Application of Molecular Imprinted Polymer Technology to detect the SARS-CoV-2 Virus through the Development of a Novel Breathalyzer-like Device

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I. ABSTRACT

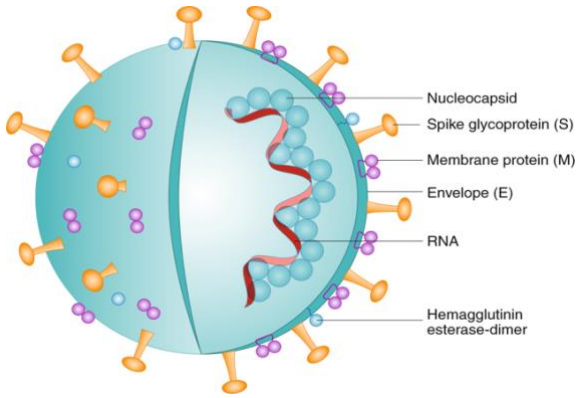
The COVID-19 pandemic has resulted in over 2 million deaths and affected around 100 million people worldwide (CDC). Across the globe, a pandemic warning system had failed when the SARS-CoV-2 virus hit. The key reason behind the ineffective response or unpreparedness is the lack of rapid diagnostic testing at the onset of the pandemic that could have been used to widely detect the presence of the virus. The lack of a highly sensitive rapid test, forces laboratories and hospitals to use conventional nucleic acid testing methods which are time consuming, labor intensive and expensive [4]. To solve this problem, we propose a 'breathalyzer-like 'device that will be able to detect the SARS-CoV-2 virus in seconds while being extremely user friendly, highly sensitive and cost effective. This breathalyzer-like device will detect SARS-CoV-2 by the binding of molecularly imprinted polymers (MIPs) to the virus's spike protein. MIPs are synthetic receptor molecules that can mimic ACE-2 receptors and offer an inexpensive, rapid, sensitive, easy-to-use, and highly selective receptor for electrochemical biosensors [8]. MIP based detection has been widely used in literature in the detection of human viral or bacterial pathogens which has shown >90% accuracy.

II. INTRODUCTION

The Covid-19 pandemic is an ongoing global health crisis, with a staggering death toll that continues to increase. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified in Wuhan, China in December 2019. The viral outbreak was categorized as a pandemic in March, 2020 by the World Health Organization (WHO). As of now, according to the numbers reported by every country to the WHO, the total number of cases has surpassed 122 million and over 2.7 million deaths have been attributed to it. The pandemic has had an adverse impact on the international community, not only affecting the global economy, but also, mental health, employment, work, education, and political aspects as well. In order to counter the spread of the disease many forms of restrictions were implemented globally, including regional and country wide lockdowns, quarantines, travel bans, and evacuation of foreign citizens.

Figure 1: [14] Schematic representation of the SARS-CoV-2 virus. An enveloped positive-sense RNA strand with spike (S) and membrane (M) glycoproteins, as well as envelope (E) and nucleocapsid (N) proteins.

All coronaviruses have an animal origin, either from bats or rodents. The SARS-CoV-2 virus either originated through natural selection in an animal host before zoonotic transfer to humans or natural selection in humans after zoonotic transfer [15]. The pangolin is believed to be the intermediate host of SARS-CoV-2 originating from bats and finally transferring to humans as it was illegally sold in the Chinese wet markets. The SARS-CoV-2 virus, based on sequence alignment and



evolutionary tree analysis, has been deduced to be a member of the group B viruses, of genus Betacoronavirus in the family of Coronaviridae, of the order Nidovirales [16]. It is an enveloped, single-stranded RNA virus. The genomic sequence of this newly

emerged virus is similar to that of SARS-CoV with a 79.6 percent sequence identity [20] It consists of four structural proteins, spike, envelope, membrane and nucleocapsid proteins [15]

The spread of the virus occurs via droplet transmission that is produced when coughing or sneezing, through personal contact, or by coming in contact with contaminated surfaces [12]. It may also be transmitted in the form of aerosol particles, by liquid droplets that convert into numerous smaller particles [20]. The virus binds to epithelial cells in the respiratory tract, replicates and begins to migrate down to the airways, entering alveolar cells. This rapid replication may trigger a strong immune response [23]. The incubation period of the onset of symptoms ranges from 1 to 14 days. It has a broad effect on people, ranging from an asymptomatic infection or a mild illness of the respiratory tract, and may go up to severe pneumonia, respiratory failure, and eventually death [15]. Patients with a median age of 59 and above, with people with pre-existing disease are at a greater risk [23]. According to a study, there was a high viral load in the upper respiratory tract, during the first week of symptoms, thus the transmissibility was high due to virus shedding, and it was also recorded to be high during mild infections or asymptomatic stages [23]. The mortality of the virus depends on factors such as sex, ethnicity, and outdoor environment, such as the level of air pollution [25].

