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Highly-sensitive and rapid detection of COVID-19 in wastewater: a comprehensive review based on graphene field effect transistor biosensor

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The novel Corona Virus Disease 2019 (COVID-19) has been spread from the Wuhan city of China has now affected many countries; it is still circulating worldwide. Consecutive studies of finding the RNA of this virus in sewage systems increase renewed interest about COVID-19 faucal transmission and its pathogenic issue on sanitation and wastewater systems. Municipal wastewater is typically remarked as one of the major end routes of different types of emerging contaminants such as pharmaceuticals, endocrine disruptors, antibiotics, micro plastics, pesticide and heavy metal residues associated with antimicrobial resistance. Currently all available, antibodies based and molecular base testing have some limitations for this purpose: whole coronavirus particles instead of pure antigen proteins need to be tested in a short time and take control of the pandemic of COVID-19. The current study helped in understanding, concept and demonstrated the potential of graphene Field Effect Transistor (FET) technology for sensitive and rapid detection of corona viruses. Therefore, extra trustworthy, quick response, economical and broadly accessible analytical devices or diagnostic approaches are crucially required. We have critically reviewed and argued the biomarkers and indicators used for COVID-19 diagnostics or SARS-CoV-2 detection. In this regard, ultrasensitive graphene FET biosensors are powerful tools in early diagnosis of COVID-19 infection *via* targeting virus S1 protein to assess the clinical progress and offer awareness on severity and critical trends of infection.

Keywords: COVID-19; Graphene; Field Effect Transistor and SARS-CoV-2

INTRODUCTION

The novel COVID-19 has been spread from the Wuhan city of China since late in December 2019 has now affected nearly 200 countries worldwide creating a colossal dysfunction of various activities all around the world (Giri et al.

2020). Evoke the disease COVID- 19, and in many countries, it is still circulating at high speed (Coccia, 2020). Typically, spreading the small droplets of coughs, saliva, or breathing out of COVID-19 patients is the main cause of the virus transmission. As saliva can act as a contagion transmittance object between personnel via contact with its spread droplets, it can be used as a reliable, suitable, and non-invasive analyte for the laboratory diagnosis of infections caused by SARS-CoV-2 as an acute respiratory syndrome (Peng et al. 2020).

Coronaviruses constitute the subfamily *Orthocoronavirinae*. These are enfold viruses, positive-sense single-stranded RNA genome and a nucleocapsid of helical symmetry with genome size ranging from approximately 26 to 32 kilobases, one of the largest among RNA viruses (Liu et al. 2020). Resembling other coronaviruses, SARS-CoV-2 primarily has four structural proteins, namely, the spike (S), membrane (M), envelop (E), and nucleocapsid (N) proteins, respectively (Wrapp et al. 2020). The S protein of virus from full advantageous to tie angiotensin convert enzyme 2 (ACE2) on the human cell surface to get entrance in a host cell. The N protein supports the viral genome but also confound in the host cellular response to viral infection. S, E, and M proteins co jointly make the viral outer protecting membrane (He et al. 2020). Both proteins (e.g. S protein) and viral RNA can be used as targets area for COVID-19 detection. Alternatively, antibodies such as IgM and IgG from patient samples could also be detected for recognizing the infection history. SARS-CoV-2 RNA is detectable 2–3 days before onset of indication and can last up to 25–50 days afterwards, depending on disease severity (Hart et al. 2020).

To date, no comprehensive review is available on the use of graphene biomarker for the rapid detection of novel corona diseases.Therefore, the present review highlight the safe use of grapehene biosensors as a powerful tools in early diagnosis of COVID-19 infection *via* targeting virus S1 protein.

