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Patented Sensitive Nano-biosensor for DNA Hybridization Detection of Low Covid-19 Viral Concentrations in Saliva

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Abstract

The novel Severe Acute Respiratory Syndrome- Coronavirus-2, the virus causing the Coronavirus disease(Covid-19), since its discovery in December 2019, disseminated very fast resulting in a global pandemic. Following that, various testing procedures have been shown to detect the presence of the virus. The current molecular diagnostic tests are reverse transcription Polymerase Chain Reaction which looks for the viral gene in the fluid sample and the antigen test detects specific proteins in the virus. The pitfalls of these tests are high cost, low sensitivity, complexity, high time consumption, reproducibility, specificity, false negativity and late viral detection. This paper seeks to introduce an efficient, sensitive, low-cost, non- invasive, early viral detection molecular diagnostic technology for detecting low concentration Covid-19 Ribonucleic acid copies with a patented approved nano-biosensor with 95% accuracy in twenty minutes. Here, the technology detects Deoxyribonucleic acid hybridization of the complementary Covid-19 viral pathogen of specific viral gene even for low concentration of viral particles. Due to its low cost and compatibility, it can be deployed as a point of care rapid test for Coronavirus, influenza A and B or any viral/ bacterial infection in low infrastructure areas or countries, providing a powerful tool for sensitive detection of pathogens by taking advantage of the unique properties of nano-biosensors. Detection of low viral concentration can help to diagnose an early stage of an infection and can also can diagnose asymptomatic patients which prevents the rapid spread of an infection drastically.

Keywords: Covid-19, Nano-biosensor, SARS-CoV-2, reverse transcription Polymerase Chain Reaction (rt-PCR), Hybridization, Coronavirus Disease, Hybridization, Molecular, Diagnostics, ssDNA, RNA, DNA, Pathogen

Introduction

Covid-19 is the Coronavirus disease which is caused by the newly found SARS-CoV-2 virus from the Coronavirus family. The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) virus was first discovered in Hubei and Wuhan, China in November-December, 2019 after large cases of respiratory lung infections were reported in hospitals with Flu-like symptoms [1]. The World Health Organization, WHO declared it a global pandemic after many cases were detected in other countries because of its high infectious rate [2].

The two major molecular diagnostic tests for Coronavirus are the reverse transcription - Polymerase Chain Reaction and antigen tests. The rt-PCR test copies the genetic material from a sample and compares it with the specific viral gene and the antigen test shows the protein causing the disease [1].

The test probes in the rt-PCR for SARS-Cov-2(virus causing covid-19) become contaminated thereby producing many false negative results. These occur through the contamination of reagents with target sequences and cross-contamination of samples which all together due to the complexity of the test can affect its specificity [3].

The use of this molecular technology, provides a more advantageous process over the traditional reverse transcription Polymerase Chain Reaction (rt-PCR) and antigen tests. It serves as a low cost, sensitive, rapid point of care detection of Coronavirus that can diagnose asymptomatic patients and provide an early detection of the virus after viral exposure.

Materials

In this paper, the hybridization of Covid-19 RNA and complementary DNA in saliva sample is to be detected on a patented composition formulated with carbon nanotube and graphene (nano- biosensor).

The change in impedance is significant due to the high specificity

of the carbon nanotube in detecting the charge produced by very specific DNA/RNA hybridization during the hybridization process. Graphene sheet plays a critical role in transporting these charges without any loss and thus making the measurement less sensitive to other environmental parameters. The rapid diagnostic system comprises of an electrical circuit unit with eight sensing chips connected to a computer to keep it low cost. Results were analyzed using Microsoft Excel. Saliva samples were taken from forty-three patients for Coronavirus screening.

Methods

Here, with serial dilution, different concentrations of Covid-19 ssDNA ranging from 10 pico Molar to 1 femto Molar were added to form a solution with Tris-EDTA Buffer or water (100nM to 10pM). The voltage and resistance across each biosensor were measured and monitored using a self-designed electrical circuitry with a microcontroller enclosed in the 3-D printed case.

