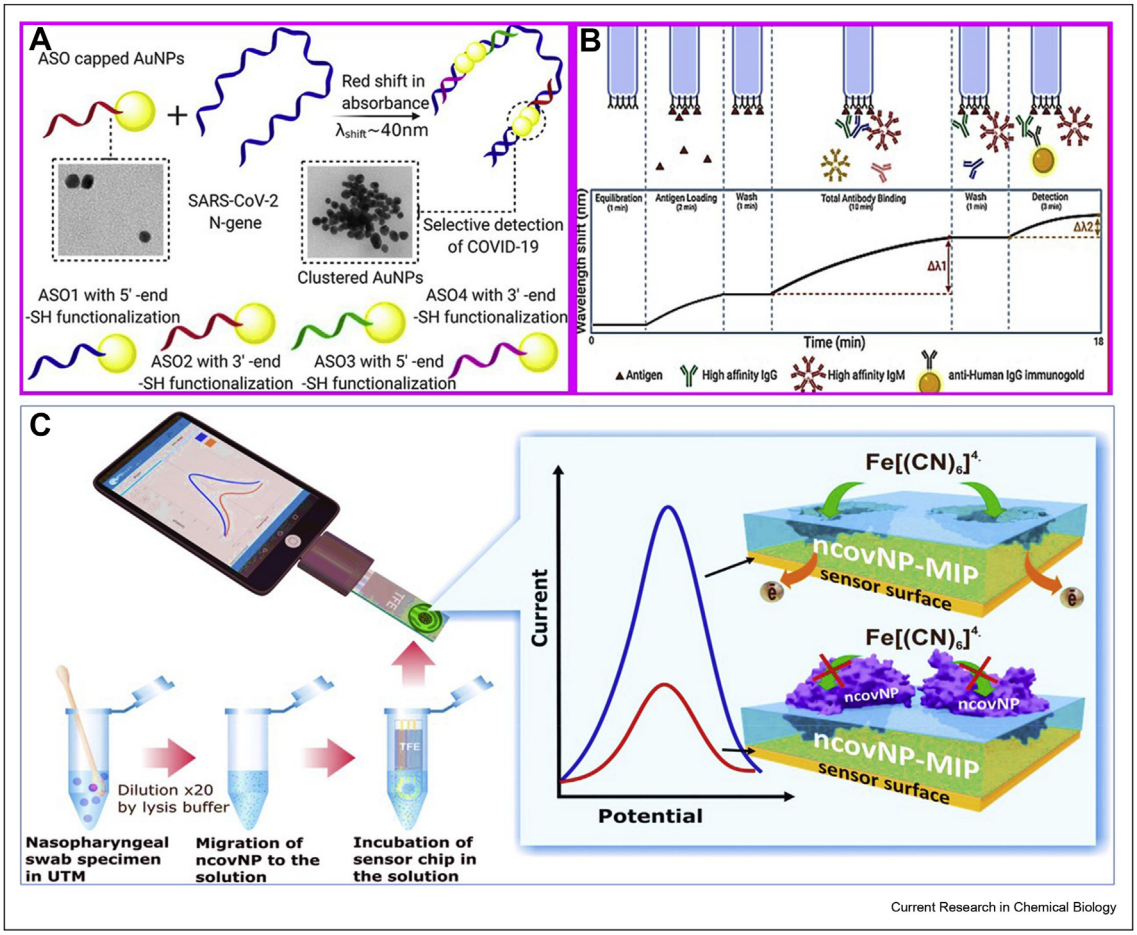

Fig. 2. (A) Functionalized ASO sequence and schematic illustration of ASO capped Au NPS agglomeration, Reprinted with permission (Moitra et al., 2020) (B) summary of SARS-CoV-2 determination from sample loading to detection step, Reprinted with permission (Dzimianski et al., 2020) (C) Schematic of working principle for SARS-CoV-2 antigen detection from nasopharynx samples. Reprinted with permission (Raziq et al., 2021).

incubated in the sample solution obtained from the nasopharynx swab of infected people and potentiostat measured a drop in charge transfer intensity of $Fe(CN)_6]^{3-/4-}$ redox probe. The developed sensor detected SARS-CoV-2 antigen with 50 fM LOD with high selectivity and reported 9 weeks storage stability.