Transmission of SARS-COV-2 in waste water system

Consecutive studies of finding the RNA of this virus in sewage systems increase renewed interest about COVID-19 faucal transmission and its pathogenic issue on sanitation and wastewater systems (Ahmed et al. 2020). Municipal wastewater is typically remark as one of the major end routes of different types of emerging contaminants such as pharmaceuticals, endocrine disruptors, antibiotics, micro plastics, pesticide and heavy metal residues associated with antimicrobial resistance (Kitajima et al. 2020). Sewage flow from the municipal system, hold a large number of fractious compounds, which are commonly eliminated by humans and other activities. Despite global hotspots for drug-infested countries where drug deals and usages are widespread are usually discovered through the sewage systems (Randazzo et al. 2020). There are numerous measures surrounding the movement restriction of about 93% global population to monitor the COVID-19 circulation and advisory interventions (Ahmed et al. 2020). As such, extensive testing is insisting on knowing the potential routes of the viral transmission, which is very expensive. Therefore, monitoring the spread of COVID-19 is time in communities through the wastewater-based epidemiology (WBE) approach is useful (Kitajima et al. 2020; Randazzo et al. 2020). Additionally, this could possibly provide quick results for effectual and urgent interference in the combat against COVID-19.

The WHO declared, as of 29 May 2020, over 5 million cases of COVID-19 cases were identified globally with 362,614 deaths and 2,596,004 people recovering (Daughton et al. 2020). Eventually, SARS-CoV-2 prevention interference which consists of personal cleanliness, accurate sanitation, hand washing and sanitizing cannot be separated from a safe supply of water. Hence, water and wastewater industries amidst of addressing this global pandemic are going to revel in widespread economic impacts (Vera et al. 2014).

The COVID-19 pandemic has led to a paradigm shift of the world's societal and vital activities with a deep transformation of human life, which mystifies a global threat to socio-economic growth and development (Ahmed et al. 2020; 1Vera et al. 2014). The risk and routine of waste collection and wastewater as an ecological response to address COVID-19 complications in water and wastewater settings has received little attention (Annalaura et al. 2020). In response, this study make known WBE to monitor the COVID-19 spread and its threats to public health, while examining the populated pooled wastewater to extenuate COVID-19 complexity.

In the past years, over 1500 pathogens have already been discovered, with almost 40 of them considered to appear from communicable

diseases with a major effect on communities (Annalaura et al. 2020; Nghiem et al 2020). These comprise severe and acute respiratory syndromes (SARS) (2002–2003), H1N1flu/swine flu (2009–2010), Ebola (2014–2016), Zika virus (2015–2016), and COVID-19 (2019–2020) (Venugopal et al. 2020). As it stands, the search for vaccines for recurrence of this COVID-19 is seems far-reached. The COVID-19 caused an international ban at various airports and seaports on travel and social gathering, practicing quarantine protocols, school and church closures, and closures of non-essential industries (Quevedo-leon et al. 2020). As a direct effect of COVID-19 to the global economic system, countries along with South Africa and the United States of America are expected to suffer from huge financial losses of billions of dollars because of revenue discounts of their water utilities (Delgado et al. 2011). Upon this foundation and many extra anticipated in days to return, studies into figuring out and developing a strong era within the water and wastewater remedy settings to diminish the COVID-19 complications are useful.

Testing methods of Corona virus

Currently, CT imaging, hematology tests and molecular methods based on viral genetic material measurements are the primary method used for clinical diagnosis of COVID-19, together with the identification of clinical symptoms to confirm infection (Jin et al. 2020). These laboratory tests are necessary to control the spread of the disease. An RNA-based metagenomic next generation sequencing (mNGS) approach was used to describe the sequence of SARS-CoV-2 without delay after the initial outbreak (Chen et al. 2020), but it is restricted by throughput, turnaround time, high cost and a requirement for high train technical expertise. Rapid detection approaches could introduce in an era of point-of-care testing (POCT) or in-field screening of viruses.