Across the globe, a pandemic warning system had failed when the SARS-CoV-2 virus hit. The key reason behind the ineffective response or unpreparedness is the lack of rapid diagnostic testing at the onset of the pandemic that could have been used to widely detect the presence of the virus. The lack of a highly sensitive rapid test, forces laboratories and hospitals to use conventional nucleic acid testing methods, which are time consuming, labor intensive and expensive. Hence our team proposes a simple handheld breathalyzer-like device that allows for a rapid detection of COVID-19 by the binding of molecularly imprinted polymers to the spike protein on the surface of the virus. The device will act as a rapid point of care or at home diagnostic test that is highly sensitive and specific while being cost effective. The proposed device will be easy to use, where the user will only need to exhale particles in a tube. The device comprises a MIP modified graphene electrode system that will detect the resistance change and a transducer will convert the physical signal to an electrical signal. This electrical signal can then be detected and the device will be able to output the results into the user's smartphone via bluetooth, resulting in a positive or negative result for the virus being detected.

1. Rapid Testing for COVID-19

Rapid COVID-19 tests are a type of COVID diagnostic that yield quick results (usually less than 15 minutes) and are most accurate in patients who have symptoms and are most contagious [25]. Rapid tests are extremely important at this stage of the COVID-19 pandemic because they are able to identify the virus when you are most likely to transmit it [21]. This is critical because people are starting to travel and spend more time with their friends and family as the pandemic progresses and the COVID-19 vaccines are being distributed. So, having a quick, accurate, affordable, and easy to perform test will help to prevent the unknowing spread of the virus to loved ones. Some of the currently available and soon to be available rapid COVID-19 tests are compared in Table 1. All of these tests have been granted Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration (FDA).

Table 1: Comparison of Different Rapid COVID-19 Tests

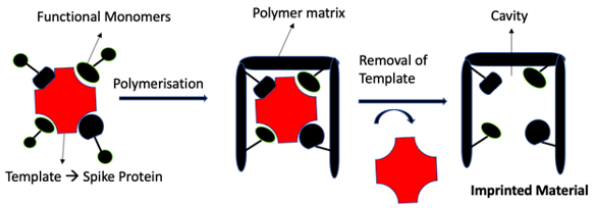
Name of Test	Type of Test	Cost (USD)	Sensitivity	Time to Results (mins)	Specificity
Ellume COVID-19 Home Test [10][11][13]	Antigen- Nasal Swab	30	91-96%	15-20	97%
BinaxNOW COVID-19 Ag Card [6]	Antigen- Nasal Swab	5	71.1%*	15	96.9%
Lucira COVID-19 All-in-One Test Kit [12]	Molecular-Nasal Swab	50	94.1%*	30	98%
Cue COVID-19 Test [7]	Molecular- Nasal Swab	Not Set	95.7%	20	97.6%
ID NOW COVID-19 [9]	Molecular- Nasal or Oral Swab	Varies	93.3%	13	98.4%

*Sensitivity was calculated for symptomatic individuals.

The tests displayed in Table 1 are able to be used as point-of-care or at-home tests and all of them require a swab sample from the respiratory tract. The device that we have proposed is also a point-of-care test, but it uses samples from the breath rather than a more invasive method. This is advantageous because many people experience discomfort with tests that require nasal swabs and thus may view a breath test as more desirable. Our device also can obtain results within 30-35 second, which is much quicker than currently available rapid tests that typically require 15 minutes for the results [3]. Furthermore, the sensitivity and specificity of our device is expected to be 90% or greater, which is comparable to the other rapid testing methods being produced (see section 3 for specifics on the proposed device).

There are also new tests being developed that use breathalyzer-like technology similar to our proposed

device. One such test being developed by Texas A&M University and Worlds Inc detects the volatile organic compounds within a patient's breath that are present when the body is fighting infection to determine whether or not they should be tested further for COVID-19. After further development of the device it also may be used as an alternative for the PCR test (which is currently the gold standard for COVID-19 testing) [28] Another



breathalyzer-like device being developed is from Scentech Medical, which is also able to detect the volatile organic compounds in an individual's breath. This device is said to have over 90% accuracy in detecting COVID-19 and is also able to differentiate between samples that are positive, negative, and those that have COVID-19 antibodies. [32] This device uses gas chromatography, mass spectrometry, and a ReCIVA collecting device to detect the presence of volatile organic compounds specific to those infected with COVID-19 [33] these tests both use volatile organic compounds to detect the presence of COVID-19 infection, while our device uses MIP technology. The use of MIPs allows our device to be highly specific to the COVID-19 virus and be very inexpensive relative to these devices. The application of this MIP technology is explained further in the next section.