The system was maintained at a specific temperature (59 degrees Celsius) since hybridization is temperature sensitive. The test began with eight primed/coated biosensing chips (as shown in Figure 1 below) with Covid-19 ssDNA and with the aid of a pipette, the saliva sample was dropped on the chips at 250-300 seconds. The test was left to run until 700seconds (12 minutes) when the results were analyzed in Microsoft excel.

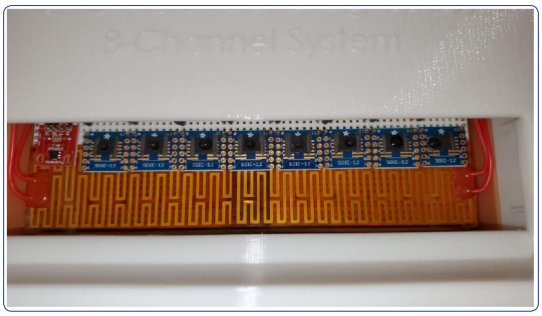

Figure 1: 8-Channel System with primed biosensing chips

Results

The hybridization process means that the Covid-19 ssDNA (referred to c-DNA) and the Covid-19 RNA virus have matched and this shows the presence of the Covid-19 viral pathogen in the saliva sample giving a positive test result as seen in Figure 2. The c-DNA is synthesized as complementary to the particular regions (ORF—Open Resource Frame) of the COVID-19 RNA. Non-hybridization depicts the absence of complementary RNA virus in the saliva sample as shown in Figure 3. This process was detected on the chips as an increase (hybridization) or decrease (non-hybridization) in voltage which can be seen on a serial monitor by the use of the Arduino software. The voltage values detected by the sensing chips were analyzed and all concentrations averaged with Microsoft Excel.

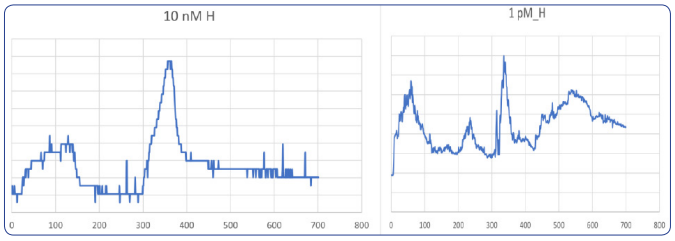

Figure 2: Hybridization graph showing a voltage change after 300 seconds with respective low Covid-19 RNA concentrations of 10nM and 1pM.

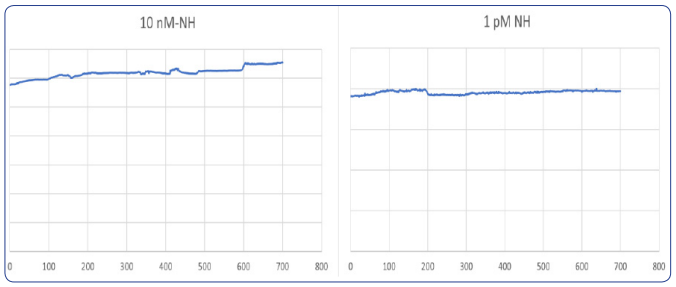

Figure 3: Non-hybridization graph showing no change in voltage after 300 seconds with respective low Covid-19 RNA concentrations of 10nM and 1pM.

Discussion

After the primed Covid-19 ssDNA chips were heated and the tests started, at 300 seconds the sudden rise in impedance stopped conductivity which resulted in an increase in the voltage. This voltage increase which signifies the hybridization of complementary Covid-19 RNA in saliva and Covid-19 ssDNA is a positive test result, which in Figure 2 is seen as a spike in the graph. In a case of no hybridization, there will be no significant change in impedance and voltage to give a negative test result as shown in Figure 3. The hybridization and non-hybridization processes were observed in all the various serially diluted Covid-19 ssDNA concentrations during the experiment. This makes the test simple and faster than the rt-PCR which needs to amplify the RNA target, making it complicated and time consuming. This technology can be used to test asymptomatic Covid -19 patients and with its low viral load requirement can aid in early detection of the virus after infection.

Conclusion

In summary, the results from the experiment show that low Covid-19 RNA copies can be detected in very low concentrations using this molecular diagnostic technology and this accounts for its high sensitivity and specificity.

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