Currently two main testing are being used for COVID-19 globally nucleic acid testing and antibody testing (Corman et al. 2020), Polymerase chain reaction (PCR)-based nucleic acid testing looks for viral RNAs in upper respiratory specimens (throat and/or nasal swabs) from an individual. The quantitative reverse transcription PCR (qRT-PCR) has gradually become the current gold standard for the diagnosis of SARS-CoV-2 infection. SARS-CoV-2 genes such as *ORF1ab* (open reading frame), *RdRp* (RNA-dependent RNA polymerase gene), *E* (envelope protein gene), and *N* (nucleocapsid protein gene) can be targeted for diagnosis. The general protocol of qRT-PCR is based on the extraction of RNA from respiratory swabs dissolved in viral transport media (VTM), and subsequent one-step reverse transcription and real-time qRT-PCR targeting one or several gene sequences from SARS-CoV-2 (Zhu et al. 2020). Researchers have tried to simplify this current protocol by avoiding the RNA extraction step based on direct nasopharyngeal swab VTM heating before the qRT-PCR, which may provide viable options to overcome any supply chain issues and help to increase the testing throughput (Alcoba-Florez et al. 2020).

Other new RNA-based methods for SARS-CoV-2 detection have also been developed to tackle this crisis, such as Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) technique (Lamb et al. 2020), evaluated a RT-LAMP assay for the SARS-COV-2 within 30 min using primers targeting *ORF1ab* and *S* (spike) genes, with a LOD of 2 × 101 copies and 2 × 102 copies of RNA per reaction, respectively. No cross reactivity was found with another 60 respiratory pathogens. The sensitivity (true positive rate) for clinical specimen diagnosis (n=130) was close to 100% (95% CI 92.3%-100%), as was its specificity (95% CI 93.7%-100%). Compared to qRT-PCR, RT-LAMP is faster and does not require prior RNA isolation from the samples. The reagents for RT-LAMP are relatively cheap and stable at room temperature, and therefore this technique holds promise for use outside of a central laboratory (in-field detection) by staff without special training and without need of advanced equipment (Park et al. 2009).

A colorimetric-LAMP method was reported using pH sensitive dyes to visualize the LAMP amplification *via* the change in pH resulting from proton accumulation due to the incorporation of deoxynucleoside triphosphates (dNTPs). A sensitivity/LOD as low as 4.8 copies/μL was achieved in testing RNA samples purified from patient respiratory swabs, and the results were in 100% agreement with those from the qRT-PCR method. This effort expands the toolbox of molecular tests beyond sophisticated diagnostic laboratories in aiming to combat and monitor the growing public health threat. Additionally, digital PCR (dPCR) has been reported to improve the LOD to at least 10-fold lower than that of RT-PCR, and overall accuracy in the clinical detection of 109 samples was reported to be 96.3%, suggesting the potential of dPCR for the detection

of asymptomatic and suspect patients (Liu et al. 2020).

Antibodies based in the patient's blood are another modality for COVID-19 detection. Antibody test kits are usually designed for the qualitative detection of IgM and/or IgG antibodies to SARS-CoV-2 in a given serum, plasma (EDTA, citrate) or venipuncture whole blood specimen from a patient. The lateral flow test strip (LFTS) or lateral flow immunoassay (LFIA) is widely used for this purpose. This is a simple cellulose-based device employing chromatographic lateral flow which is intended to detect the presence of a target analyte (antibody to SARS-CoV-19) in a liquid sample (blood/serum/plasma, etc.) without the need for specialized personal and costly equipment although lab-based equipment can be used to achieve higher sensitivity (Posthuma-Trumpie et al. 2009).

It usually contains a sample pad, a conjugate pad, a nitrocellulose membrane and an absorbent pad. The sample pad is exposed to the sample (a mixture of blood cells, vesicles, cell debris, antibodies, small molecules, etc.) and acts as a filter to promote lateral aid flow. The sample rehydrates the pre-immobilized gold conjugated recombinant antigen (i.e. spike protein or its receptor binding domain (RBD) on the conjugate pad and the antibodies bind with their matching antigens. Due to capillary force, the sample continues to flow along the nitrocellulose membrane to reach the test line and the control line. The absorbent pad will absorb excess sample fluid. Colloidal gold nanoparticles are often used for colorimetric visualization, but colored latex nanoparticles, fluorophores, etc., can also be used (Sajid et al. 2015).