Figure 2: *[1]* Formation of an MIP binding cavity. Immobilization of a template protein on a surface, formation of MIP around the template, elution of template protein to reveal empty binding cavity.

2.Application of MIPs

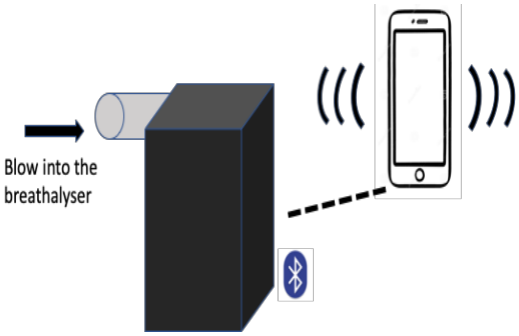
The proposed breathalyzer-like device is a rapid diagnostic test for COVID-19 that is primarily based on molecularly imprinted polymer technology. MIPs are functional synthetic receptor molecules with specialized molecular target selectivity. MIPs offer an inexpensive, rapid, sensitive, easy-to-use, and highly selective receptor for electrochemical biosensors.[8] MIPs have high selectivity and specific recognition capabilities for a target molecule called a template. The molecular imprinting process essentially involves three main steps: (i) self-assembly of template and functional monomer molecules through covalent or non-covalent bonds, (ii) polymerization of template-monomer complex with cross-linking monomers and (iii) template removal to unveil a binding cavity that is specific to the imprint molecule [1]. This study will implement self assembly, a non-covalent method for polymerisation of the MIP layer. An immunosensor may seem like an attractive alternative to an MIP-based sensor but, biomolecules used in immunosensors are highly unstable (chemically and physically) which prevents their use in harsh environments. There are several advantages of MIPs: they are physically and chemically stable, hence, can be stored for a long period of time in dry state at room temperature. They are low cost, easily reproducible and require simple preparation compared to their biological counterparts.

MIPs have been widely researched and well established in detection of human viral pathogens. MIPs are also known to detect Hepatitis A virus using resonance light scattering (RLS) as a sensing platform [3]. Electrochemiluminescence in combination with functional nanoparticles provide binding sites located on imprinted surfaces for fast recognition of the HIV virus [1]. Molecularly imprinted polymers have been used for molecular binding and screening of the influenza virus H5N1 [31]. Influenza, commonly known as the seasonal flu, is a viral infection that attacks the respiratory system. Wangchareansak et al, combined MIPs with quartz crystal microbalance (QCM) to screen the various influenza virus subtypes H5N1, H5N3, H1N1, H1N3 and H6N1 [1]. QCM transducer allows for fast signal recognition and increased sensitivity of detection. MIP layers were created for each virus subtype which reportedly showed great recognition properties to the original virus template and were offered as molecular fingerprints. Not only does the study provide a way to identify inhibitors, antibodies and substrates that reduce the functionality of the virus through a conformational change, it also allows for an alternative rapid diagnostic for influenza virus A subtypes in unknown samples with detection limits as low as 105 particles/mL [31].

Furthermore, MIPs are a popular way to detect the dengue virus. Dengue virus is a single stranded positive RNA based virus that is about 100nm in size. Recently, Arshad et al. have developed an impedimetric sensor for the early detection of dengue virus. The sensor composed of screen-printed carbon electrodes (SPCE) modified with electrospun nanofibers of polysulfone and coated with dopamine while using NS1 (non-structural protein 1—a specific and sensitive biomarker for dengue virus infection) as template during polymerization [2]. Cyclic voltammetry (CV) measurements were taken to measure the electrochemical properties of MIP modified

electrodes. The impedimetric sensor showed selective detection of NS1 concentrations with a detection limit as low as 0.3ng/mL [2]. MIP based detection has been widely used in literature in the detection of human viral and has shown 90% accuracy. Not only are MIPs a promising technology in the aqueous environment, they also show potential for detection of substances in the gas phase (exhaled breath). Emam et al, conducted a study which detects butylated hydroxytoluene (BTH) (volatile organic compound in the breath) within 60s in gas using a molecularly imprinted graphene based electrochemical sensor [27]. The binding of BTH to the MIP layer displays an increase in resistance change caused by an electron transfer to the graphene layer which can be measured within 30-35 seconds. MIPs are valuable technology that can also detect molecules in the air. The critical literature review forms the basis of the technology used in the breathalyzer-like device that will allow for viral detection in the breath.

The breathalyzer-like device that is proposed in this paper will be consist of an MIP layer on a graphene sheet which in turn will be immobilized onto a carbon electrode. (See figures 3 and 4) The electrode will be attached to an electronic sensor that will detect a



resistance change upon binding of the spike protein, this resistance change will be measured through an electrochemical detection method. The composition of the device and its working principle are described in more detail in the next section.