An aptamer-based portable and reusable sensor was developed recently for SARS-CoV-2 diagnosis in just 60 Seconds. The designed sensor was plugged into smartphone and indicated a positive test result on screen by measuring the electrical resistance of complementary virus proteins in sample. The sensor was able to detect virus in saliva, microscopic particles and on solid surfaces as well. The sensor was designed to be reused by destroying the previous virus present on it *via* heat produced by electric current. The method involved the placement of small strips on the sensor surface for sample application that eliminated the sample destruction and cross-contamination (https://www.ineffableisland.com/2020/05/portable-reusable-sensor-for phone.html). Likewise, Huang and coworkers (Huang et al., 2020) have designed a plasmonic nano biosensor unified with a typical 96-well plate for one step detection of specific (S) protein functionalized SARS-CoV-2 particles without prior sample processing. The sensor performed high sensing aptitude by 30 viral particles detection a single step within 15 min with remarkable specificity in presence of VSV, MERS and SARS particles. The virus particles were quantified in the proportional concertation range of 10^3 vp mL^{-1} to 10^6 vp mL^{-1}. Comparable sensing aptitude was also confirmed by Au NPs enhanced portable optical apparatus and recorded results showed that reported sensors might be employed quickly in clinical diagnostics of COVID-19. Moreover, the (SARS-CoV-2-Ag) antigen was detected in spiked saliva by a self-constructed EC immunosensor (eCovSens) (Mahari et al., 2020). The designed biosensors based on SPCE modified electrode detected changes in EC signals upon the interaction of (SARS-CoV-2-Ab/Ag) with 10 fM LOD in buffer. The sensing performance of (eCovSens) device was compared with conventional potentiostat based (FTO/AuNPs/SARS-CoV-2-Ab) electrode that showed high sensitivity towards (SARS-CoV-2-Ag) detection in saliva samples with (90 fM LOD), excellent selectivity, fast detection (within 30 Sec) and four-week storage capacity. Moreover, contrasting to potentiostat, the (eCovSens) device was portable, battery-operated with (1.3–3V) usage, could be used for POC diagnosis of COVID-19 patients.

A smartphone-based EC sensor for SARS-CoV-2 detection from RNA extracts of 25 COVID-19 and 8 recovery patients was reported recently (Zhao et al., 2021). The developed sensor was based on the sandwich-type assembly of capture probe on Au@Fe_3O_4 NPs. Next, a host-guest complex of calixarenes/toluidine blue as EC mediator was immobilized reduced graphene and Au@SCX8-TB-RGO-LP bioconjugate obtained. The obtained sandwich-type (capture and label probe) EC sensor produced long concatemers after the addition of an auxiliary probe. EIS and DPV techniques were employed to analyze the EC performance of the developed sensor that showed linear range of 10^{-17} to 10^{-12} with 3 aM LOD. Plasmonic sensors are favorable devices that have enabled the recognition and specific screening of various biomarkers. A downsized plasmonic sensor based on the idea of toroidal