SARS-CoV-2 antibody based test strips which are fast and at relatively low cost. For example, a rapid and point-of-care lateral flow immunoassay has been developed for the simultaneous detection of IgM and IgG antibodies against SARS-CoV-2 virus in blood within 15 min (Li et al. 2020). A chemiluminescence-immunoassay has also been reported for the detection of SARS-CoV-2 infections and surveillance of changing antibody patterns based on the recombinant nucleocapsid antigen and magnetic beads. Clinical IgG testing identified 65 SARS-CoV-2 infections from 79 confirmed patients and only two false-positive cases from the control group (n = 80) with sensitivity and specificity values reaching 82.3% and 97.5% respectively.

In addition, a colloidal gold-based immune chromatographic (ICG) strip test detecting viral IgM or IgG was carried out with 134 samples from 105 patients, and a sensitivity of 11.1% was achieved at the early stage (1-7 days after onset), 92.9% at intermediate stage (8- 14 days after onset) and 96.8% at late stage (more than 15 days) (Pan et al. 2020). However, the specificity was not evaluated for this ICG assay. Accordingly to a multi-center cross-sectional study, the positive rate for IgG from antibody testing could reach 100% at around 20 days after symptom onset, confirming the strong ability of antibody testing kits for use in late-stage infections. The positive rate of serum IgG single testing has been reported to be higher than that of IgM alone in COVID-19 detection, but the detection of both IgG and IgM was shown to be more accurate (Li et al. 2020). Antibody test kits are not yet available for home testing but do allow testing in laboratories or by healthcare workers at a point-of-care.

Antibody testing cannot confirm the presence of the virus. Positive results mean acquired immunity against COVID-19 infection, which might be ascribed to past or present infections with non-SARS-CoV-2 strains such as coronavirus HKU1. In contrast, negative results do not rule out SARS-CoV-2 infection, particularly for those who have been in contact with virus carriers. IgM was found to be detectable in patient's blood after 3-6 days post-infection, withIgG detectable after 8 days (Xie et al. 2020). Hence antibody testing is useful at the intermediate or late stages rather than the early stage of infection (Pan et al. 2020).

In a word, this rapid screening tool is more suitable as a complementary method to nucleic acid testing (especially for negative results) by providing important immunological evidence for physicians to make diagnostic and pre-treatment decisions, but not as a sole basis for the diagnosis or exclusion of COVID-19 infection. Notably, once a vaccine for COVID-19 is available and people become immunized by vaccination, antibody testing may not be able to differentiate those who acquire immunity from those infected ones (Xie et al. 2020).

These all antibodies based and molecular base testing has some limitations for this purpose: whole coronavirus particles instead of pure antigen proteins need to be tested in short time and take control of the pandemic of COVID-19. The early studies have proof, concept and demonstrated the potential of Gr-FET technology for sensitive and rapid detection of coronaviruses.

Graphene field effect transistor immuno sensor

A key message from the WHO in early March is: 'test, test, test'. Testing is particularly a quick detection which is ultimately fastidious and a potent way to monitor. Rapid testing also cooperates in effective allocation of medical resources in hospitals and secures time for frontline health workers. Specifically for low-income countries, fast, affordable, in-field and point-of-care testing can have perceptible results in controlling the spread of the disease where health systems may be weak and approach the medical treatment boundary, and handle the pandemic before vaccines or effective drugs become available. Accordingly, rapid, affable, cost-effective and easy detections for large-scale screening, in-field testing and point-of-care diagnosis of the disease are of majestic importance and urgency for quickly controlling the highly contagious and rapid spread of COVID-19 (He et al. 2020).

Accordingly, rapid, affable, cost-effective and easy detections for large-scale screening, in-field testing and point-of-care diagnosis of the disease are of majestic importance and urgency for quickly controlling the highly contagious and rapid spread of COVID-19 (Jin et al. 2020).

The roles of biosensors for quick and facile detection of SARS-CoV-2 covering viral RNAs, surface antigens/whole viruses, antibodies and potential biomarkers detection is advantageous. Biosensors as elegant analytical devices uniting specific recognition of target and sensitive readout of signals can help in rapid, facile and cost-effective detection of COVID-19 in the field and at a point-of-care.