III. METHODS

The breathalyzer-like device will detect SARS-CoV-2 by the binding of molecularly imprinted polymers (MIPs) to the virus's spike protein. The MIPs will be synthesized through polymerisation of functional monomers around a template of the spike protein via noncovalent interactions [19]. The spike protein will then be extracted, resulting in a porous polymeric network with binding cavities fitting the size, shape and functionalities of the spike protein. [22] The nature of MIPs allows the cavities to be changed based on what is needed to be detected, therefore allowing future changes to the MIPs depending on what the user wants to test for.

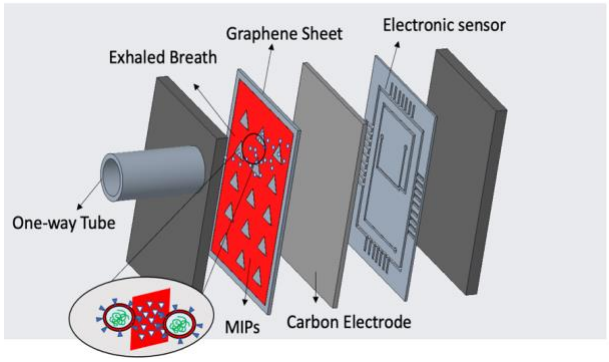


Figure 3: *Device Composition*

To use this device, a person would blow into a tube that collects their exhaled particles. Within the device, there would be a graphene sheet connected to a MIP layer. Graphene has a high electronic conductivity and high carrier mobility, which increases the sensitivity of the device. The graphene sheet will be immobilized on a glassy carbon electrode which will connect to an electronic sensor to detect resistance change. When the target molecule (viral S protein) is trapped in the MIP layer, an extra electron is transferred to the graphene layer, which causes a measurable resistance increase within 30-35 seconds, bringing an extremely fast result rate. This increase in resistance will show a positive result for SARS-CoV-2. The electronic sensor will consist of a transducer which will change the physical resistance change to a detectable electrical signal, similar to an ohmmeter. The electrical signal will be detected using an electrochemical detection method and the results will be directly linked to the smartphone of the user via bluetooth.

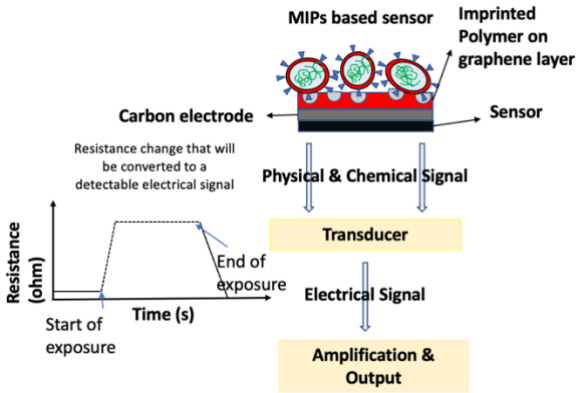


Figure 4: *The working principle behind the breathalyzer-device for COVID-19*

A breathalyzer-like test provides an extremely easy and user-friendly method of monitoring the COVID-19 virus. This device can be accessible to the public through various ways, some of the sectors which will result in in airports for instant detection of a COVID patient, it can be used by college students as a method to check Since the MIPs are similar to molds, after being removed of prior particle bounds, the breathalyzer can be reused for regular testing of individuals. The device does not require any training or a testing facility to obtain results, allowing for the general public to test themselves without prior research knowledge. In order to test the device, green fluorescent protein is going to be used to test the affinity of the MIPs to detect the protein. With this data, we can conclude whether or not the spike protein will be able to be detected through the use of MIPs in the same fashion.

Conclusion

This paper clearly identifies the urgency of a rapid test with a high level of sensitivity and accuracy. There are already a vast number of rapid tests that exist which are efficient and cost effective but the breathalyzer device will be much more desirable as it will be patient friendly in terms of price, comfort and the accuracy of results in just a few seconds. The tests can be widely accessible and can help potential consumers in the transport sector, such as airports, bus stops and even students in school or university campuses. There will be no prior experience needed to use this device and it will be reusable. It will allow for rapid detection and close monitoring of the virus in areas of high transmissibility. With new coronavirus variants increasing day by day, it has become important for any test to be highly sensitive and adaptable. One of the advantages of using MIPs is that they can be easily molded according to the template of the molecule we want to detect. If new variants of the coronavirus require us to detect any molecule other than the spike protein, we will be able to create an MIP layer for that particular molecule in a similar fashion outlined in this paper. Future research studies can pave way for detection of the different variants of the virus through a single device with the incorporation of different MIP layers. MIPs are diverse molecules that present a promising technology. Their application to the coronavirus can help in the development of cutting-edge medical devices or tests that allow for rapid detection of virus.

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