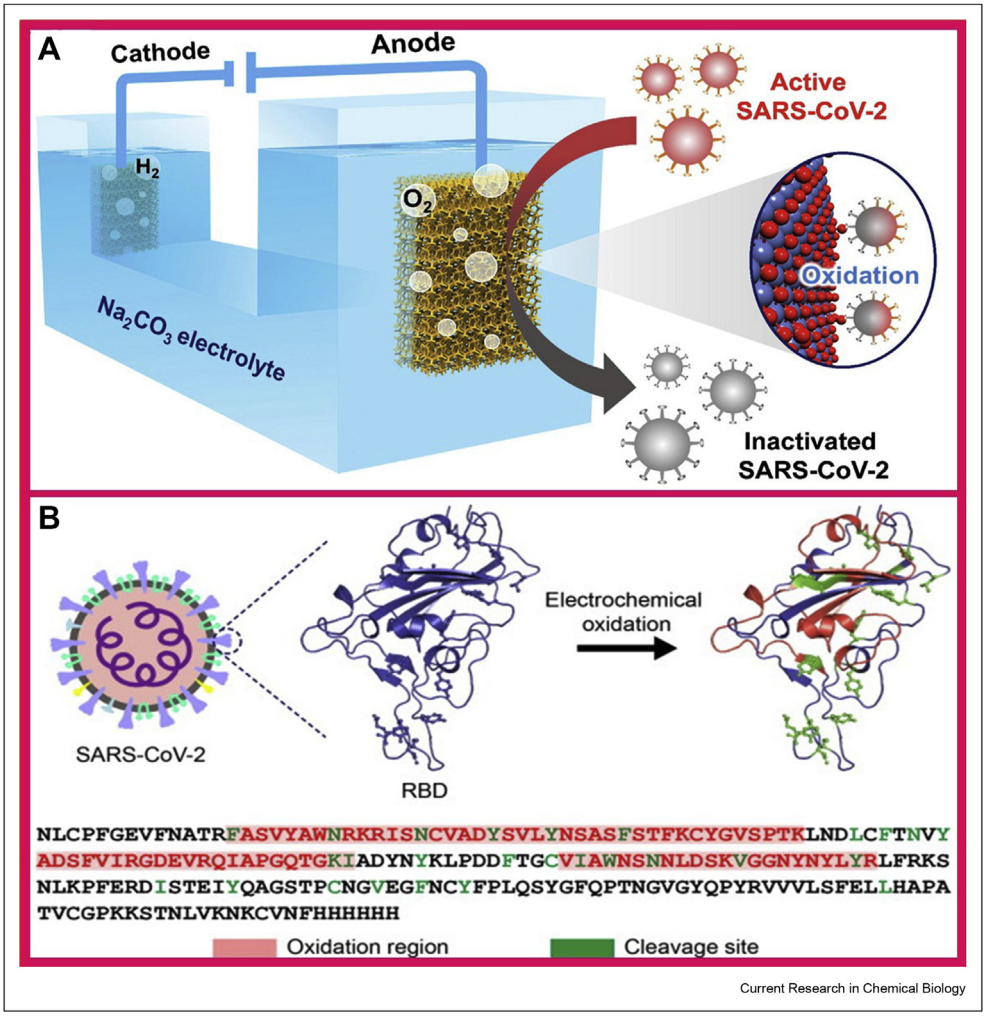

Fig. 3. (A) Graphic illustration of the EC inactivation method for SARS-CoV-2, (B) The mechanism SARS-CoV-2 inactivation with oxidation sequence regions and EC oxidative cleavage positions in the RBD. Reprinted with permission (Tu et al., 2020).

electrodynamics that can withstand the limited plasmonic approaches through ultranarrow lines in terahertz frequency was fabricated for SARS-CoV-2 S protein detection (Ahmadivand et al., 2020). Owing to the advantage of increased sensitivity of toroidal reverberations to environmental agitations aided the development of a metasensing podium with 4.2 fM lowest LOD in 80 min. These investigations proved that developed sensors can be employed for the detection of SARS-CoV-2 specific proteins, antibodies and antigens in diverse biological samples for early disease diagnosis.

3. Inactivating SARS-CoV-2 by EC method: a teaser

Advanced disinfection and supplementary preventive measures towards SARS-CoV-2 have been implemented globally to restrict its spread. Since SARS-CoV-2 is a biosafety level 3 agent and a novel virus, decontamination data for this particular virus is rare. The complete deactivation of SARS-CoV-2 is currently accomplished by various techniques using unsafe antiseptics (*i-e* chlorination). Therefore, it is important to develop sustainable approaches for proficiently disinfecting virus. Currently, SARS-CoV-2 inactivation was achieved electrochemically oxidation on a highly stable Ni-foam (Tu et al., 2020). They set up two-electrode-based EC cell using Na_2CO_3 electrolyte, Ni foam and *in-situ* formed (NiOOH) as cathode and anode respectively (Fig. 3A). The Ni foam possesses high conductivity, pore size and highly active site that promoted SARS-CoV-2 diffusion during the EC oxidation reaction. The inactivation of SARS-CoV-2 in a tissue culture reached 99% within 5 min at an applied voltage of 5 V and ($\leq$37 °C). Theoretical calculations and mass spectrometric investigations revealed that reactive oxygen species produced on the anode surface-induced the peptide backbone cleavage by oxidizing peptide chains of RBD of (S) protein, and thus deactivated the virus. The results suggested that high fraction of amino acids in the RBD sequence made it easy to be attacked and decomposed (Fig. 3B). This approach delivered a well-organized and sustainable methodology for the decontamination of the (SARS-CoV-2) viruliferous vaporizers and effluents.

4. Conclusion and future outlook

EC sensors can be well thought-out as advanced tools that can lead to life-saving decisions on treatment and understanding of disease strains. In conclusion, a framework of different sensing techniques and up-to-date sensors developed for SARS-CoV-2 detection is provided in this review. These EC sensors are frequently based on virus protein/antigen/antibody detection for COVID-19 diagnosis by employing portable SPR, FET, cell-based, chip and aptamer-based, optical sensors and Au NPs based EC sensors and POC devices. Furthermore, complete deactivation of SARS-CoV-2 by EC oxidation approach has been explained. These state-of-the-art sensing platforms could pave the way for swift, accurate, specific, sensitive and onsite detection together with analytical devices for COVID-19 and other unparalleled pandemics.

Though EC sensing platforms are extremely promising, but they still face various challenges to move from bench to their use in POC. In our opinion, the problem associated with EC sensors presenting their lesser availability in device setup may be the concerned matter of least usability for SARS-CoV-2 detection by clinicians. The development of NPs based EC sensors as a diagnostic probe is substantial in the detection of viruses. There should be progress in nanomaterials based advance EC sensors with first-rate selectivity and sensitivity which allow machine-based signal handing out for sensing of viral antigens/other indicators. Other commercially available kits and products (such as ELISA, Abbot's well-known ID NOW™ kit and RT-PCR system) are sensitive, precise and often provide quantifiable detection of the SARS-CoV-2. Though, they are complicated, expensive and slow to transport. Despite the recent advancements in POC devices, it is significantly desirable to authenticate the consistency of these apparatuses with actual medical samples to evaluate their accurate clinical suitability. Moreover, to overcome the restrictions linked with discrete sample preparation, minimal user contact to the virus and device engineering scale-up is required. Great efforts are needed to deliver reusable and portable devices proficient in discriminating viruses with high selectivity and sensitivity echelons.

Declaration of competing interest

The authors declare no conflict of interest.

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