In addition, the quick detection of viral genetic components in swabs using biosensors, it would be interesting to appliance the direct and facile sensing of the whole virus particle or it's harmonized surface antigen epitope. This scheme could be applied for the development of a diagnostic or screening tool for COVID-19. There has been some applicable research studies reported so far. For example, an ultrasensitive graphene field-effect transistor (Gr-FET) immuno sensor was reported earlier as an effort towards simple and rapid screening for COVID-19 (Chen et al. 2020). The graphene surface was functionalized with SARS-COV-2 spike S1 subunit protein antibody (CSAb) or ACE2 receptor. The hybridization of the slightly positively charged S1 protein (which contains a receptor binding domain, RBD) with the immobilized CSAb/ACE2 receptors modify its conductance/resistance *via* field effect, which can be electrically read out in a perceptive way. This Gr-FET immunosensor can rapidly identify (in about 2 min) and accurately snatch the COVID-19 spike protein S1 at a LOD down to 0.2 pM, in a real-time and label-free manner. An early proof of concept study and prove the possibility of Gr-FET technology for sensitive and rapid detection of coronaviruses. Interestingly, another field-effect transistor (FET)-based graphene bio sensing device coated with a specific antibody against SARS-CoV-2 spike protein has been reported for direct detection of SARS-CoV-2. The introduced virus particles onto the antibody coated graphene surface generated readable electric changes (Figure 1).

This COVID-19 FET sensor not only detects SARS-CoV-2 antigen protein transport medium for swab samples, but also detects cultured viruses and viruses in clinical samples (Seo et al. 2020). Therefore, testing based on antigen/whole virus by graphene FET is needed to develop as early as possible and increase the detection of COVID-19 in a short time.

Increased access to COVID-19 testing has allowed increased monitoring of the community spread, but several diagnostic challenges remain. Currently, the standard testing method is viral nucleic acid real-time polymerase chain reaction (RT-PCR), which is a slow process and requires expensive equipment and trained technicians for nasopharyngeal swab sample collection and analysis (Li et al. 2020). In addition, the sheer volume of testing required is overwhelming the ability for healthcare systems to report RT-PCR results to patients, causing, in some states, delays of ~7-10 days to inform positive10 findings and enact isolation and monitoring protocols. Despite the recent advances on point-of-care (POC) rapid RT-PCR test, 11–15 nucleic acid tests are also known to produce false negatives, which may limit containment strategies and access to treatment (Yang et al. 2020). An additional consideration for RT-PCR is that it only identifies active carriers of the virus. Identifying convalescent persons based on COVID-19 antibody presentation is equally important as it may provide health officials with crucial information regarding the potential impact of reopening measures (Sethuraman et al. 2020). Serologic assays detect circulating antibodies specific to SARS-CoV-2 antigens, including the nucleocapsid protein and the outer spike protein (Li et al. 2020; Lisboa Bastos et al. 2020).

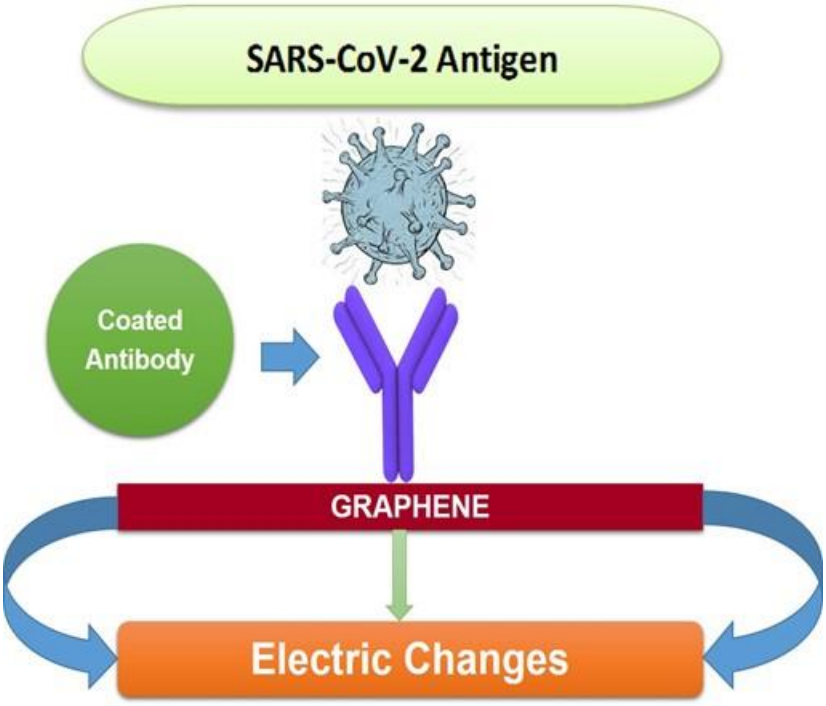

Figure 1: Basic design of graphene field effect transistor (Gr-FET) immunosensor for SARS-CoV-2 antigen detection

However, it is not possible to differentiate between asymptomatic carriers and immune persons using antibody detection. Therefore, to effectively mitigate the risks of COVID-19 community spread, systems are required that determine simultaneously both the viral and serologic status of an individual. There is a clear and urgent need for a highly sensitive, rapid, inexpensive, telemedicine COVID-19 test that can identify a patient's past and present infection status (Morales-Narvaez et al. 2020).The detection of SARS-CoV-2 in medical samples was performed using a field-effect transistor (FET)-based bio sensing gadget, and its performance/efficacy was evaluated by applying a cultured virus, antigen protein and nasopharyngeal swab samples from COVID-19 patients. The FET biosensor exhibited a suitable sensitivity for the identification of COVID-19 with no sample pretreatment or labeling, but different materials may be considered for improvement of the signal-to-noise ratio. Importantly, the device showed no measurable cross reactivity with the Middle East respiratory syndrome coronavirus (MERS-CoV) antigen, demonstrating the remarkable potential of this sensor to identify the SARS-CoV-2 antigen protein from that of MERS-CoV (Seo et al. 2020). Considering the availability of current diagnostic approaches, field-effect transistor (FET) based biosensing platforms have many promising benefits such as the capability to be very sensitive and to detect small volumes of target analyte instantaneously. These biosensors have potential use in clinical analysis, point-of-care tests, and on-site diagnostics (Liu et al. 2019). Graphene with the hexagonal carbon atoms exposed on its surface, being electronically conductive, having high charge mobility and specific surface area, has proved to be ultrasensitive in sensing systems owing to its capability to detect nearby variations on their surface and to provide an ideal sensing platform. Therefore, graphene based FET biosensors are very important to carry out the immunological diagnosis with high sensitivity. In this regard, they have successfully fabricated a device based on FET technology for the detection of SARS-CoV-2 in clinical specimens. The FET biosensor was able to detect SARSCoV-2 spike protein 1 fg/mL in phosphate buffer saline and 100 fg/mL clinical transports medium. Additionally, FET biosensor performed very well in detection of SARCoV-2 in self-cultured medium and nasopharyngeal swab samples with detection limits of 1.6 × 101 plaque-forming units/mL (pfu/mL) and 2.42 × 102

copies/mL. Interestingly, the fabricated bio-sensing device showed no quantifiable cross-reactivity with MERS-CoV antigen (Seo et al. 2020; Asif et al. 2019), early detection and diagnosis techniques are mandatory in order to enhance the control on infection, treatment, and new vaccine discovery.

CONCLUSION

The current study helped to explain, conceptualize and demonstrate the capacity for responsive and rapid identification of coronaviruses by graphene Field Effect Transistor (FET) technology. Therefore, extra accurate, rapid response, cost-effective and readily available analytical methods or diagnostic approaches are crucially needed. The biomarkers and measures used for COVID-19 diagnostics or the diagnosis of SARS-CoV-2 have been objectively reviewed and argued. Ultra-sensitive grapehene FET biosensors are powerful instruments in the early detection of COVID-19 infection to measure clinical success and to increase understanding of the seriousness and vital patterns of infection through targeting S1 virus protein.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All the authors contributed equally.

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